Quality and quality changes in fresh fish

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

H. H. Huss
Technological Laboratory
Ministry of Agriculture and Fisheries
Denmark

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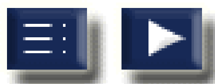

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In 1988, FAO published "Fresh fish - quality and quality changes" (FAO Collection: Fisheries, No. 29), authored by Professor Hans H. Huss. It has been published by FAO in English, French and Spanish, and apart from the original Danish version, it has been translated and published by FAO field projects into Arabic, Chinese and Vietnamese. It has served for the training of thousands of fish technologists throughout the world, in particular during the development of the FAO/DANIDA Training Project on Fish Technology and Quality Control. The broad experience gained during the training activities has been extremely useful to improve, update and enlarge whenever necessary the above-mentioned document.

After seven years, the need became apparent for a new publication on the subject. Professor Huss prepared the new document with the assistance and contribution of colleagues and experts in the various subject fields involved, as acknowledged below:

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Address:

Technological Laboratory, Danish Ministry of Agriculture and Fisheries, Technical University, Building 221, DK-2800 Lyngby, Denmark

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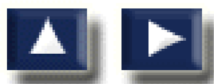


1. INTRODUCTION

World catches of fish have increased in the 1970s and 1980s but seem to have stabilized since 1988 to just under 100 million t. As the human population is ever increasing, it means that less fish will be available *per caput* every year. Nevertheless, a large part of this valuable commodity is wasted: it has been estimated by FAO that post-harvest losses (discards at sea and losses due to deterioration) remain at a staggering 25 % of the total catch. Better utilization of the aquatic resources should therefore aim primarily at reducing these enormous losses by improving the quality and preservation of fish and fish products and by upgrading discarded low value fish to food products. Very often, ignorance and lack of skill in fish handling or in the administration of fisheries are among the causes for lack of progress in this direction.

FAO has long recognized the need for training in fish technology, and since 1971 a series of training courses, financed by the Danish International Development Agency (DANIDA), has been conducted in the developing countries. In 1988 a training manual entitled "Fresh fish - quality and quality changes" was published. This book has been extensively used and is now out of print. This present book is a revised and updated version of the first publication. It still only deals with fresh fish, as it is felt that a solid background knowledge of the raw material is essential for further development in preservation of and adding value to the product. In the context of this book, fresh fish is either fish kept alive until it is consumed, or dead fish preserved only by cold water or ice.

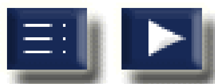
The book describes fundamentals in fish biology, chemical composition of fish and *post mortem* changes, with a view to explaining the rationale for optimal catch handling procedures and obtaining maximum shelf life. The effect of various factors (temperature, atmosphere, etc.) on fresh fish quality is discussed as are the various sensory, chemical and micro-biological methods for assessing fish quality. Wherever possible, data on tropical fish have been included.

Two new chapters, not included in the first publication, have been added. One is a description of the practical application of new and improved fish handling methods (Chapter 7) and the other is the application of the Hazard Analysis Critical Control Point (HACCP) system in a quality assurance programme for fresh and frozen fish (Chapter 9).

Fresh fish handling procedures encompass all the operations aimed at maintaining food safety and quality characteristics from the time fish is caught until it is consumed. In practice, it means reducing the spoilage rate as much as possible, preventing contamination with undesirable microorganisms, substances and foreign bodies and avoiding physical damage of edible parts.

The immediate effect of fish handling procedures (e.g., washing, gutting, chilling) on quality can easily be assessed by sensory methods. Fish quality, in terms of safety and keeping time, is highly influenced by non-visible factors such as autolysis and contamination and growth of microorganisms. These effects can only be assessed long after the damage has occurred, and the proper procedures must thus be based on knowledge about the effects of the many different factors involved. Large or small improvements are usually feasible when analysing current fish handling methods.

It is hoped that the reading of this book, combined with practical training, will be helpful in providing the stimulus which is often necessary to promote development in fisheries.



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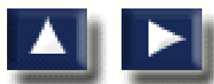


2. AQUATIC RESOURCES AND THEIR UTILIZATION

More than two-thirds of the world's surface is covered by water and the total yearly production of organic material in the aquatic environment has been estimated at about 40 000 million t (Moeller Christensen, 1968). Tiny microscopic plants, the phytoplankton, are the primary producers of organic material using the energy supplied by the sun (see Figure 2.1).

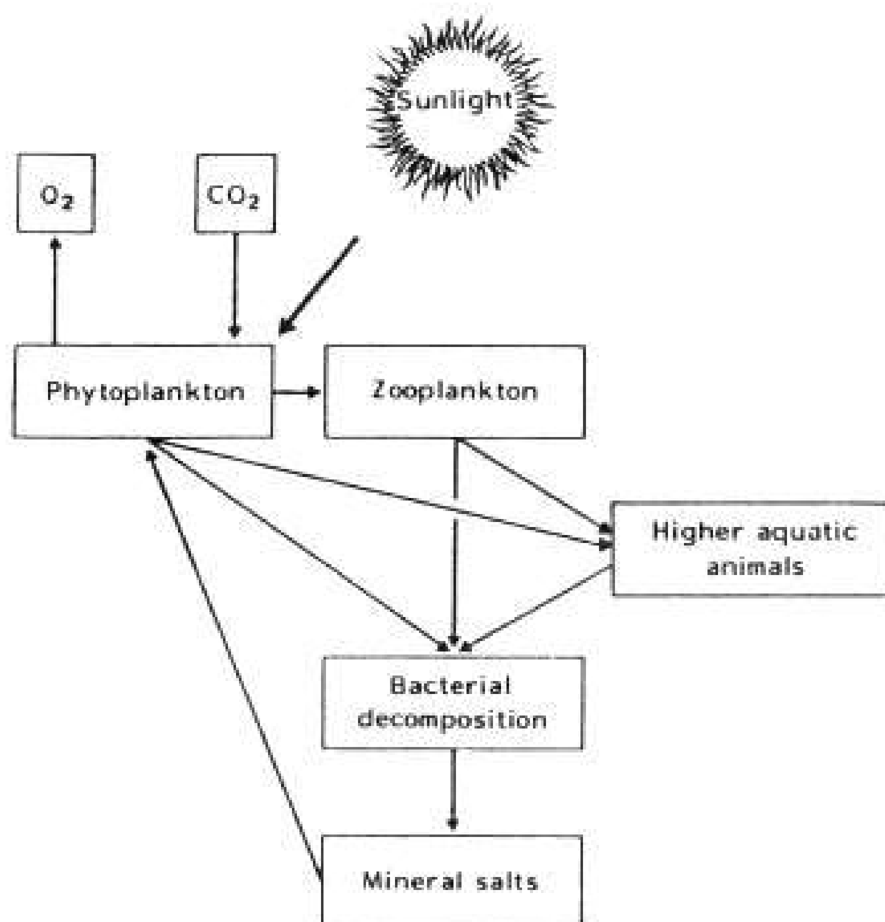


Figure 2.1 The annual aquatic production of organic material is estimated at 40 000 million t (Moeller Christensen, 1968)

This enormous primary production is the first link in the food chain and forms the basis for all life in the sea. How much harvestable fish results from this primary production has been the subject of much speculation. However, there are great difficulties in estimating the ecological efficiency, i.e., the ratio of total production at each successive trophic level. Gulland (1971) reports a range from 10 to 25 % but suggests 25 % as the absolute upper limit of ecological efficiency; for example, not all of the production at one trophic level is consumed by the next. Ecological efficiency also varies between levels, being higher at the lower levels of the food chain with smaller organisms using proportionally more of their food intake for growth rather than for maintenance. Diseases, mortality, pollution, etc. may also influence ecological efficiency. As an example, the conditions in the North Sea, an area with very rich fishing waters, are shown in Figure 2.2.

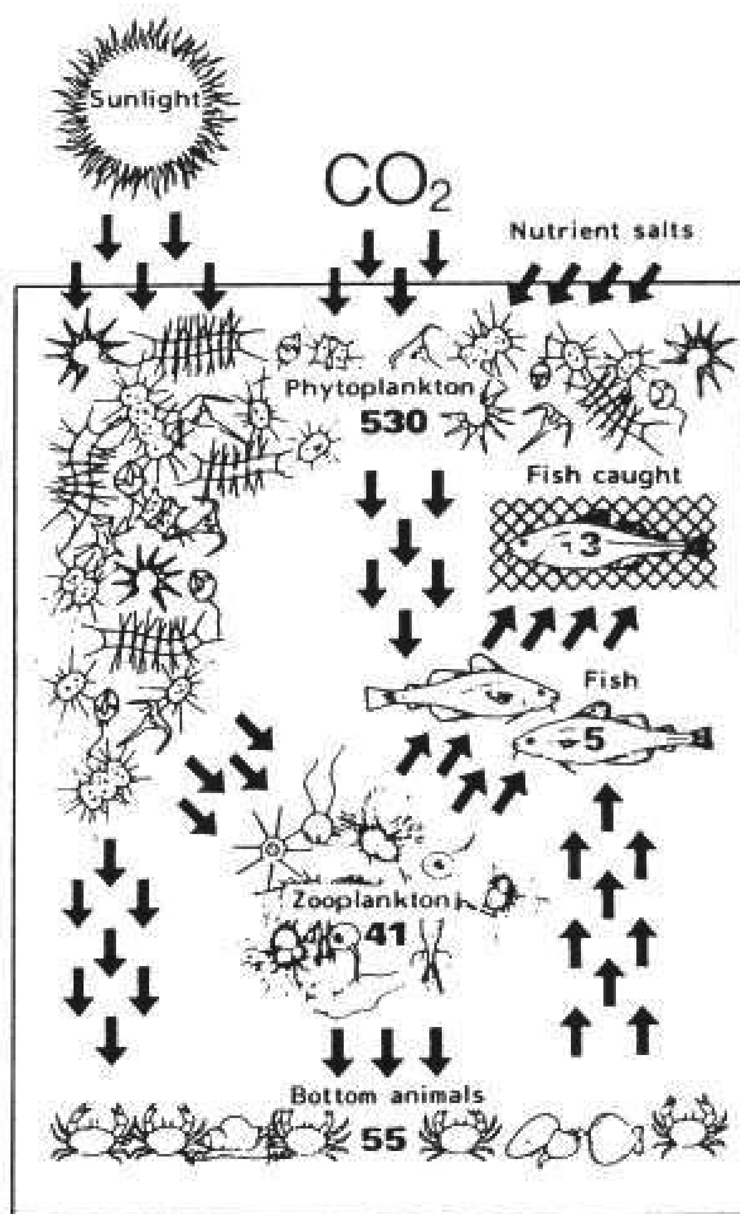


Figure 2.2 Annual production (in million t) in the North Sea, one of the richest

fishing grounds in the world (Moeller Christensen and Nystroem, 1977)

Since production is greater in the early stages of the food chain, the potential catch is also greater if harvesting is carried out at these stages.

Up to 1970, the world catch of marine fish continued to rise at an overall rate of 6 percent per year, according to FAO statistics. Great optimism was expressed by various authors who estimated the potential world catch to be somewhere between 200 million t/year to 2 thousand t/year (Gulland, 1971); most of this wide variation being due to uncertainties concerning the trophic level at which the harvest would be taken. The world fish catch since 1970 is shown in Figure 2.3.

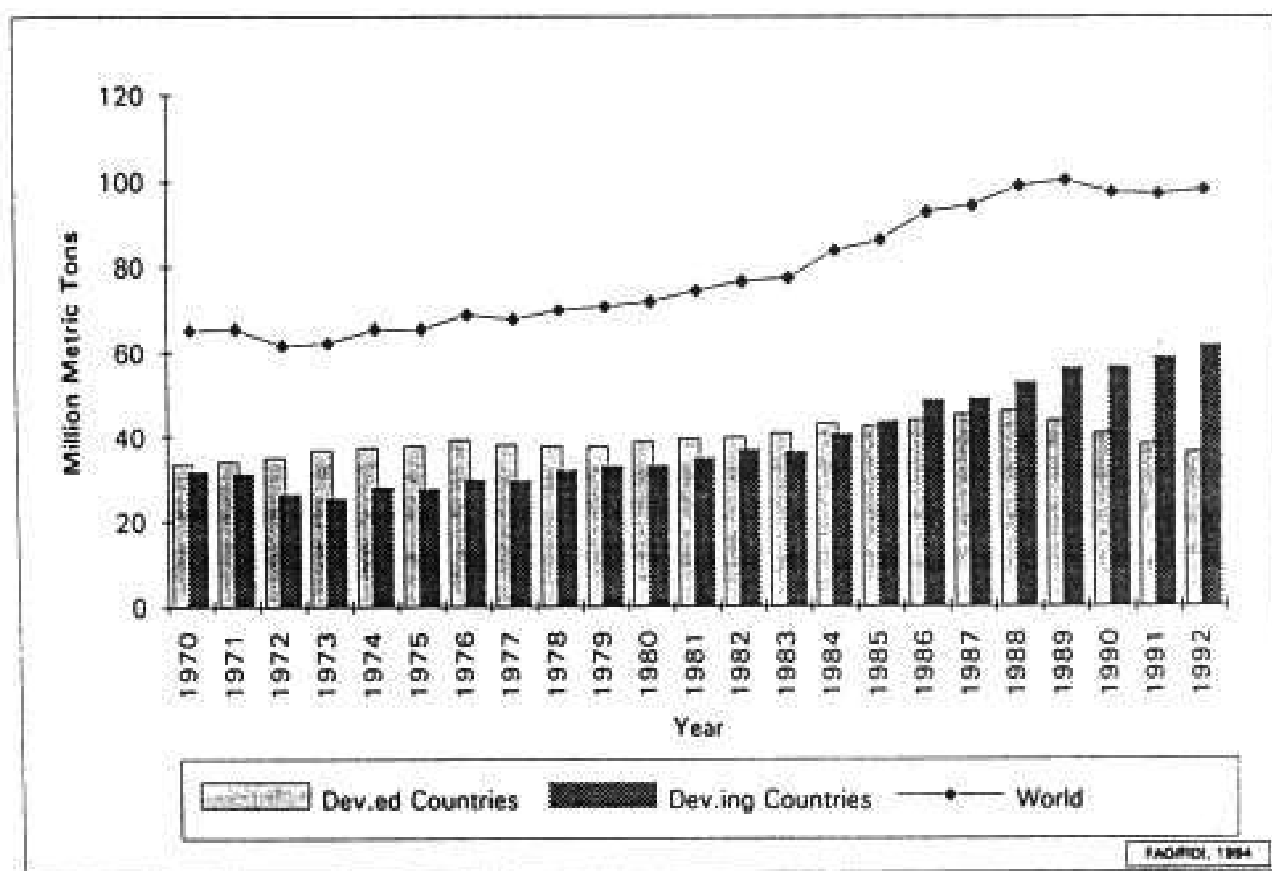


Figure 2.3 Total world fish catch from 1970 to 1992 (FAO, 1994 a)

It is clear from Figure 2.3 that the yearly increase in catches has slowed down since 1970, and the total catch reached a peak of 100 million t in 1989. Since then it has started to drop as a number of fish stocks have begun to collapse, in many cases due to overfishing. However, a slight upward trend is noticed for 1992 and for 1993 world catch is estimated to reach 101 million t. While total catch has started to decline since the peak in 1989, the catch from developing

countries as a group is still increasing and since 1985 has exceeded that from developed countries. Thus in 1992 little more than 60 % of the total world catch was taken by developing countries, and it is estimated that this figure will increase to 66% in 1993. This also means that an increasing part of the world fish catch is taken from warm tropical waters.

Are we then reaching the limits of production from "wild" aquatic resources now or do the optimistic predictions from the 1970s still hold? The answer to this question is not only in the affirmative, but for many resources the limit was reached decades earlier than the peak in global landings (FAO, 1993 a). A combination of factors has helped to mark the depletion of many conventional resources. One of these is that continued investments in fishing fleets throughout the world has meant that although catch rates and abundance of high value fish species have often declined, the overall level of fishing effort has increased so that roughly similar levels of landings are being taken at much greater cost to many fishing nations.

The real problems with decreasing fish stocks are familiar. First there is "the tragedy of the commons" - whatever lacks a known owner, whether buffalo or fish - which everyone will race to exploit and ultimately destroy.

The next problem which can be identified is the exceptionally poor management of the aquatic resources. What has been done has been too late and too little. The 1982 Law of the Sea, which extended the territorial seas from 12 to 200 miles, gave the coastal States an opportunity to take a protective interest in their fishing grounds. Instead, many of them rushed to plunder the resources by offering generous subsidies and tax relief for new vessels. Also, the much used quota-system is subject to severe criticism. Often, the net result is increased fishing and increased waste, as perfectly good fish are thrown overboard if quotas are already reached. Many fish stocks (such as pollack, haddock and halibut off New England) are now considered "commercially extinct"; that is, there are now too few fish to warrant catching.

The typical history of the use of a single fish stock has been illustrated as shown in Figure 2.4.

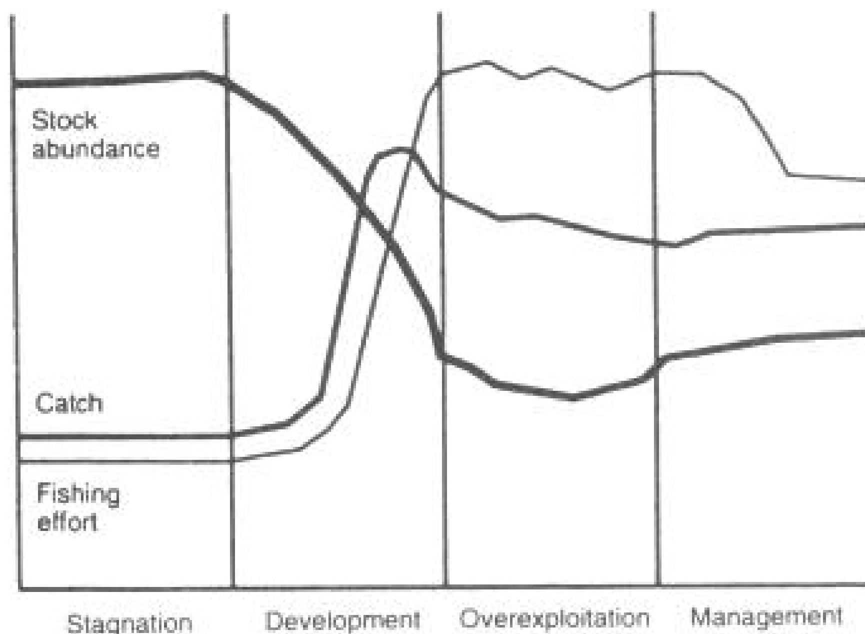


Figure 2.4 Schematic changes in stock abundance, catch and fishing effort in situations of development, overexploitation and management of fisheries. (SOURCE: Danish International Development Agency, DANIDA, 1989)

From an initial stage of under-utilization the fishing passes through a phase of rapid expansion until the limit of the resource is reached. This is then followed by a period o overfishing with high fishing effort, but reduced catches until finally - and hopefully - a phase of proper management is reached. Details on resource management are beyond the scope of this book, but should include the concept of sustainability, environmental aspects and responsible fishing. However, in an FAO publication (FAO, 1994) it is stated that change from a focus on short-term development of fishing fleets to proper management is a necessary, but insufficient condition for sustainable development. In the same report it is further stated that "Sustainable Development" as promoted at the United Nations Conference on Environment and Development (UNCED) in 1992 cannot be achieved under open-access regimes, whether these are within or outside national territorial waters.

In contrast, the world aquaculture production inclusive of aquatic plants has steadily increased over the last decade totalling 19.3 million t in 1992, almost half of this (49% is produced in marine aquaculture, 44% in inland aquaculture, and the rest in brackish environment. About 49% of world aquaculture production are fish. Production of aquati plants is increasing rapidly and reached 5.4 million t in 1992, while smaller increases if production of molluscs and crustaceans are seen (Figure 2.5). The total value of the aquaculture production is estimated to more than $US 32.5 billion in 1992.

To summarize, it can be said that further increases in supply of fish can be

expected from better utilization/ reduction of losses and further expansion of aquaculture.

Table 2.1 shows the breakdown of world fish production.

Table 2.1 Breakdown of world fish production (percentage of world total in live weight) (FAO, 1993 a)

Year	For human consumption					Other purposes Animal Feed
	Total	Fresh	Freezing	Curing	Canning	
1982	71.1	19.4	25.3	12.8	13.6	28.9
1992	72.8	27.0	24.1	9.3	12.4	27.2

Table 2.1 shows relatively modest differences in the breakdown of the fish production during the decade 1982-92. However, there was a significant increase in fresh fish consumption. Total fish for human consumption increased by 1.2% while fish used for curing and canning continued to decrease.

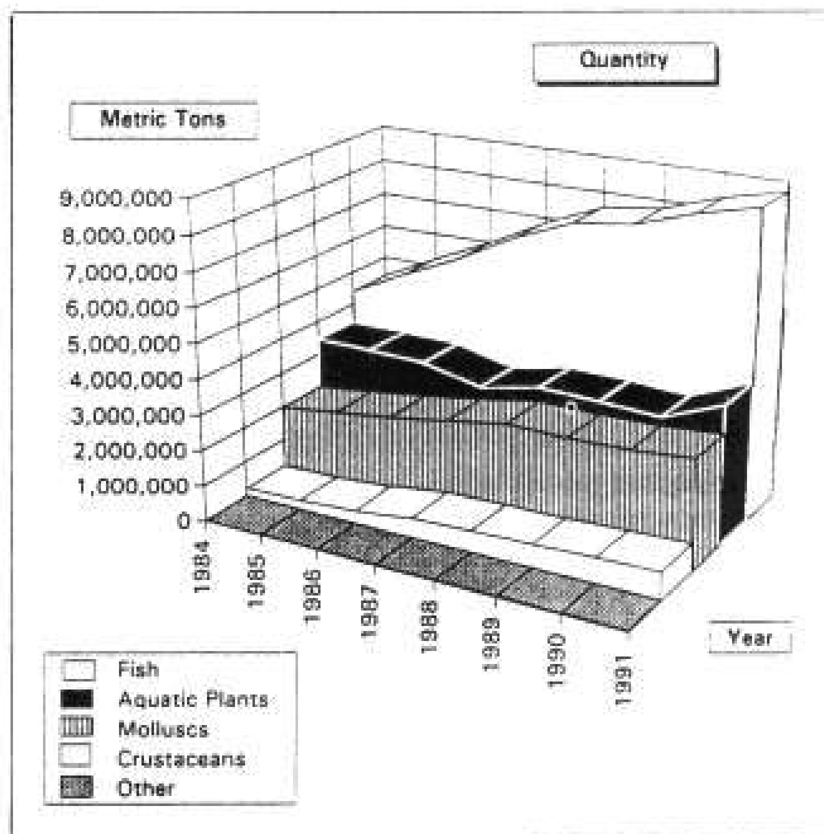
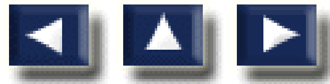


Figure 2.5 World Aquaculture Production by species category, 1984-91 (FAO, 1993 c)

In value terms, fishery exports reached an estimated $US 40.1 billion in 1993 (FISHDAB, 1994). Exports of fish and fishery products from developing countries continued to increase reaching a total value of $US 19.4 billion in 1993. In the same year exports from developed countries dropped by 5% to an estimated total value of $US 20.7 billion. Developing countries recorded an increasingly positive trade balance in fish trade, which reached $US 12.7 billion in 1993 (FISHDAB, 1994).

It should be noted that Table 2.1 does not give a true picture of the amount of fish available for human food. An enormous amount of fish is wasted due to discards on board or post-harvest losses during processing and distribution. It has been estimated that the global amount of discards is in the range of 17-39 million t/year with an average of 27 million t/year (Alverson et al., 1994). It has been further estimated that the total post-harvest losses in fish products are about 10 % (James, D., personal communication 1994). These high losses are mainly due to problems of fisheries management, and lack of proper technology and of economic incentives.



3. BIOLOGICAL ASPECTS

3.1. Classification
3.2. Anatomy and physiology
3.3. Growth and reproduction

3.1 Classification

Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Thurman and Webber, 1984).

Five vertebrate classes have species which could be called fish, but only two of these groups - the sharks and rays, and the bonyfish - are generally important and widely distributed in the aquatic environment. The evolutionary relationship between the various groups of fish is shown in Figure 3.1.

Fish are the most numerous of the vertebrates, with at least 20 000 known species, and more than half (58 %) are found in the marine environment. They are most common in the warm and temperate waters of the continental shelves (some 8 000 species). In the cold polar waters about 1 100 species are found. In the oceanic pelagic environment well away from the effect of land, there are only some 225 species. Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1 000 m depth) the number of species increases. There are some 1 000 species of so-called mid- water fish (Thurman and Webber, 1984).

Classifying all these organisms into a system is not an easy task, but the taxonomist groups organisms into natural units that reflect evolutionary relationships. The smallest unit is the species. Each species is identified by a scientific name which has two parts the genus and the specific epithet (binominal nomenclature). The genus name is always capitalized and both are italicized. As an example, the scientific (species) name of the common dolphin is *Delphinus delphis.* The genus is a category that contains one or more species, while the next step in the hierarchy is

the family which may contain one or more genus. Thus the total hierarchical system is: Kingdom: Phylum: Class: Order: Family: Genus: Species.

The use of common or local names often creates confusion since the same species may have different names in different regions or, conversely, the same name is ascribed to several different species, sometimes with different technological properties. As a point of reference the scientific name should, therefore, be given in any kind of publication or report the first time a particular species is referred to by its common name. For further information see the International Council for the Exploration of the Sea "List of names of Fish and Shellfish" (ICES, 1966); the "Multilingual Dictionary of Fish and Fish Products" prepared by the Organisation for Economic Cooperation and Development (OECD, 1990) and the "Multilingual Illustrated Dictionary of Aquatic Animals and Plants" (Commission of the European Communities, 1993).

The classification of fish into cartilaginous and bony (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently (section 5) and vary with regard to chemical composition (section 4).

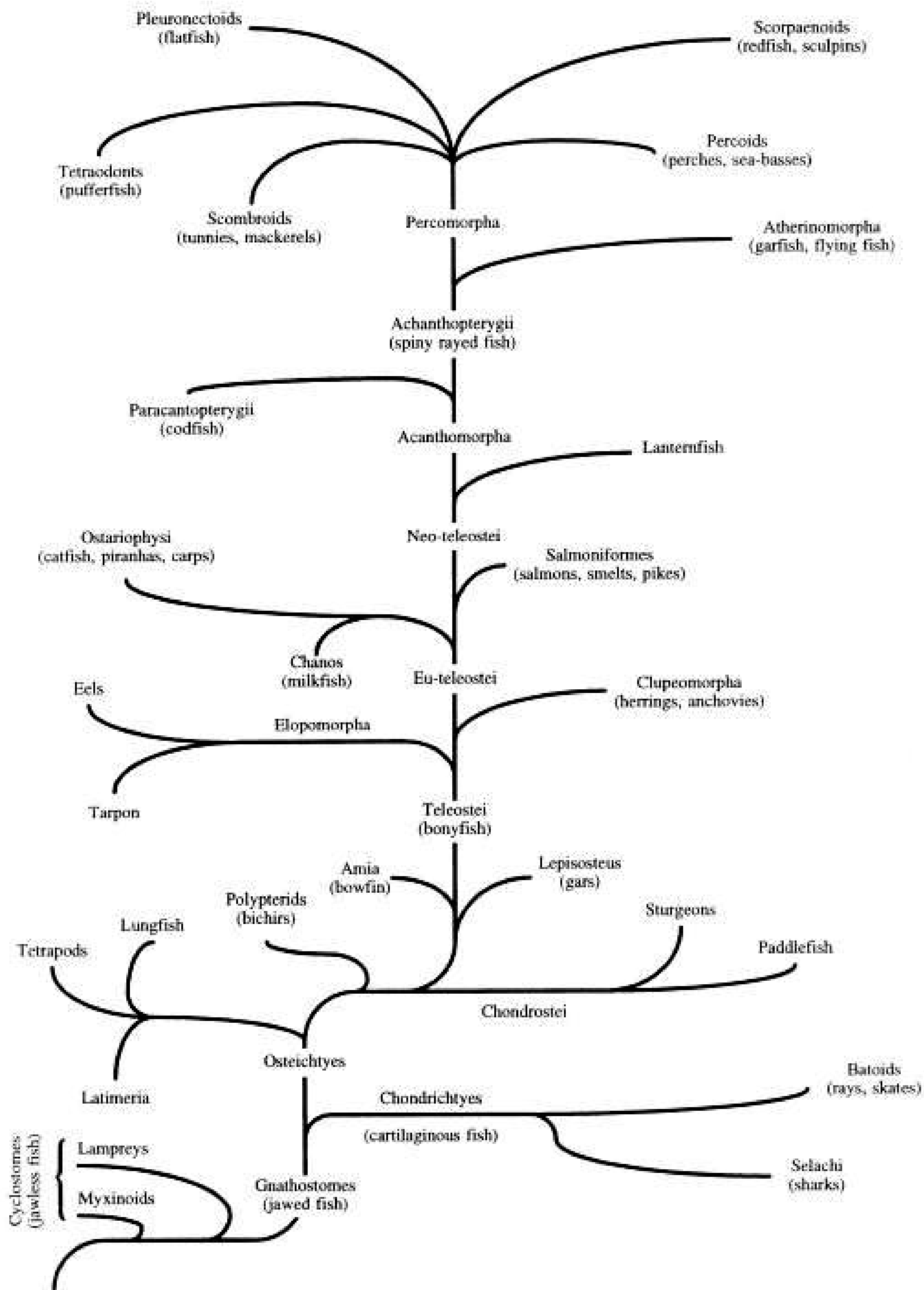


Figure 3.1 Simplified phylogenetic tree of the fishes. (Examples of food-fish, using common English names are shown in parantheses). (SOURCE: N. Bonde (1994),

Geological Inst., Copenhagen)

Furthermore, fish can be divided into fatty and lean species, but this type of classification is based on biological and technological characteristics as shown in Table 3.1.

Table 3.1 Classification of fish

Scientific grouping	Biological characteristics	Technological characteristics	Examples
Cyclostomes	jawless fish		lampreys, slime-eels
Chondrichthyes	cartilaginous fish	high urea content in muscle	sharks, skate, rays
Teleostei or bony fish	pelagic fish	fatty fish (store lipids in body tissue)	herring, mackerel, sardine tuna, sprat
	demersal fish	lean (white) fish (store lipids in liver only)	cod, haddock, hake grouper, seabass

3.2 Anatomy and physiology

The skeleton

Being vertebrates, fish have a vertebral column - the backbone - and a cranium covering the brain. The backbone runs from the head to the tail fin and is composed of segments (vertebrae). These vertebrae are extended dorsally to form neural spines, and in the trunk region they have lateral processes that bear ribs (Figure 3.2). The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes) (see also Figure 3.3). Usually, there is also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise prepared for food.

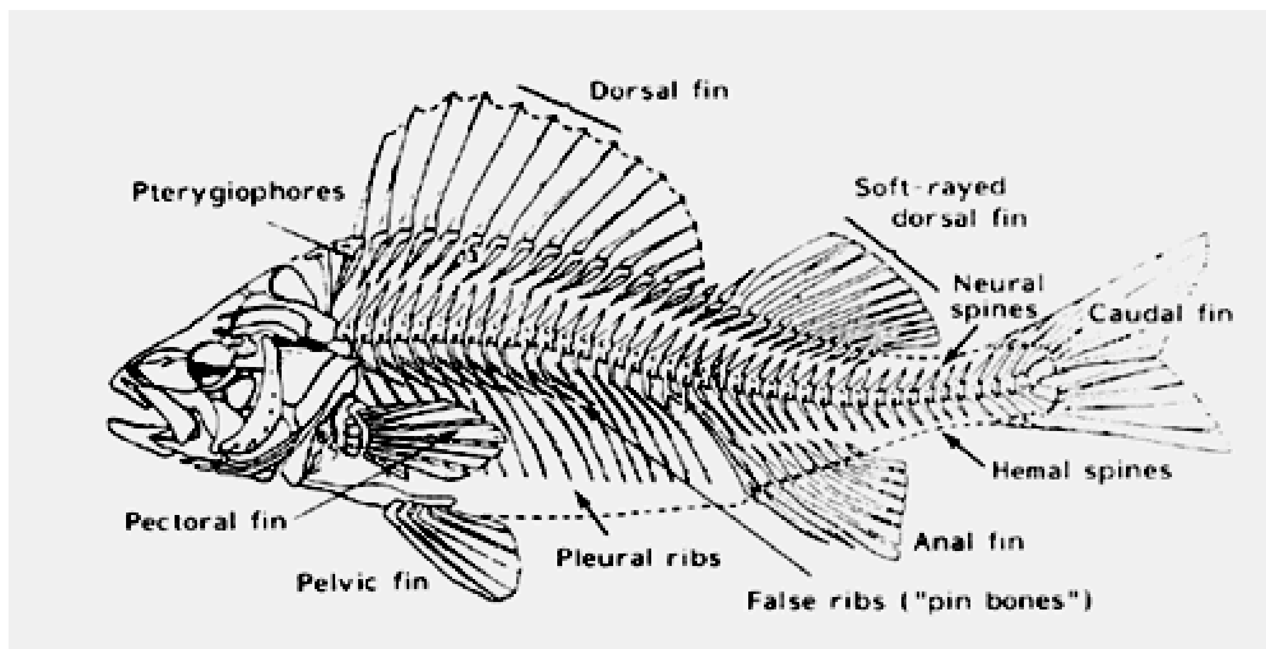


Figure 3.2 Skeleton of bonyfish (Eriksson and Johnson, 1979)

Muscle anatomy and function

The anatomy of fish muscle is different from the anatomy of terrestrial mammals, in that the fish lacks the tendinous system connecting muscle bundles **to the skeleton of the** animal. Instead, fish has muscle cells running in parallel and connected to sheaths of connective tissue (myocommata), which are anchored to the skeleton and the skin. The bundles of parallel muscle cells are called myotomes (Figure 3.3).

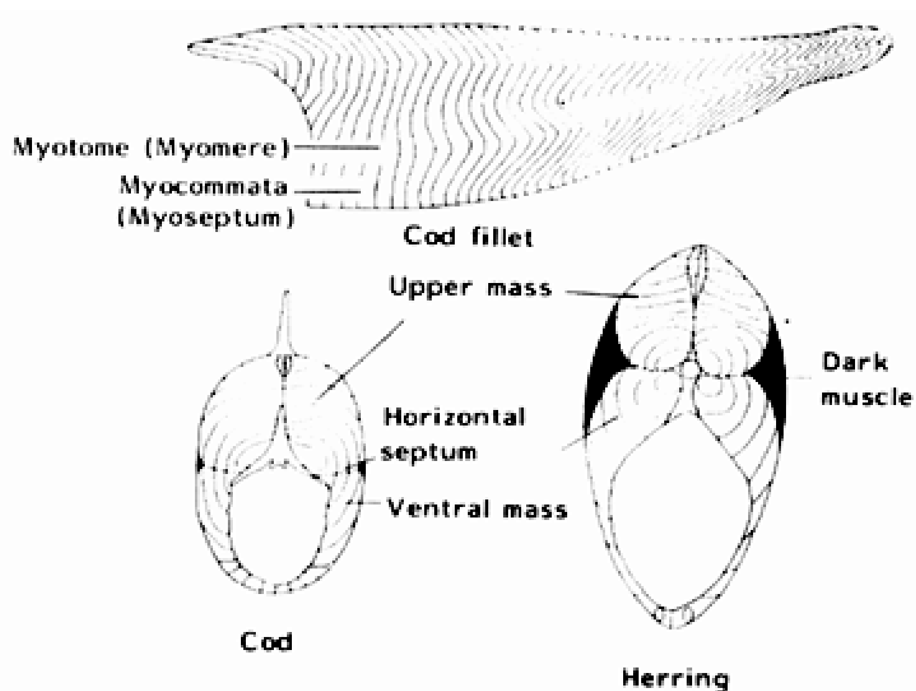


Figure 3.3 Skeletal musculature of fish (Knorr, 1974)

All muscle cells extend the full length between two myocommata, and run parallel with the longitudinal direction of the fish. The muscle mass on each side of the fish makes up the fillet, of which the upper part is termed the dorsal muscle and the lower part the ventral muscle.

The fillet is heterogenous in that the length of the muscle cells vary from the head end (anterior) to the tail end (posterior). The longest muscle cells in cod are found at about the twelfth myotome counting from the head, with an average length around 10 mm in a fish that is 60 cm long (Love, 1970). The diameter of the cells also vary, being widest in the ventral part of the fillet.

The myocommata run in an oblique, almost "plow-like" pattern perpendicular to the long axis of the fish, from the skin to the spine. This anatomy is ideally suited for the flexing muscle movements necessary for propelling the fish through the water.

As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, i.e., the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc., and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating system making the muscle appear striated upon microscopic examination (Figure 3.4).

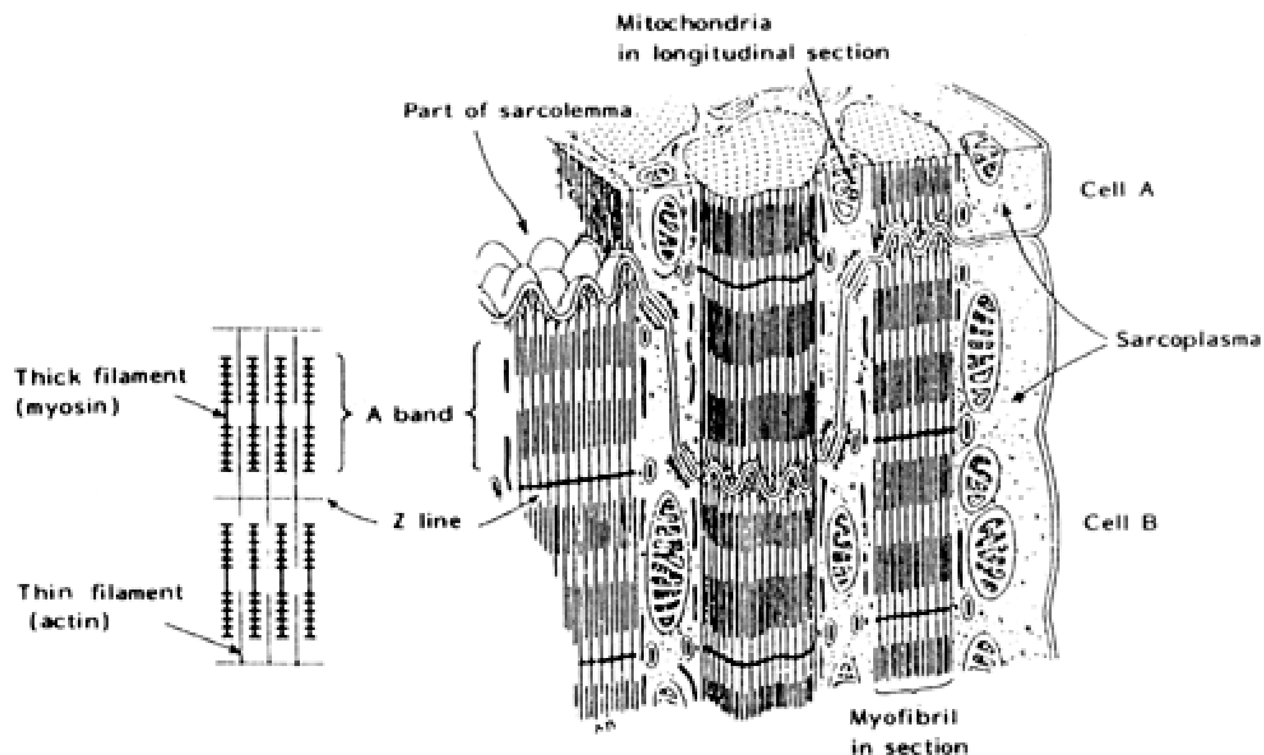


Figure 3.4 Section of a cell showing various structures including the myofibrils (Bell *et al., 1976)*

Most fish muscle tissue is white but, depending on the species, many fish will have a certain amount of dark tissue of a brown or reddish colour. The dark muscle is located just under the skin along the side of the body.

The proportion of dark to light muscle varies with the activity of the fish. In pelagic fish, i.e., species such as herring and mackerel which swim more or less continuously, up to 48 % of the body weight may consist of dark muscle (Love, *1970).* In demersal fish, i.e., species which feed on the bottom and only move periodically, the amount of dark muscle is very small.

There are many differences in the chemical composition of the two muscle types, some of the more noteworthy being higher levels of lipids and myoglobin in the dark muscle.

From a technological point of view, the high lipid content of dark muscle is important because of problems with rancidity.

The reddish meat colour found in salmon and sea trout does not originate from myoglobin but is due to the red carotenoid, astaxanthin. The function of this pigment has not been clearly established, but it has been proposed that the carotenoid may play a role as an antioxidant. Further, the accumulation in the muscle may function as a depot for pigment needed at the time of spawning when the male develops a strong red colour in the skin and the female transport carotenoids into the eggs. The latter seems to depend heavily on the amount of carotenoids for proper development after fertilization. It is clearly seen that the muscle colour of salmonids fades at the time of spawning.

The fish cannot synthesize astaxanthin and is thus dependent on ingestion of the pigment through the feed. Some salmonids live in waters where the natural prey does not contain much carotenoid, e.g., in the Baltic Sea, thus resulting in a muscle colour less red than salmonids from other waters. This may be taken as an indication that the proposed physiological function of astaxanthin in salmonids explained above may be less important.

In salmon aquaculture, astaxanthin is included in the feed, as the red colour of the flesh is one of the most important quality criteria for this species.

Muscle contraction starts when a nervous impulse sets off a release of Ca + + from the sarcoplasmic reticulum to the myofibrils. When the Ca + + concentration increases at the active enzyme site on the myosin filament, the enzyme ATP-ase is activated. This ATP-ase splits the ATP found between the actin and myosin filaments, causing a release of energy. Most of this energy is used as contractile

energy making the actin filaments slide in between the myosin filaments in a telescopic fashion, thereby contracting the muscle fibre. When the reaction is reversed (i.e., when the Ca + + is pumped back, the contractile ATP-ase activity stops and the filaments are allowed to slip passively past each other), the muscle is relaxed.

The energy source for ATP generation in the light muscle is glycogen, whereas the dark muscle may also use lipids. A major difference is, further, that the dark muscle contains much more mitochondria than light muscle, thus enabling the dark muscle to operate an extensive aerobic energy metabolism resulting in CO_2 and H_2O as the end products. The light muscle, mostly generating energy by the anaerobic metabolism, accumulates lactic acid which has to be transported to the liver for further metabolization. In addition, the dark muscle is reported to possess functions similar to those are found in the liver.

The different metabolic patterns found in the two muscle types makes the light muscle excellently fitted for strong, short muscle bursts, whereas the dark muscle is designed for continual, although not so strong muscle movements.

Post mortem the biochemical and physiological regulatory functions operating in vivo ceases, and the energy resources in the muscle are depleted. When the level of ATP reaches its minimum, myosin and actin are interconnected irreversibly, resulting in rigor mortis. This phenomenon is further described in section 5.

The cardiovascular system

The cardiovascular system is of considerable interest to the fish technologist since it is important in some species to bleed the fish (i.e., remove most of the blood) after capture.

The fish heart is constructed for single circulation (Figure 3.5). In bony fish it consists of two consecutive chambers pumping venous blood toward the gills via the ventral aorta.

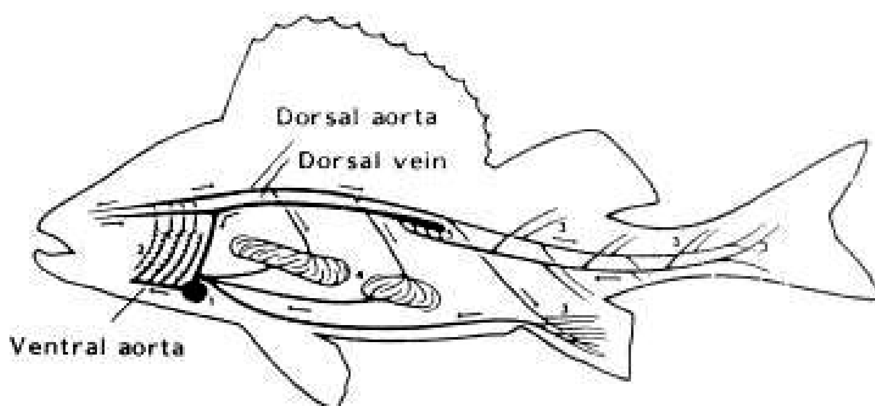


Figure 3.5 Blood circulation in fish (Eriksson and Johnson, 1979)

Notes:

1. The heart pumps blood toward the gills.
2. The blood is aerated in the gills.
3. Arterial blood is dispersed into the capillaries where the transfer of oxygen and nutrients to the surrounding tissue takes place.
4. The nutrients from ingested food are absorbed from the intestines, then transported to the liver and later dispersed in the blood throughout the body.
5. In the kidneys the blood is "purified" and waste products are excreted via the urine.

After being aerated in the gills, the arterial blood is collected in the dorsal aorta running just beneath the vertebral column and from here it is dispersed into the different tissues via the capillaries. The venous blood returns to the heart, flowing in veins of increasingly larger size (the biggest is the dorsal vein which is also located beneath the vertebral column). The veins all gather into one blood vessel before entering the heart. The total volume of the blood in fish ranges from 1.5 to 3.0 % of the body weight. Most of it is located in the internal organs while the muscular tissues, constituting two- thirds of the body weight, contain only 20 % of the blood volume. This distribution is not changed during exercise since the light muscle in particular is not very vascularized.

During blood circulation the blood pressure drops from around 30 mg Hg in the ventral aorta to 0 when entering the heart (Randall, 1970). After the blood has passed through the gills, the blood pressure derived from the pumping activity of the heart is already greatly decreased. Muscle contractions are important in pumping the blood back to the heart and counterflow is prevented by a system of paired valves inside the veins.

Clearly, the single circulation of fish is fundamentally different from the system in mammals (Figure 3.6), where the blood passes through the heart twice and is propelled out into the body under high pressure due to the contractions of the heart.

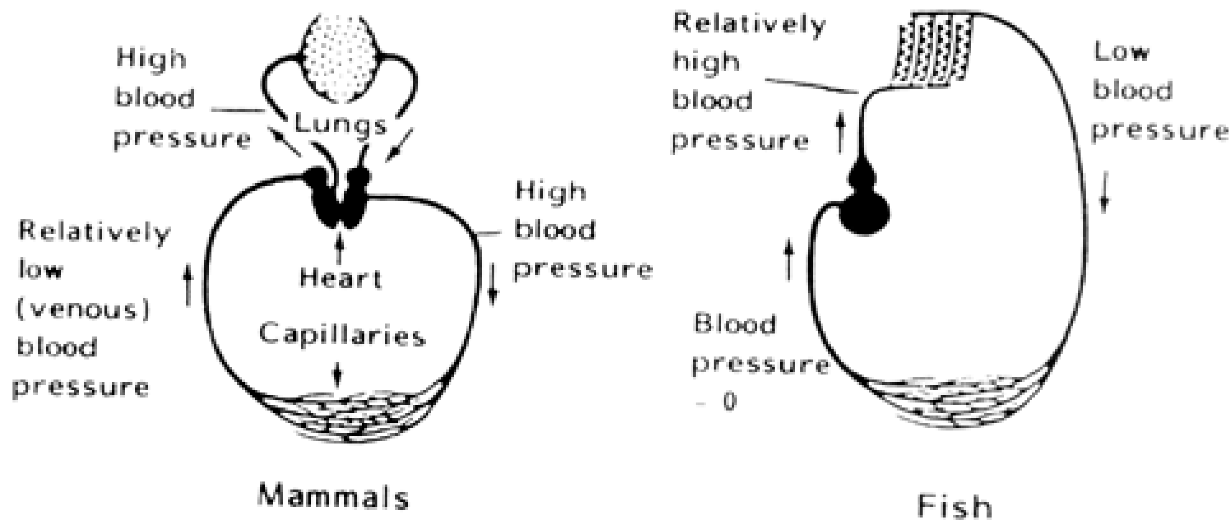


Figure 3.6 Blood circulation in fish and mammals (Eriksson and Johnson, 1979)

In fish, the heart does not play an important role in the transportation of blood from the capillaries back to the heart. This has been confirmed in an experiment where the impact of different bleeding procedures on the colour of cod fillets was examined. No difference could be found regardless of whether the fish had been bled by means of cutting the throat in front of or behind the heart before gutting, or had not been cut at all before slaughter.

In some fisheries, bleeding of the fish is very important as a uniform white fillet is desirable. In order to obtain this, a number of countries have recommended that fish are bled for a period (15-20 min) prior to being gutted. This means that throat cutting and gutting must be carried out in two separate operations and that special arrangements (bleeding tanks) must be provided on deck. This complicates the working process (two operations instead of one), time-consuming for the fishermen and increases the time-lag before the fish is chilled. Furthermore it requires extra space on an otherwise crowded working deck.

Several researchers have questioned the necessity of handling the fish in a two-step procedure involving a special bleeding period (Botta *et al.,* 1986; Huss and Asenjo, 1977 a; Valdimarsson *et al.* 1984). There seems to be general agreement about the following:

- bleeding is more affected by time onboard prior to bleeding/gutting than by the actual bleeding/gutting procedure.
- best bleeding is obtained if live fish are handled, but it is of major importance to cut the fish before it enters rigor mortis since it is the muscle contractions that force the blood out of the tissues.

Disagreement exists as to the cutting method. Huss and Asenjo (1977 a) found best

bleeding if a deep throat cut including the dorsal aorta was applied, but this was not confirmed in the work of Botta *et al.* (1986). The latter also recommended to include a bleeding period (two-step procedure) when live fish were handled (fishing with pound net, trap, seine, longline or jigging), while Valdimarsson *et al.* (1984) found that the quality of dead cod (4 h after being brought onboard) was slightly improved using the two-step procedure. However, it should be pointed out that the effect of bleeding should also be weighted against the advantages of having a fast and effective handling procedure resulting in rapid chilling of the catch.

Discoloration of the fillet may also be a result of rough handling during catch and catch handling while the fish is still alive. Physical mishandling in the net (long trawling time, very large catches) or on the deck (fishermen stepping on the fish or throwing boxes, containers and other items on top of the fish) may cause bruises, rupture of blood vessels and blood oozing into the muscle tissue (haematoma).

Heavy pressure on dead fish, when the blood is clotted (e.g., overloading of fish boxes) does not cause discoloration, but the fish may suffer a serious weight loss.

Other organs

Among the other organs, only the roe and liver play a major role as foodstuffs. Their size depends on the fish species and varies with life cycle, feed intake and season. In cod the weight of the roe varies from a few percent up to 27 % of the body weight and the weight of the liver ranges from 1 to 4.5 %. Likewise, the composition can change and the oil content of the liver vary from 15 to 75 %, with the highest values being found during autumn (Jangaard *et al.,* 1967).

3.3 Growth and reproduction

During growth it is the size of each muscle cell that increases rather than the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a size characteristic of the species and is this not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to rear female fish in aquaculture.

Every year mature fish use energy to build up the gonads (the roe and milk). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake (Figure 3.7).

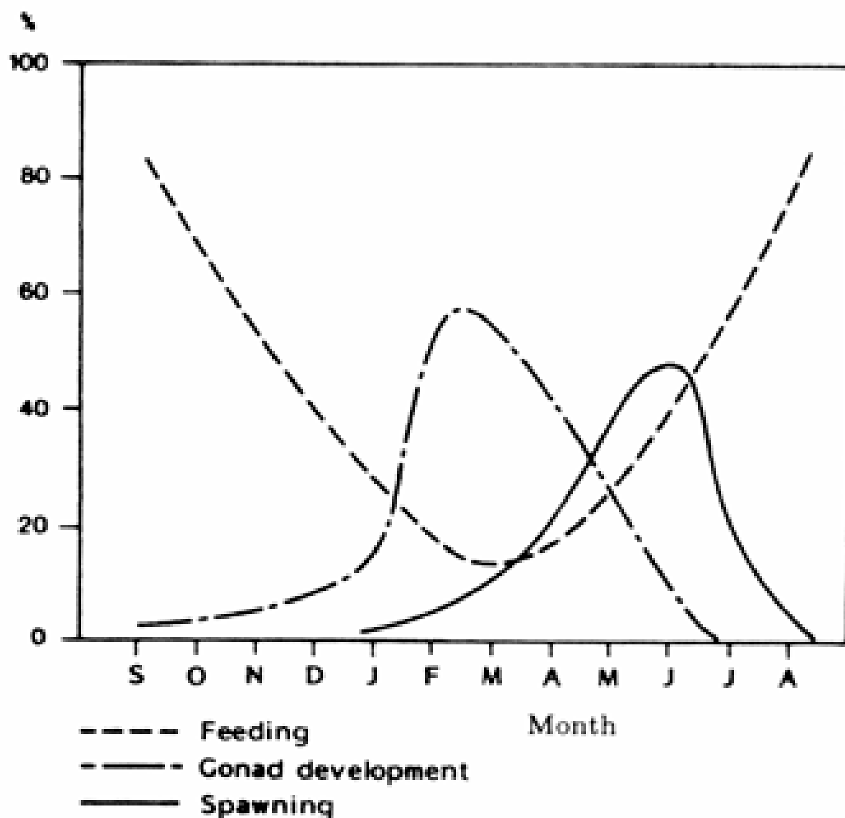


Figure 3.7 Relation between feeding cycle (percentage sample with food in stomach) and reproductive cycle (gonad development), percentage fish with ripening gonads (spawning, percentage ripe fish) of haddock *(Melanogrammus aeglefinus).* It should be noted that the development of the gonads takes place while the fish is starving (Hoar, 1957).

In North Sea cod it was found that prior to spawning the water content of the muscle increases (Figure 3.8) and the protein content decreases. In extreme cases the water content of very large cod can attain 87 % of the body weight prior to spawning (Love, 1970).

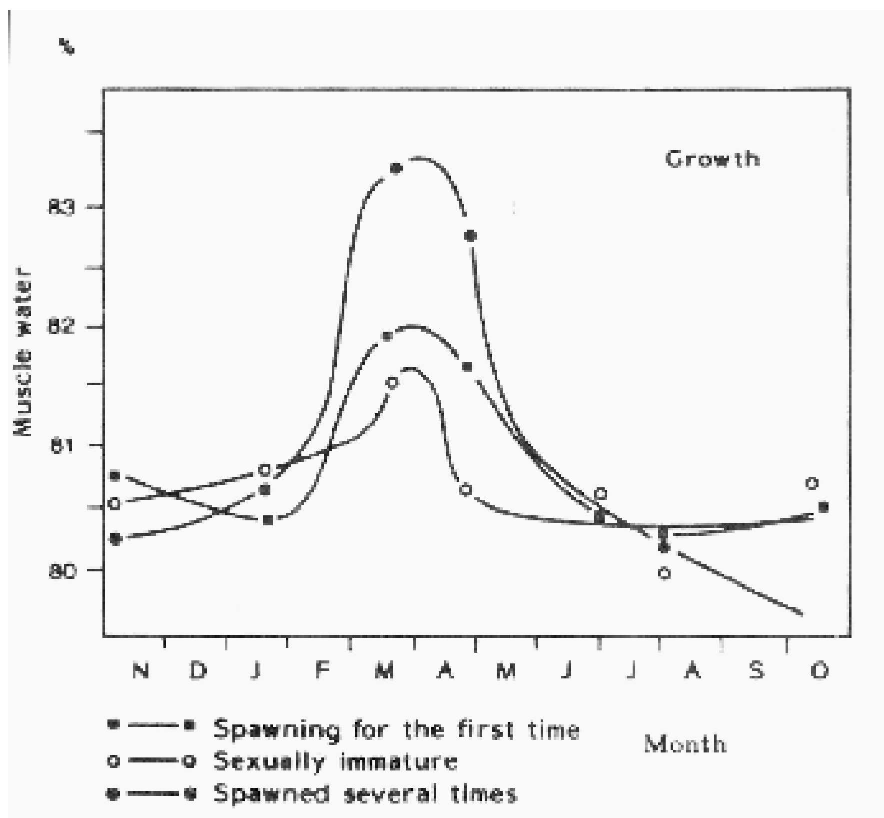
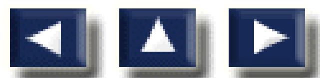


Figure 3.8 Water content of cod muscle *(Gadus morhua)* (Love, 1970)

The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity (Figure 3.7), while some have ripe ovaries for nearly the whole year.

The depletion of the reserves of the fish during gonadal development can be extremely severe, especially if reproduction is combined with migration to the breeding grounds. Some species, e.g., Pacific salmon *(Oncorhynchus* spp.), eel *(Anguilla anguilla)* and others, manage to migrate only once, after which they degenerate and die. This is partly because these species do not eat during migration so that, in the case of a salmon, it can lose up to 92 % of its lipid, 72 % of its protein and 63 % of its ash content during migration and reproduction (Love, 1970).

On the other hand, other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about eight years before spawning causes its death, and other species can live even longer (Cushing, 1975). In former times, 25-year-old herring *(Clupea harengus)* were not unusual in the Norwegian Sea, and plaice *(Pleuronectes platessa) up* to 35 years old have been found. One of the oldest fish reported was a sturgeon *(Acipenser sturio)* from Lake Winnebago in Wisconsin. According to the number of rings in the otolith, it was over 100 years old.



4. CHEMICAL COMPOSITION

4.1. Principal constituents
4.2. Lipids
4.3. Proteins
4.4. N-containing extractives
4.5. Vitamins and minerals

4.1 Principal constituents

The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment and season.

The principal constituents of fish and mammals may be divided into the same categories, and examples of the variation between the constituents in fish are shown in Table 4.1. The composition of beef muscle has been included for comparison.

Table 4.1 Principal constituents (percentage) of fish and beef muscle

Constituent	Fish (fillet)			Beef (isolated muscle)
	Min.	Normal variation	Max.	
Protein	6	16-21	28	20

Lipid	0.1	0.2-25	67	3
carboydrate		<0.5		1
Ash	0.4	1.2-1.5	105	1
Water	28	66-81	96	75

SOURCES: Stansby, 1962; Love, 1970

As can be seen from Table 4.1, a substantial normal variation is observed for the constituents of fish muscle. The minimum and maximum values listed are rather extreme and encountered more rarely.

The variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Fish will have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Usually spawning, whether occurring after long migrations or not, calls for higher levels of energy. Fish having energy depots in the form of lipids will rely on this. Species performing long migrations before they reach specific spawning grounds or rivers may utilize protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish. Most species, in addition, do usually not ingest much food during spawning migration and are therefore not able to supply energy through feeding.

During periods of heavy feeding, at first the protein content of the muscle tissue will increase to an extent depending upon how much it has been depleted, e.g., in relation to spawning migration. Then the lipid content will show a marked and rapid increase. After spawning the fish resumes feeding behaviour and often migrates to find suitable sources of food. Plankton-eating species such as herring will then naturally experience another seasonal variation than that caused by spawning, since plankton production depends on the season and various physical parameters in the oceans.

The lipid fraction is the component showing the greatest variation. Often, the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of spawning. Figure 4.1 shows the characteristic variations in the North Sea herring (4.1a) and mackerel (4.1b).

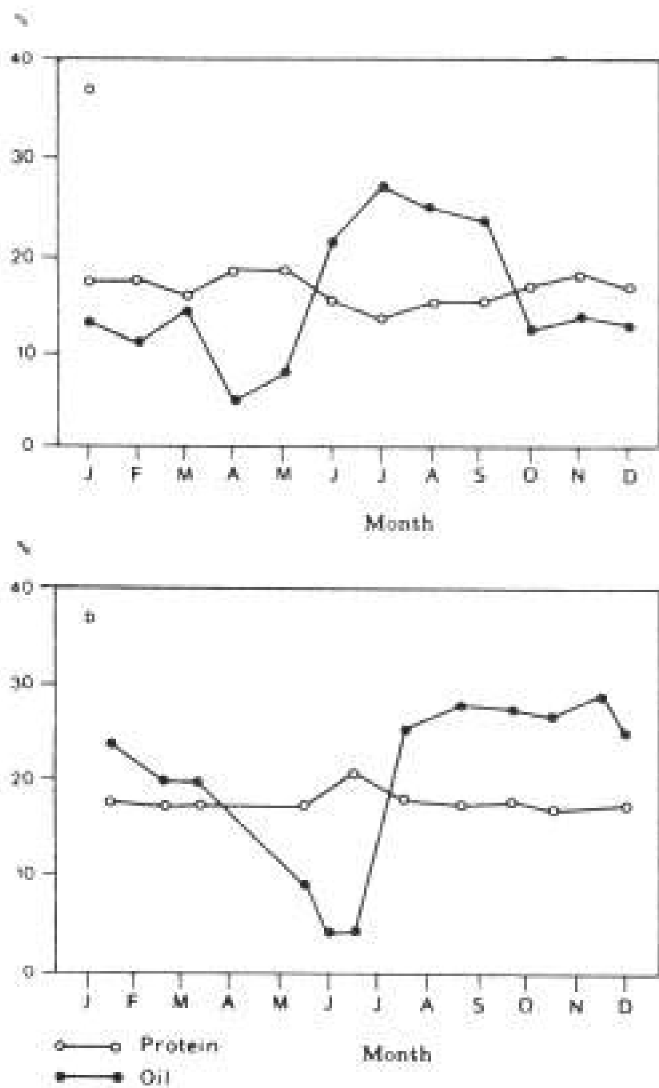


Figure 4.1 Seasonal variation in the chemical composition of (a) herring fillets *(Clupea harengus)* and (b) mackerel fillets *(Scomber scombrus).* Each point indicates the mean value of eight fillets

Although the protein fraction is rather constant in most species, variations have been observed such as protein reduction occuring in salmon during long spawning migrations (Ando *et al.,* 1985 b; Ando and Hatano, 1986) and in Baltic cod during the spawning season, which for this species extends from January to June/July (Borresen, 1992). The latter variation is illustrated in Figure 4.2.

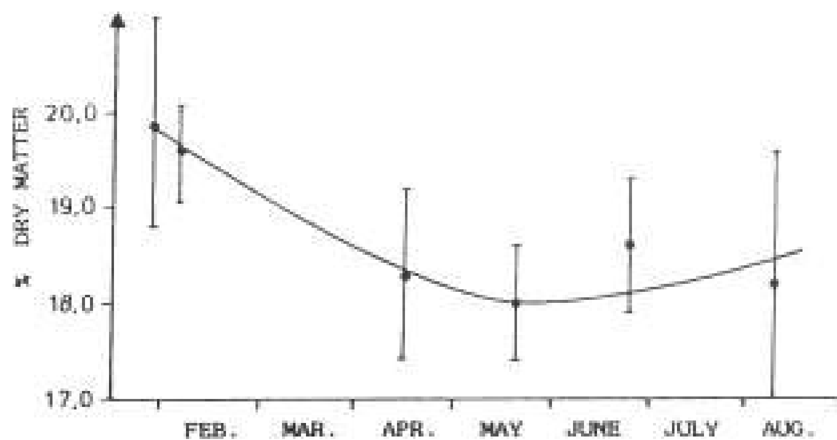


Figure 4.2 Variation in percentage dry matter in muscle of Baltic cod Vertical bars represent standard deviation of the mean value. (Borresen, 1992)

Some tropical fish also show a marked seasonal I variation in chemical composition. West African shad *(Ethmalosa dorsalis)* shows a range in fat content of 2-7 % (wet weight) over the year with a maximum in July (Watts, 1957). Corvina *(Micropogon furnieri)* and pescada-foguete*(Marodon ancylodon)* captured off the Brazilian coast had a fat content range of 0.2-8.7 % and 0.1-5.4 % respectively (Ito and Watanabe, 1968). It has also been observed that the oil content of these species varies with size, larger fish containing about 1 % more oil than smaller ones. Watanabe (1971) examined freshwater fish from Zambia and found a variation from 0.1 to 5.0 % in oil content of four species including both pelagics and demersals.

A possible method for discriminating lean from fatty fish species is to term fish that store lipids only in the liver as lean, and fish storing lipids in fat cells distributed in other body tissues as fatty fish. Typical lean species are the bottom-dwelling ground fish like cod, saithe and hake. Fatty species include the pelagics like herring, mackerel and sprat. Some species store lipids in limited parts of their body tissues only, or in lower quantities than typical fatty species, and are consequently termed semi- fatty species (e.g., barracuda, mullet and shark).

The lipid content of fillets from lean fish is low and stable whereas the lipid content in fillets from fatty species varies considerably. However, the variation in the percentage of fat is reflected in the percentage of water, since fat and water normally constitute around 80 % of the fillet. As a rule of thumb, this can be used to estimate the fat content from an analysis of the amount of water in the fillet. In fact, this principle is being utilized with success in a fat-analysing instrument called the Torry Fish Fat Meter, where it is the water content that is actually being measured (Kent et *al.,* 1992).

Whether a fish is lean or fatty the actual fat content has consequences for the technological characteristics postmortem. The changes taking place in fresh lean fish may be predicted from knowledge of biochemical reactions in the protein fraction, whereas in fatty species changes in the lipid fractions have to be included. The implication may be that the storage time is reduced due to lipid oxidation, or special precautions have to be taken to avoid this.

The variations in water, lipid and protein contents in various fish species are shown in Table 4.2.

Table 4.2 Chemical composition of the fillets of various fish species

Species	Scientific name	Water %	Lipid %	Protein %	Energy value(kJ/ 100 g)
Blue whiting a)	Micromesistius poutassou	79-80	1.9-3.0	13.8-15.9	314-388
Cod a)	Gadus morhua	78-83	0.1-0.9	15.0-19.0	295-332
Eel a)	Anguilla anguilla	60-71	8.0-31.0	14.4	
Herring a)	Clupea harengus	60-80	0.4-22.0	16.0-19.0	
Plaice a)	Pleuronectes platessa	81	1.1-3.6	15.7-17.8	332-452
Salmon a)	Salmo salar	67-77	0.3-14.0	21.5	
Trout a)	Salmo trutta	70-79	1.2-10.8	18.8-19.1	
Tuna a)	Thunnus spp.	71	4.1	25.2	581
Norway lobster a)	Nephrops norvegicus	77	0.6-2.0	19.5	369
Pejerrey b)	Basilichthys bornariensis	80	0.7-3.6	17.3-17.9	
Carp b)	Cyprinus carpio	81.6	2.1	16.0	
Sabalo c)	Prochilodus platensis	67.0	4.3	23.4	
Pacu c)	Colossoma macropomum	67.1	18.0	14.1	
Tambaqui c)	Colossoma brachypomum	69.3	15.6	15.8	
Chincuiña c)	Pseudoplatystoma tigrinum	70.8	8.9	15.8	
Corvina c)	Plagioscion squamosissimus	67.9	5.9	21.7	
Bagré c)	Ageneiosus spp.	79.0	3.7	14.8	

SOURCES: a) Murray and Burt, 1969, b)Poulter and Nicolaides, 1995 a. c) Poulter and Nicolaides, 1985 b

The carbohydrate content in fish muscle is very low, usually below 0.5 %. This

is typical for striated muscle, where carbohydrate occurs in glycogen and as part of the chemical constituents of nucleotides. The latter is the Source of ribose liberated as a consequence of the autolytic changes *post mortem*.

As demonstrated above, the chemical composition of the different fish species will show variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. These factors are observed in wild, free-living fishes in the open sea and inland waters. Fish raised in aquaculture may also show variation in chemical composition, but in this case several factors are controlled, thus the chemical composition may be predicted. To a certain extent the fish farmer is able to design the composition of the fish by selecting the farming conditions. It has been reported that factors such as feed composition, environment, fish size, and genetic traits all have an impact on the composition and quality of the aquacultured fish (Reinitz et al., 1979).

The single factors having the most pronounced Impact on the chemical composition is considered to be the feed composition. The fish farmer is interested in making the fish grow as fast as possible on a minimum amount of feed, as the feed is the major cost component in aquaculture. The growth potential is highest when the fish is fed a diet with a high lipid content for energy purposes and a high amount of protein containing a well balanced composition of amino acids.

However, the basic metabolic pattern of the fish sets some limits as to how much lipid can be metabolized relative to protein. Because protein is a much more expensive feed ingredient than lipid, numerous experiments have been performed in order to substitute as much protein as possible with lipids. Among the literature that may be consulted is the following: Watanabe *et al., 1979;* Watanabe, *1982;* Wilson and Halver, *1986;* and Watanabe *et al., 1987.*

Usually most fish species will use some of the protein for energy purposes regardless of the lipid content. When the lipid content exceeds the maximum that can be metabolized for energy purposes, the remainder will be deposited in the tissues, resulting in a fish with very high fat content. Apart from having a negative impact on the overall quality, it may also decrease the yield, as most surplus fat will be stored in depots in the belly cavity, thus being discarded as waste after evisceration and filleting.

A normal way of reducing the fat content of aquacultured fish before harvesting is to starve the fish for a period. It has been demonstrated for both fatty and lean fish species that this affects the lipid content (see, e.g., Reinitz, 1983; Johansson and Kiessling, 1991*;* Lie and Huse, 1992).

It should be mentioned that in addition to allowing for the possibility of, within certain limits, predetermining the fish composition in aquaculture operations, keeping fish in captivity under controlled conditions also offers the possibility of conducting experiments in which variation in chemical composition observed in wild fish may be provoked. The experiments may be designed such that the mechanisms behind the variations observed in wild fish may be elucidated.

4.2 Lipids

The lipids present in teleost fish species may be divided into two major groups: the phospholipids and the triglycerides. The phospholipids make up the integral structure of the unit membranes in the cells; thus, they are often called structural lipids. The triglycerides are lipids used for storage of energy in fat depots, usually within special fat cells surrounded by a phospholipid membrane and a rather weak collagen network. The triglycerides are often termed depot fat. A few fish have wax esters as part of their depot fats.

The white muscle of a typical lean fish such as cod contains less than 1 % lipids. Of this, the phospholipids make up about 90 % *(Ackman, 1980).* The phospholipid fraction in a lean fish muscle consists of about 69 % phosphatidyl-choline, 19 % *phosphatidyl-ethanolamine* and 5 % phosphatidyl-serine. In addition, there are several other phospholipids occurring in minor quantities.

The phospholipids are all contained in membrane structures, including the outer cell membrane, the endoplasmic reticulum and other intracellular tubule systems, as well as membranes of the organelles like mitochondria. In addition to phospholipids, the membranes also contain cholesterol, contributing to the membrane rigidity. In lean fish muscle cholesterol may be found in a quantity of about 6 % of the total lipids. This level is similar to that found in mammalian muscle.

As already explained, fish species may be categorized as lean or fatty depending on how they store lipids for energy. Lean fish use the liver as their energy depot, and the fatty species store lipids in fat cells througout the body.

The fat cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail. In some species which store extraordinarily high amounts of lipids the fat may also be deposited in the belly cavity. Depending on the amount of polyunsaturated fatty acids, most fish fats are more or less liquid at low temperature.

Finally, fat depots are also typically found spread throughout the muscle

structure. The concentration of fat cells appears to be highest close to the myocommata and in the region between the light and dark muscle (Kiessling *et al.,* 1991). The dark muscle contains some triglycerides inside the muscle cells even in lean fish, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism.

In dark muscle the energy reserves are completely catabolized to CO_2 and water, whereas in light muscle lactic acid is formed. The mobilization of energy is much faster in light muscle than in dark muscle, but the formation of lactic acid creates fatigue, leaving the muscle unable to work for long periods at maximum speed. Thus, the dark muscle is used for continuous swimming activities and the light muscle for quick bursts, such as when the fish is about to catch a prey or to escape a predator.

An example of the seasonal variation in fat deposition in mackerel and capelin is shown in Figure 4.3, where it is seen that the lipid content in the different tissues varies considerably. The lipid stores are typically used for long spawning migrations and when building up gonads (Ando *et al.,* 1985 a). When the lipids are mobilized for these purposes there are questions as to whether the different fatty acids present in the triglyceride are utilized selectively. This is apparently not the case in salmon, but in cod a selective utilization of $C_{22:6}$ has been observed (Takama *et al.,* 1985).

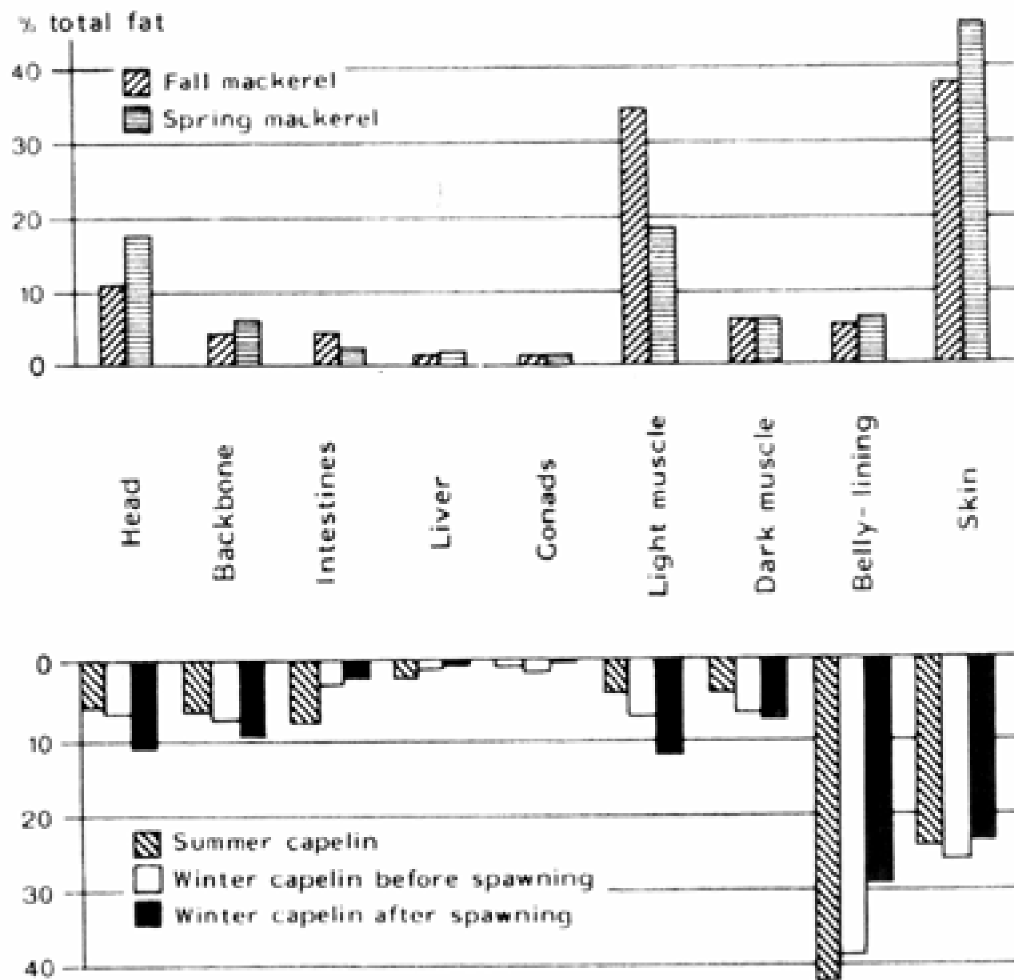


Figure 4.3 Distribution of the total fatin various parts of the body of mackerel (top) and capelin (bottom) of Norwegian origin (Lohne, 1976)

The phospholipids may also be mobilized to a certain extent during sustained migrations (Love, 1970), although this lipid fraction is considered to be conserved much more than the triglycerides.

In elasmobranchs, such as sharks, a significant quantity of the lipid is stored in the liver and may consist of fats like diacyl-alkyl-glyceryl esters or squalene. Some sharks may have liver oils with a minimum of 80 % of the lipid as unsaponifiable substance, mostly in the form of squalene (Buranudeen and Richards-Rajadurai, 1986).

Fish lipids differ from mammalian lipids. The main difference is that fish lipids include up to 40% of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two double bonds per fatty acid molecule while the depot fats of fish contain several fatty acids with five or six double bonds (Stansby and Hall, 1967).

The percentage of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in the polyunsaturated fatty acids of lipids from freshwater fish (approximately 70 %) than in the corresponding lipids from marine fish (approximately 88 %), (Stansby and Hall, 1967). However, the composition of the lipids is not completely fixed but can vary with the feed intake and season.

In human nutrition fatty acids such as linoleic and linolenic acid are regarded as essential since they cannot be synthesized by the organism. In marine fish, these fatty acids constitute only around 2 % of the total lipids, which is a small percentage compared with many vegetable oils. However, fish oils contain other polyunsaturated fatty acids which are "essential" to prevent skin diseases in the same way as linoleic and arachidonic acid. As members of the linolenic acid family (first double bond in the third position, w-3 counted from the terminal methyl group), they will also have neurological benefits in growing children. One of these fatty acids, eicosapentaenoic acid (C20:5 w 3), has recently attracted considerable attention because Danish scientists have found this acid high in the diet of a group of Greenland Eskimos virtually free from arteriosclerosis. Investigations in the United Kingdom and elsewhere have documented that eicosapen-taenoic acid in the blood is an extremely potent antithrombotic factor (Simopoulos et al., 1991).

4.3 Proteins

The proteins in fish muscle tissue can be divided into the following three groups:

1. Structural proteins (actin, myosin, tropormyosin and actomyosin), which constitute 70-80 % of the total protein content (compared with 40 % in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength (^{3}0.5 M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength ($<$0.15 M). This fraction constitutes 25-30 % of the protein.
3. Connective tissue proteins (collagen), which constitute approximately 3 % of the -protein in teleostei and about 10 % in elasmobranchii (compared with 17 % in mammals).

The structural proteins make up the contractile apparatus responsible for the muscle movement as explained in section 3.2. The amino-acid composition is approximately the same as for the corresponding proteins in mammaliam muscle, although the physical properties may be slightly different. The

isoelectric point (pI) is around pH 4.5-5.5. At the corresponding pH values the proteins have their lowest solublity, as illustrated in Figure 4.4.

The conformational structure of fish proteins is easily changed by changing the physical environment. Figure 4.4 shows how the solubility characteristics of the myofibrillar proteins are changed after freeze-drying. Treatment with high salt concentrations or heat may lead to denaturation, after which the native protein structure has been irreversibly changed.

When the proteins are denatured under controlled conditions their properties may be utilized for technological purposes. A good example is the production of surimi-based products, in which the gel forming ability of the myofibrillar proteins is used. After salt and stabilizers are added to a washed, minced preparation of muscle proteins, and after a controlled heating and cooling procedure the proteins form a very strong gel (Suzuki, 1981).

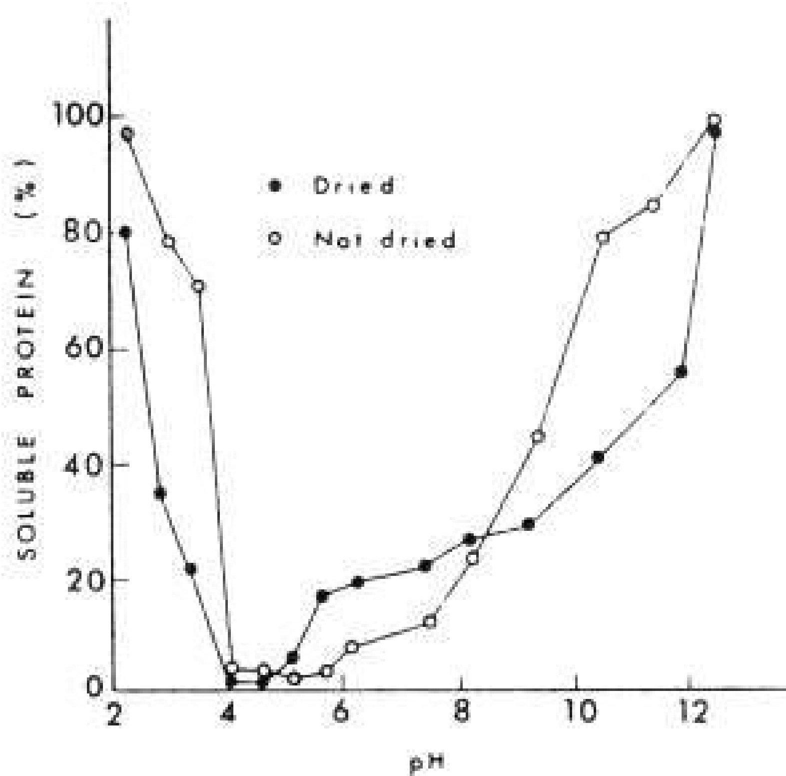


Figure 4.4 Solubility of myofibrillar protein before and after freeze drying at pH values ranging from 2 to 12 (Spinelli *et al.,*1972)

The majority of the sarcoplasmic proteins are enzymes participating in the cell metabolism, such as the anaerobic energy conversion from glycogen to ATP. If the organelles within the muscle cells are broken, this protein fraction may also contain the metabolic enzymes localized inside the endoplasmatic reticulum, mitochondria and lysosomes.

The fact that the composition of the sarcoplasmic protein fraction changes when the organelles are broken was suggested as a method for differentiating fresh from frozen fish, under the assumption that the organelles were intact until freezing (Rehbein *et al.,* 1978*,* Rehbein, 1979, Salfi *et al.,* 1985). However, it was later stated that these methods should be used with great caution, as some of the enzymes are liberated from the organelles also during iced storage of fish (Rehbein, 1992).

The proteins in the sarcoplasmic fraction are excellently suited to distinguishing between different fish species, as all the different species have their characteristic band pattern when separated by the isoelectric focusing method. The method was succesfully introduced by Lundstrom (1980) and has been used by many laboratories and for many fish species. A review of the literature is given by Rehbein (1990)*.*

The chemical and physical properties of collagen proteins are different in tissues such as skin, swim bladder and the myocommata in muscle (Mohr, 1971)*.* In general, collagen fibrils form a delicate network structure with varying complexity in the different connective tissues in a pattern similar to that found in mammals. However, the collagen in fish is much more thermolabile and contains fewer but more labile cross-links than collagen from warm-blooded vertebrates. The hydroxyprolin content is in general lower in fish than in mammals, although a total variation between 4.7 and 10 % of the collagen has been observed (Sato *et at,* 1989).

Different fish species contain varying amounts of collagen in the body tissues. This has led to a theory that the distribution of collagen may reflect the swimming behaviour of the species (Yoshinaka *et at, 1988).* Further, the varying amounts and varying types of collagen in different fishes may also have an influence on the textural properties of fish muscle (Montero and Borderias, 1989). Borresen (1976) developed a method for isolation of the collagenous network surrounding each individual muscle cell. The structure and composition of these structures has been further characterized in cod by Almaas (1982).

The role of collagen in fish was reviewed by Sikorsky *et al.* (1984). An excellent, more recent review is given by Bremner (1992), in which the most recent literature of the different types of collagen found in fish is presented.

Fish proteins contain all the essential amino-acids and, like milk, eggs and mammalian meat proteins, have a very high biological value (Table 4.3).

Table 4.3 Essential amino-acids (percentage) in various proteins

Amino-acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

SOURCES: Braekkan, 1976; Moustgard, 1957

Cereal grains are ususally low in lysine and/or the sulphur-containing amino-acids (methionine and cysteine), whereas fish protein is an excellent source of these aminoacids. In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly.

In addition to the fish proteins already mentioned there is a renewed interest in specific protein fractions that may be recovered from by-products, particularly in the viscera. One such example is the basic protein or protamines found in the milt of the male fish. The molecular weight is usually below 10 000 kD and the pI is higher than 10. This is a result of the extreme amino-acid composition that may show as much as 65 % arginine.

The presence of the basic proteins has long been known, and it is also known that they are not present in all fish species (Kossel, 1928). The best sources are salmonids and herring, whereas ground fish like cod are not found to contain protamines.

The extreme basic character of protamines makes them interesting for several reasons. They will adhere to most other proteins less basic. Thus they have the effect of enhancing functional properties of other food proteins (Poole *et al.,* 1987; Phillips *et al.,* 1989). However, there is a problem in removing all lipids present in the milt from the protein preparation, as this results in an off-flavour in the concentrations to be used in foods.

Another interesting feature of the basic proteins is their ability to prevent growth of microorganisms (Braekkan and Boge, 1964; Kamal *et al.,* 1986). This appears to be the most promising use of these basic proteins in the future.

4.4 N-containing extractives

The N-containing extractives can be defined as the water-soluble, low molecular weight, nitrogen- containing compounds of non-protein nature. This NPN-fraction (non-protein nitrogen) constitutes from 9 to 18 % of the total nitrogen in teleosts.

The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino-acids, nucleotides and purine bases, and, in the case of cartilaginous fish, urea.

Table 4.4 lists some of the components in the NPN-fraction of various fish, poultry meat and mammalian meat.

Table 4.4 Major differences in muscle extractives

Compound in mg/100 wet weight[1)]	Fish Cod	Fish Herring	Fish Shark species	Crustaceans Lobster	Poutry Leg muscle	Mammalian muscle
1) Total extractives	1200	1200	3000	5500	1200	3500
2) Total free amino-acids:	75	300	100	3000	440	350

Arginine	<10	<10	<10	750	<20	<10
Glycine	20	20	20	100-1000	<20	<10
Glutamic acid	<10	<10	<10	270	55	36
Histidine	<1.0	86	<1.0	-	<10	<10
Proline	<1.0	<1.0	<1.0	750	<10	<10
3) Creatine	400	400	300	0	-	550
4) Betaine	0	0	150	100	-	-
5) Trimethylamine oxide	350	250	500-1000	100	0	0
6) Anserine	150	0	0	0	280	150
7) Carnosine	0	0	0	0	180	200
8) Urea	0	0	2000	-	-	35

[1] It should be noted that the unit in this table refers to the total molecular weight of the compound

SOURCE: Shewan, 1974

An example of the distribution of the different compounds in the NPN-fraction in freshwater and marine fish is shown in Figure 4.5. It should be noted that the composition varies not only from species to species, but also within the species depending on size, season, muscle sample, etc.

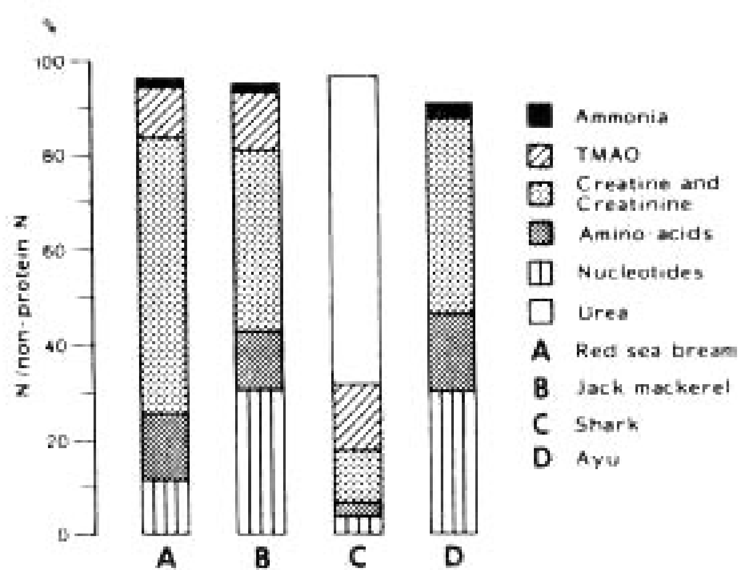


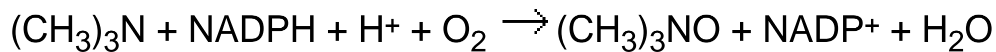
Figure 4.5 Distribution of non-protein nitrogen in fish muscles of two marine bonyfish (A,B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama *et al.,* 1977)

TMAO constitutes a characteristic and important part of the NPN-fraction in marine species and deserves further mention. This component is found in all marine fish species in quantities from 1 to 5 % of the muscle tissue (dry weight) but is virtually absent from freshwater species and from terrestrial organisms (Anderson and Fellers, 1952; Hebard *et al.,* 1982).

One exception was recently found in a study of Nile perch and tilapia from Lake Victoria, where as much as 150-200 mg TMAO/100 g of fresh fish was found (Gram *et al.,* 1989).

Although much work has been conducted on the origin and role of TMAO, there is still much to be clarified. Stroem *et al.* (1979) have shown that TMAO is formed by biosynthesis in certain zooplankton species. These organisms possess an enzyme (TMA mono-oxygenase) which oxidizes TMA to TMAO. TMA is commonly found in marine plants as are many other methylated amines (monomethylamine and dimethylamine). Plankton-eating fish may obtain their TMAO from feeding on these zooplankton (exogenous origin). Belinski (1964) and Agustsson and Stroem (1981) have shown that certain fish species are able to synthesize TMAO from TMA, but this synthesis is regarded as being of minor importance.

The TMA-oxidase system is found in the microsomes of the cells and is dependent on the presence of Nicotinamide ademine denucleotide phosphate (NADPH):



It is puzzling that this mono-oxygenase can be widely found in mammals (where it is thought to function as a detoxifier), while most fish have low or no detectable actitity of this enzyme.

Japanese research (Kawabata, 1953) indicates that there is a TMAO-reducing system present in the dark muscle of certain pelagic fishes.

The amount of TMAO in the muscle tissue depends on the species, season, fishing ground, etc. In general, the highest amount is found in elasmobranchs and squid (75-250 mg N/100 g); cod have somewhat less (60-120 mg N/100 g) while flatfish and pelagic fish have the least. An extensive compilation of data is given by Hebard *et* al. (1982). According to Tokunaga (1970), pelagic fish (sardines, tuna, mackerel) have their highest concentration of TMAO in the dark muscle while demersal, white-fleshed fish have a much higher content in the white muscle.

In elasmobranchs, TMAO seems to play a role in osmoregulation, and it has been shown that a transfer of small rays to a mixture of fresh and sea water (1:1) will result in a 50 % reduction of intracellular TMAO. The role of TMAO in teleosts is more uncertain.

Several hypotheses for the role of TMAO have been proposed:

- TMAO is essentially a waste product, a detoxified form of TMA
- TMAO is an osmoregulator
- TMAO functions as an "anti-freeze"
- TMAO has no significant function. It is accumulated in the muscle when the fish is fed a TMAO-containing diet

According to Stroem (1984), it is now generally believed that TMAO has an osmoregulatory role.

As the occurrence of TMAO had previously been found virtually only in marine species until the observation published by Gram *et al.* (1989), it was speculated that TMAO together with high amounts of taurine could have additional effects, at least in fresh water fish (Anthoni *et al.,* 1990 a).

Quantitatively, the main component of the NPN-fraction is creatine. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction.

The NPN-fraction also contains a fair amount of free amino-acids. These constitute 630 mg/ 100 g light muscle in mackerel *(Scomber scombrus),* 350-420 mg/ 100 g in herring *(Clupea harengus)* and 310-370 mg/100 g in capelin *(Mallotus villosus).* The relative importance of the different amino- acids varies with species. Taurine, alanine, glycine and imidazole-containing amino-acids seem to dominate in most fish. Of the imidazole-containing amino-acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine.

The amount of nucleotides and nucleotide fragments in dead fish depends on the state of the fish and is discussed in section 5.

4.5 Vitamins and minerals

The amount of vitamins and minerals is species-specific and can furthermore vary with season. In general, fish meat is a good source of the B vitamins and, in the case of fatty species, also of the A and D vitamins. Some freshwater species such as carp have high thiaminase actitity so the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular but also of iron, copper and selenium. Saltwater fish have a high content of iodine. In Tables 4.5 and 4.6 some of the vitamin and mineral contents are listed. Because of the natural variation of these constituents, it is impossible to give accurate figures.

Table 4.5 Vitamins in fish

Fish	A (IU/g)	D (IU/g)	B_1 (thiamine) (μ/g)	B_2 (riboflavin) (μ/g)	Niacin (μ/g)	Pantothenic acid (μ/g)	B_6 (μ/g)
Cod fillet	0-50	0	0.7	0.8	20	1.7	1.7
Herring fillet	20-400	300-1000	0.4	3.0	40	10	4.5
Cod-liver oil	200-10.000	20-300	-	[1] 3.4	[1] 15	[1] 4.3	-

1) Whole liver

SOURCE: Murray and Burt, 1969

Table 4.6 Some mineral constituents of fish muscle

Element	Average value (mg/100 g)	Range (mg/100 g)
Sodium	72	30 -134
Potassium	278	19 -502
Calcium	79	19 -881
Magnesium	38	4.5-452
Phosphorus	190	68-550

SOURCE: Murray and Burt, 1969

The vitamin content is comparable to that of mammals except in the case of the A and D vitamins which are found in large amounts in the meat of fatty species and in abundance in the liver of species such as cod and halibut. It should be noted that the sodium content of fish meat is relatively low which makes it suitable for low-sodium diets.

In aquacultured fish, the contents of vitamins and minerals are considered to reflect the composition of the corresponding components in the fish feed, although the observed data should be interpreted with great caution (Maage *et al.,* 1991). In order to protect the n-3 polyunsaturated fatty acids, considered of great importance both for fish and human health, vitamin E may be added to the fish feed as an antioxidant. It has been shown that the resulting level of vitamin E in the fish tissue corresponds to the concentration in the feed (Waagbo *et al.,* 1991).

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3. BIOLOGICAL ASPECTS

3.1. Classification
3.2. Anatomy and physiology
3.3. Growth and reproduction

3.1 Classification

Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Thurman and Webber, 1984).

Five vertebrate classes have species which could be called fish, but only two of these groups - the sharks and rays, and the bonyfish - are generally important and widely distributed in the aquatic environment. The evolutionary relationship between the various groups of fish is shown in Figure 3.1.

Fish are the most numerous of the vertebrates, with at least 20 000 known species, and more than half (58 %) are found in the marine environment. They are most common in the warm and temperate waters of the continental shelves (some 8 000 species). In the cold polar waters about 1 100 species are found. In the oceanic pelagic environment well away from the effect of land, there are only some 225 species. Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1 000 m depth) the number of species increases. There are some 1 000 species of so-called mid- water fish (Thurman and Webber, 1984).

Classifying all these organisms into a system is not an easy task, but the taxonomist groups organisms into natural units that reflect evolutionary relationships. The smallest unit is the species. Each species is identified by a scientific name which has two parts the genus and the specific epithet (binominal nomenclature). The genus name is always capitalized and both are italicized. As an example, the scientific (species) name of the common dolphin is *Delphinus delphis.* The genus is a category that contains one or more species, while the next step in the hierarchy is

the family which may contain one or more genus. Thus the total hierarchical system is: Kingdom: Phylum: Class: Order: Family: Genus: Species.

The use of common or local names often creates confusion since the same species may have different names in different regions or, conversely, the same name is ascribed to several different species, sometimes with different technological properties. As a point of reference the scientific name should, therefore, be given in any kind of publication or report the first time a particular species is referred to by its common name. For further information see the International Council for the Exploration of the Sea "List of names of Fish and Shellfish" (ICES, 1966); the "Multilingual Dictionary of Fish and Fish Products" prepared by the Organisation for Economic Cooperation and Development (OECD, 1990) and the "Multilingual Illustrated Dictionary of Aquatic Animals and Plants" (Commission of the European Communities, 1993).

The classification of fish into cartilaginous and bony (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently (section 5) and vary with regard to chemical composition (section 4).

Figure 3.1 Simplified phylogenetic tree of the fishes. (Examples of food-fish, using common English names are shown in parantheses). (SOURCE: N. Bonde (1994),

Geological Inst., Copenhagen)

Furthermore, fish can be divided into fatty and lean species, but this type of classification is based on biological and technological characteristics as shown in Table 3.1.

Table 3.1 Classification of fish

Scientific grouping	Biological characteristics	Technological characteristics	Examples
Cyclostomes	jawless fish		lampreys, slime-eels
Chondrichthyes	cartilaginous fish	high urea content in muscle	sharks, skate, rays
Teleostei or bony fish	pelagic fish	fatty fish (store lipids in body tissue)	herring, mackerel, sardine tuna, sprat
	demersal fish	lean (white) fish (store lipids in liver only)	cod, haddock, hake grouper, seabass

3.2 Anatomy and physiology

The skeleton

Being vertebrates, fish have a vertebral column - the backbone - and a cranium covering the brain. The backbone runs from the head to the tail fin and is composed of segments (vertebrae). These vertebrae are extended dorsally to form neural spines, and in the trunk region they have lateral processes that bear ribs (Figure 3.2). The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes) (see also Figure 3.3). Usually, there is also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise prepared for food.

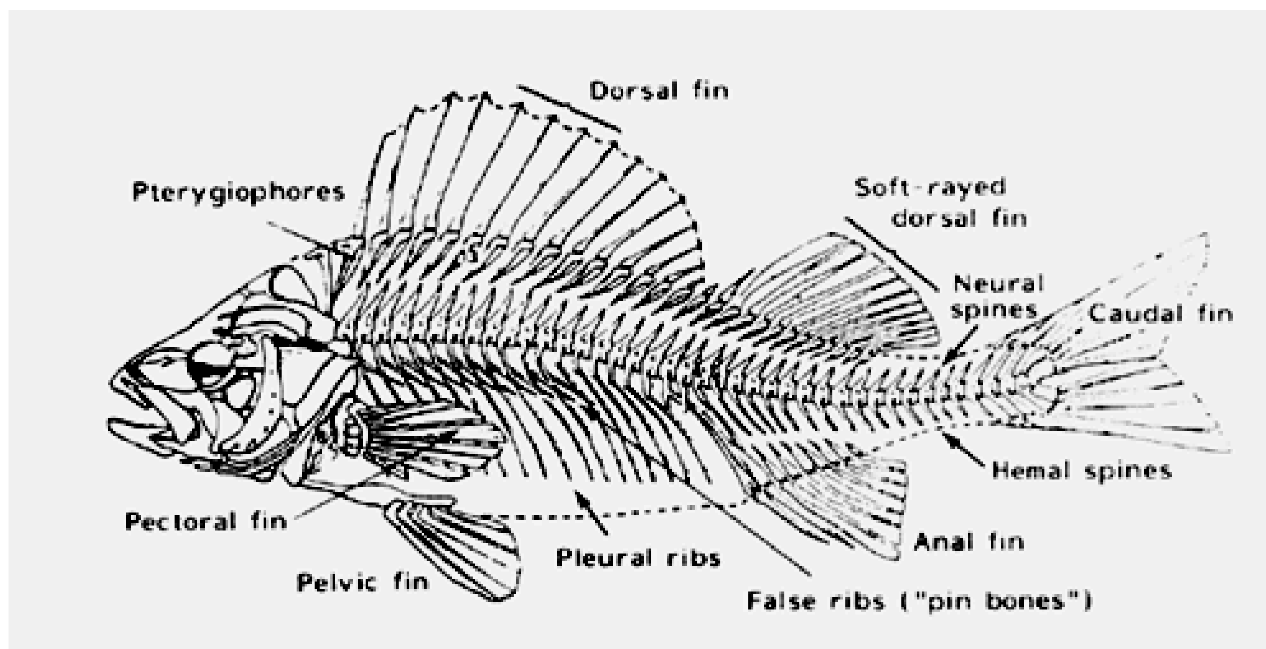


Figure 3.2 Skeleton of bonyfish (Eriksson and Johnson, 1979)

Muscle anatomy and function

The anatomy of fish muscle is different from the anatomy of terrestrial mammals, in that the fish lacks the tendinous system connecting muscle bundles **to the skeleton of the** animal. Instead, fish has muscle cells running in parallel and connected to sheaths of connective tissue (myocommata), which are anchored to the skeleton and the skin. The bundles of parallel muscle cells are called myotomes (Figure 3.3).

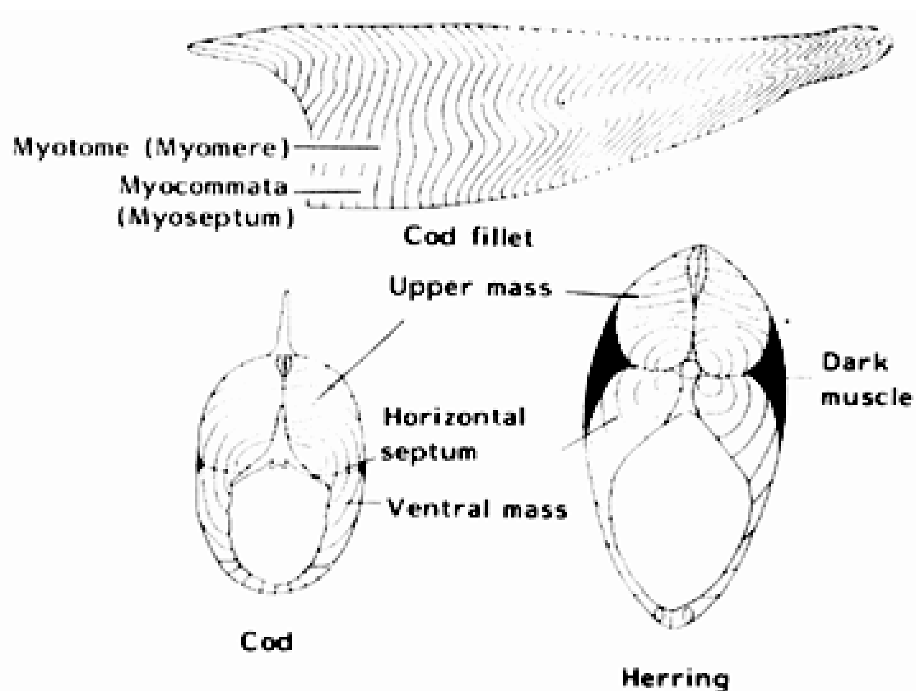


Figure 3.3 Skeletal musculature of fish (Knorr, 1974)

All muscle cells extend the full length between two myocommata, and run parallel with the longitudinal direction of the fish. The muscle mass on each side of the fish makes up the fillet, of which the upper part is termed the dorsal muscle and the lower part the ventral muscle.

The fillet is heterogenous in that the length of the muscle cells vary from the head end (anterior) to the tail end (posterior). The longest muscle cells in cod are found at about the twelfth myotome counting from the head, with an average length around 10 mm in a fish that is 60 cm long (Love, 1970). The diameter of the cells also vary, being widest in the ventral part of the fillet.

The myocommata run in an oblique, almost "plow-like" pattern perpendicular to the long axis of the fish, from the skin to the spine. This anatomy is ideally suited for the flexing muscle movements necessary for propelling the fish through the water.

As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, i.e., the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc., and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating system making the muscle appear striated upon microscopic examination (Figure 3.4).

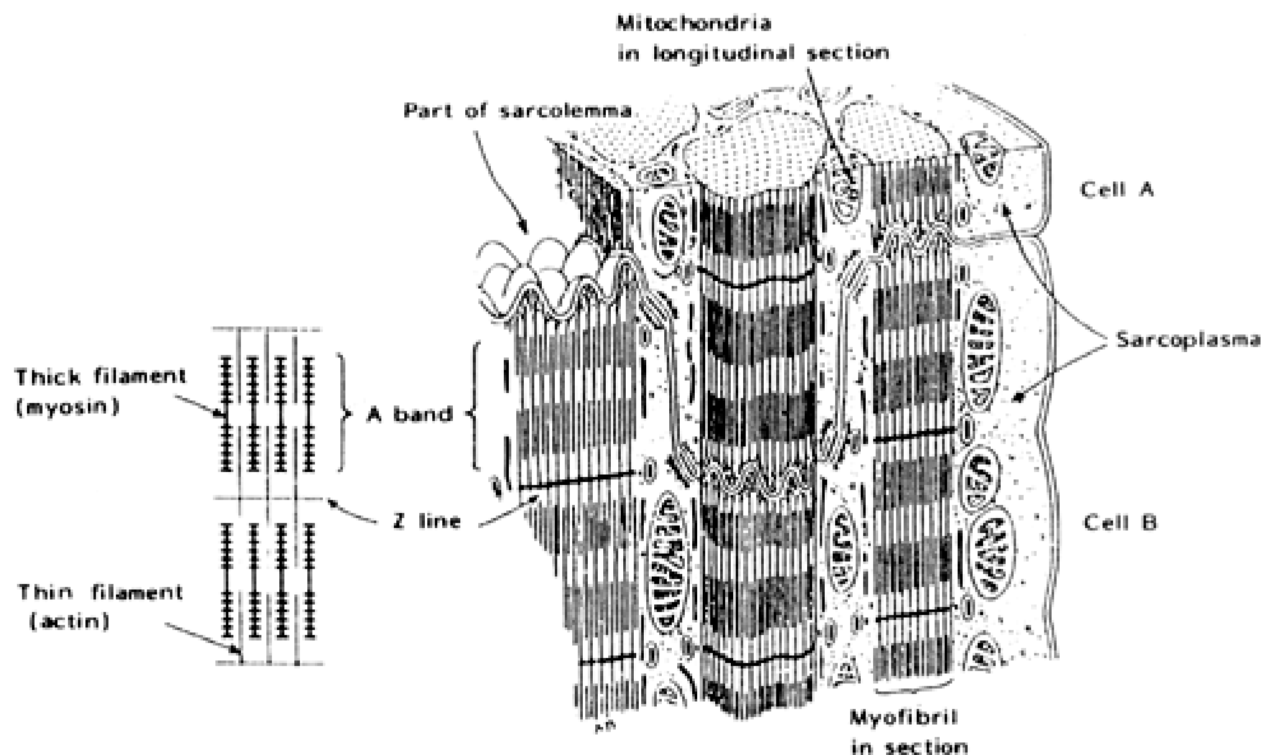


Figure 3.4 Section of a cell showing various structures including the myofibrils (Bell *et al., 1976)*

Most fish muscle tissue is white but, depending on the species, many fish will have a certain amount of dark tissue of a brown or reddish colour. The dark muscle is located just under the skin along the side of the body.

The proportion of dark to light muscle varies with the activity of the fish. In pelagic fish, i.e., species such as herring and mackerel which swim more or less continuously, up to 48 % of the body weight may consist of dark muscle (Love, *1970).* In demersal fish, i.e., species which feed on the bottom and only move periodically, the amount of dark muscle is very small.

There are many differences in the chemical composition of the two muscle types, some of the more noteworthy being higher levels of lipids and myoglobin in the dark muscle.

From a technological point of view, the high lipid content of dark muscle is important because of problems with rancidity.

The reddish meat colour found in salmon and sea trout does not originate from myoglobin but is due to the red carotenoid, astaxanthin. The function of this pigment has not been clearly established, but it has been proposed that the carotenoid may play a role as an antioxidant. Further, the accumulation in the muscle may function as a depot for pigment needed at the time of spawning when the male develops a strong red colour in the skin and the female transport carotenoids into the eggs. The latter seems to depend heavily on the amount of carotenoids for proper development after fertilization. It is clearly seen that the muscle colour of salmonids fades at the time of spawning.

The fish cannot synthesize astaxanthin and is thus dependent on ingestion of the pigment through the feed. Some salmonids live in waters where the natural prey does not contain much carotenoid, e.g., in the Baltic Sea, thus resulting in a muscle colour less red than salmonids from other waters. This may be taken as an indication that the proposed physiological function of astaxanthin in salmonids explained above may be less important.

In salmon aquaculture, astaxanthin is included in the feed, as the red colour of the flesh is one of the most important quality criteria for this species.

Muscle contraction starts when a nervous impulse sets off a release of Ca + + from the sarcoplasmic reticulum to the myofibrils. When the Ca + + concentration increases at the active enzyme site on the myosin filament, the enzyme ATP-ase is activated. This ATP-ase splits the ATP found between the actin and myosin filaments, causing a release of energy. Most of this energy is used as contractile

energy making the actin filaments slide in between the myosin filaments in a telescopic fashion, thereby contracting the muscle fibre. When the reaction is reversed (i.e., when the Ca + + is pumped back, the contractile ATP-ase activity stops and the filaments are allowed to slip passively past each other), the muscle is relaxed.

The energy source for ATP generation in the light muscle is glycogen, whereas the dark muscle may also use lipids. A major difference is, further, that the dark muscle contains much more mitochondria than light muscle, thus enabling the dark muscle to operate an extensive aerobic energy metabolism resulting in CO_2 and H_2O as the end products. The light muscle, mostly generating energy by the anaerobic metabolism, accumulates lactic acid which has to be transported to the liver for further metabolization. In addition, the dark muscle is reported to possess functions similar to those are found in the liver.

The different metabolic patterns found in the two muscle types makes the light muscle excellently fitted for strong, short muscle bursts, whereas the dark muscle is designed for continual, although not so strong muscle movements.

Post mortem the biochemical and physiological regulatory functions operating in vivo ceases, and the energy resources in the muscle are depleted. When the level of ATP reaches its minimum, myosin and actin are interconnected irreversibly, resulting in rigor mortis. This phenomenon is further described in section 5.

The cardiovascular system

The cardiovascular system is of considerable interest to the fish technologist since it is important in some species to bleed the fish (i.e., remove most of the blood) after capture.

The fish heart is constructed for single circulation (Figure 3.5). In bony fish it consists of two consecutive chambers pumping venous blood toward the gills via the ventral aorta.

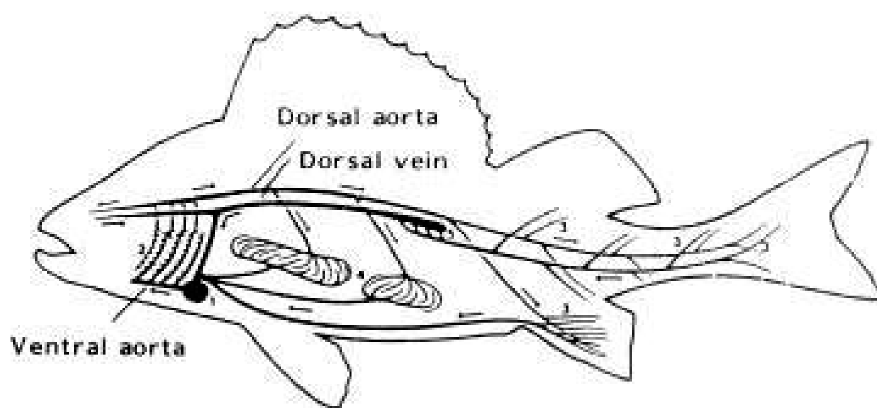


Figure 3.5 Blood circulation in fish (Eriksson and Johnson, 1979)

Notes:

1. The heart pumps blood toward the gills.
2. The blood is aerated in the gills.
3. Arterial blood is dispersed into the capillaries where the transfer of oxygen and nutrients to the surrounding tissue takes place.
4. The nutrients from ingested food are absorbed from the intestines, then transported to the liver and later dispersed in the blood throughout the body.
5. In the kidneys the blood is "purified" and waste products are excreted via the urine.

After being aerated in the gills, the arterial blood is collected in the dorsal aorta running just beneath the vertebral column and from here it is dispersed into the different tissues via the capillaries. The venous blood returns to the heart, flowing in veins of increasingly larger size (the biggest is the dorsal vein which is also located beneath the vertebral column). The veins all gather into one blood vessel before entering the heart. The total volume of the blood in fish ranges from 1.5 to 3.0 % of the body weight. Most of it is located in the internal organs while the muscular tissues, constituting two- thirds of the body weight, contain only 20 % of the blood volume. This distribution is not changed during exercise since the light muscle in particular is not very vascularized.

During blood circulation the blood pressure drops from around 30 mg Hg in the ventral aorta to 0 when entering the heart (Randall, 1970). After the blood has passed through the gills, the blood pressure derived from the pumping activity of the heart is already greatly decreased. Muscle contractions are important in pumping the blood back to the heart and counterflow is prevented by a system of paired valves inside the veins.

Clearly, the single circulation of fish is fundamentally different from the system in mammals (Figure 3.6), where the blood passes through the heart twice and is propelled out into the body under high pressure due to the contractions of the heart.

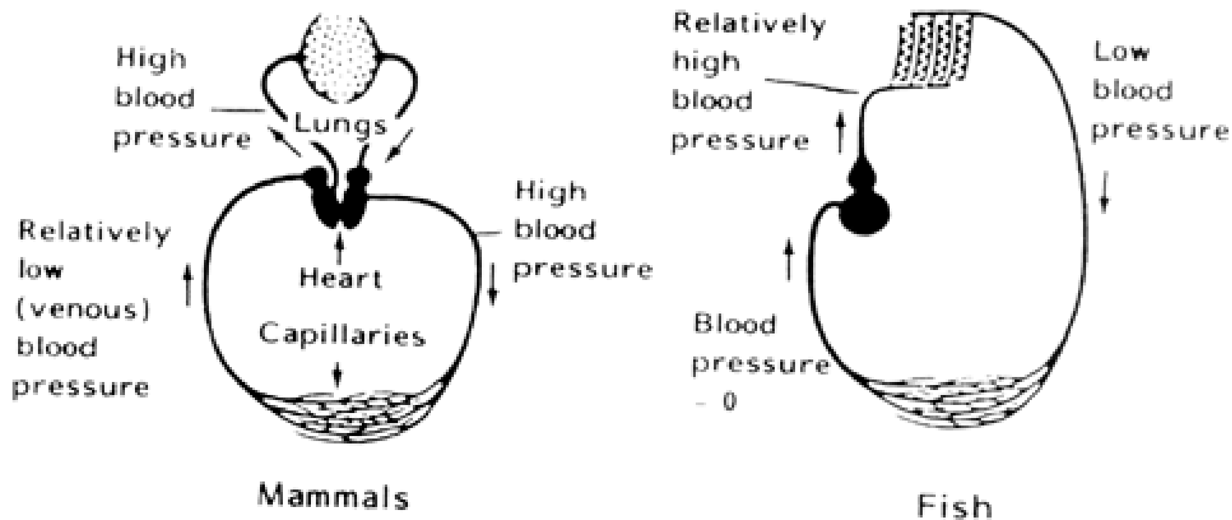


Figure 3.6 Blood circulation in fish and mammals (Eriksson and Johnson, 1979)

In fish, the heart does not play an important role in the transportation of blood from the capillaries back to the heart. This has been confirmed in an experiment where the impact of different bleeding procedures on the colour of cod fillets was examined. No difference could be found regardless of whether the fish had been bled by means of cutting the throat in front of or behind the heart before gutting, or had not been cut at all before slaughter.

In some fisheries, bleeding of the fish is very important as a uniform white fillet is desirable. In order to obtain this, a number of countries have recommended that fish are bled for a period (15-20 min) prior to being gutted. This means that throat cutting and gutting must be carried out in two separate operations and that special arrangements (bleeding tanks) must be provided on deck. This complicates the working process (two operations instead of one), time-consuming for the fishermen and increases the time-lag before the fish is chilled. Furthermore it requires extra space on an otherwise crowded working deck.

Several researchers have questioned the necessity of handling the fish in a two-step procedure involving a special bleeding period (Botta *et al.,* 1986; Huss and Asenjo, 1977 a; Valdimarsson *et al.* 1984). There seems to be general agreement about the following:

- bleeding is more affected by time onboard prior to bleeding/gutting than by the actual bleeding/gutting procedure.
- best bleeding is obtained if live fish are handled, but it is of major importance to cut the fish before it enters rigor mortis since it is the muscle contractions that force the blood out of the tissues.

Disagreement exists as to the cutting method. Huss and Asenjo (1977 a) found best

bleeding if a deep throat cut including the dorsal aorta was applied, but this was not confirmed in the work of Botta *et al.* (1986). The latter also recommended to include a bleeding period (two-step procedure) when live fish were handled (fishing with pound net, trap, seine, longline or jigging), while Valdimarsson *et al.* (1984) found that the quality of dead cod (4 h after being brought onboard) was slightly improved using the two-step procedure. However, it should be pointed out that the effect of bleeding should also be weighted against the advantages of having a fast and effective handling procedure resulting in rapid chilling of the catch.

Discoloration of the fillet may also be a result of rough handling during catch and catch handling while the fish is still alive. Physical mishandling in the net (long trawling time, very large catches) or on the deck (fishermen stepping on the fish or throwing boxes, containers and other items on top of the fish) may cause bruises, rupture of blood vessels and blood oozing into the muscle tissue (haematoma).

Heavy pressure on dead fish, when the blood is clotted (e.g., overloading of fish boxes) does not cause discoloration, but the fish may suffer a serious weight loss.

Other organs

Among the other organs, only the roe and liver play a major role as foodstuffs. Their size depends on the fish species and varies with life cycle, feed intake and season. In cod the weight of the roe varies from a few percent up to 27 % of the body weight and the weight of the liver ranges from 1 to 4.5 %. Likewise, the composition can change and the oil content of the liver vary from 15 to 75 %, with the highest values being found during autumn (Jangaard *et al.,* 1967).

3.3 Growth and reproduction

During growth it is the size of each muscle cell that increases rather than the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a size characteristic of the species and is this not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to rear female fish in aquaculture.

Every year mature fish use energy to build up the gonads (the roe and milk). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake (Figure 3.7).

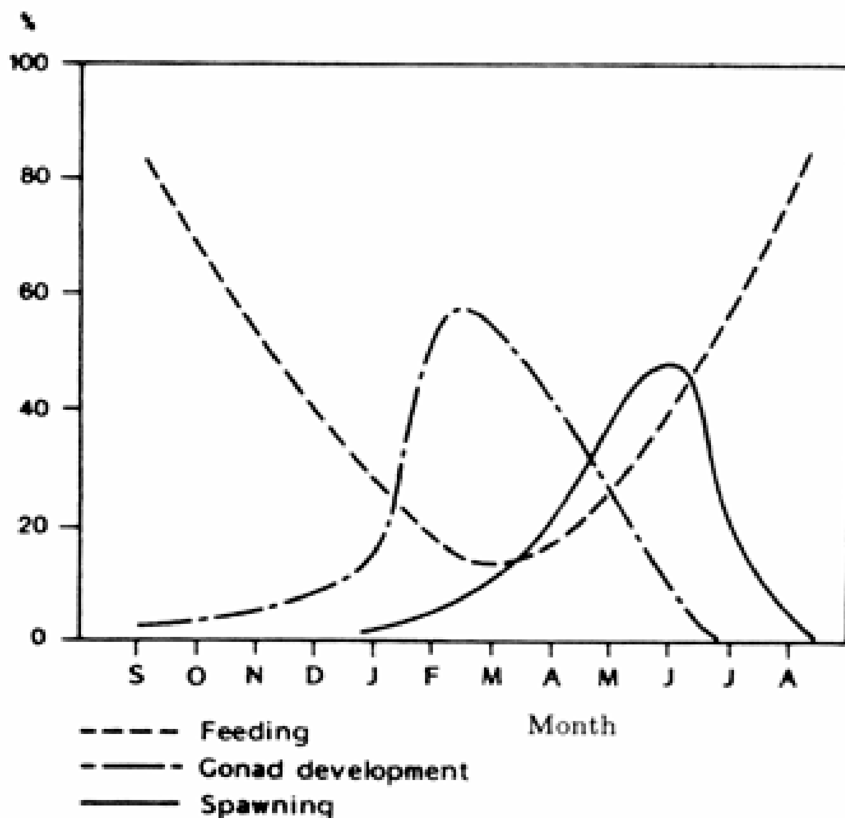


Figure 3.7 Relation between feeding cycle (percentage sample with food in stomach) and reproductive cycle (gonad development), percentage fish with ripening gonads (spawning, percentage ripe fish) of haddock *(Melanogrammus aeglefinus).* It should be noted that the development of the gonads takes place while the fish is starving (Hoar, 1957).

In North Sea cod it was found that prior to spawning the water content of the muscle increases (Figure 3.8) and the protein content decreases. In extreme cases the water content of very large cod can attain 87 % of the body weight prior to spawning (Love, 1970).

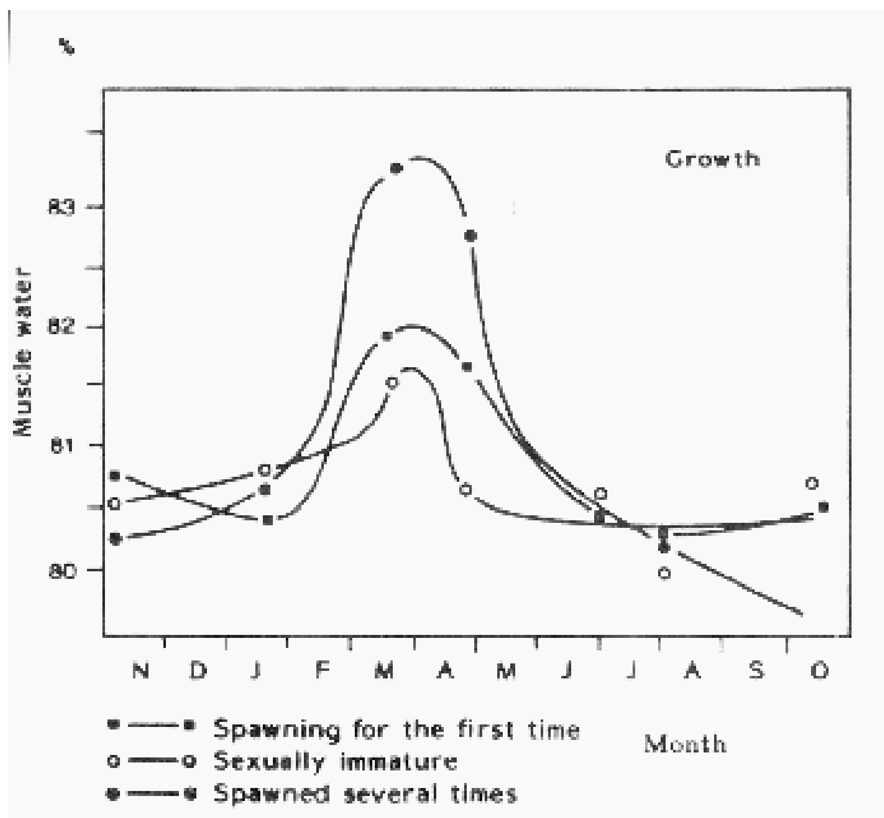
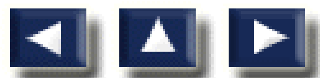


Figure 3.8 Water content of cod muscle *(Gadus morhua)* (Love, 1970)

The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity (Figure 3.7), while some have ripe ovaries for nearly the whole year.

The depletion of the reserves of the fish during gonadal development can be extremely severe, especially if reproduction is combined with migration to the breeding grounds. Some species, e.g., Pacific salmon *(Oncorhynchus* spp.), eel *(Anguilla anguilla)* and others, manage to migrate only once, after which they degenerate and die. This is partly because these species do not eat during migration so that, in the case of a salmon, it can lose up to 92 % of its lipid, 72 % of its protein and 63 % of its ash content during migration and reproduction (Love, 1970).

On the other hand, other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about eight years before spawning causes its death, and other species can live even longer (Cushing, 1975). In former times, 25-year-old herring *(Clupea harengus)* were not unusual in the Norwegian Sea, and plaice *(Pleuronectes platessa) up* to 35 years old have been found. One of the oldest fish reported was a sturgeon *(Acipenser sturio)* from Lake Winnebago in Wisconsin. According to the number of rings in the otolith, it was over 100 years old.



5. POSTMORTEM CHANGES IN FISH

5.1. Sensory changes
5.2. Autolytic changes
5.3. Bacteriological changes
5.4. Lipid oxidation and hydrolysis

5.1 Sensory changes

Sensory changes are those perceived with the senses, i.e., appearance, odour, texture and taste.

Changes in raw fresh fish

The first sensory changes of fish during storage are concerned with appearance and texture. The characteristic taste of the species is normally developed the first couple of days during storage in ice.

The most dramatic change is onset of rigor mortis. Immediately after death the muscle is totally relaxed and the limp elastic texture usually persists for some hours, whereafter the muscle will contract. When it becomes hard and stiff the whole body becomes inflexible and the fish is in rigor mortis This condition usually lasts for a day or more and then rigor resolves. The resolution of rigor mortis makes the muscle relax again and it becomes limp, but no longer as elastic as before rigor. The rate in onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish (Table 5.1).

The effect of temperature on rigor is not uniform. In the case of cod, high temperatures give a fast onset and a very strong rigor mortis. This should be avoided as strong rigor tensions may cause gaping, i.e., weakening of the connective tissue and rupture of the fillet.

It has generally been accepted that the onset and duration of rigor mortis are more rapid at high temperatures, but observations, especially on tropical fish show the opposite effect of temperature with regard to the onset of rigor. It is evident that in these species

the onset of rigor is accelerated at 0°C compared to 10°C, which is in good correlation with a stimulation of biochemical changes at 0°C (Poulter *et al.,* 1982; Iwamoto *et al.,* 1987). However, an explanation for this has been suggested by Abe and Okuma (1991) who have shown that onset of rigor mortis in carp (Cyprinus *carpio)* depends on the difference in sea temperature and storage temperature. When the difference is large the time from death to onset of rigor is short and *vice versa.*

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 hours (Azam *et al.,* 1990; Proctor *et al.,* 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor the fish body will be completely stiff; the filleting yield will be very poor, and rough handling can cause gaping. If the fillets are removed from the bone pre-rigor the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink up to 52 % and white muscle up to 15 % of the original length (Buttkus, 1963). If the fish is cooked pre-rigor the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Post-rigor the flesh will become firm, succulent and elastic.

Table 5.1 Onset and duration of rigor mortis in various fish species

Species	**Condition**	**Temperature °C**	**Time from death to onset of *rigor* (hours)**	**Time from death to end of *rigor* (hours)**
Cod *(Gadus morhua)*	Stressed	0	2-8	20-65
	Stressed	10-12	1	20-30
	Stressed	30	0.5	1-2
	Unstressed	0	14-15	72-96
Grouper *(Epinephelus malabaricus)*	Unstressed	2	2	18
Blue Tilapia *(Areochromis aureus)*	Stressed	0	1	
	Unstressed	0	6	
Tilapia *(Tilapia mossanibica)* small 60g	Unstressed	0-2	2-9	26.5

Grenadier *(Macrourus whitson)*	Stressed	0	<1	35-55
Anchovy *(Engraulis anchoita)*	Stressed	0	20-30	18
Plaice *(Pleuronectes platessa)*	Stressed	0	7-11	54-55
Coalfish *(Pollachius virens)*	Stressed	0	18	110
Redfish *(Sebastes* spp.)	Stressed	0	22	120
Japanese flounder *(Paralichthys olivaceus)*		0	3	>72
		5	12	>72
		10	6	72
		15	6	48
		20	6	24
Carp *(Cyprinus carpio)*		0	8	
		10	60	
		20	16	
	Stressed	0	1	
	Unstressed	0	6	

SOURCES: Hwang *et al.,* 1991; Iwamoto *et al., 1987;* Korhonen *et al.,* 1990;Nakayama *et al.,* 1992; Nazir and Magar, 1963; Partmann, 1965; Pawar and Magar, 1965; Stroud, 196; Trucco *et al.,* 1982

Whole fish and fillets frozen pre-rigor can give good products if they are carefully thawed at a low temperature in order to give *rigor mortis* time to pass while the muscle is still frozen.

The sensory evaluation of raw fish in markets and landing sites is done by assessing the appearance, texture and odour. The sensory attributes for fish are listed in Table 5.2. Most scoring systems are based upon changes taking place during storage in melting

ice. It should be remembered that the characteristic changes vary depending on the storage method. The appearance of fish stored under chilled condition without ice does not change as much as for iced fish, but the fish spoil more rapidly and an evaluation of cooked flavour will be necessary. A knowledge of the time /temperature history of the fish should therefore be essential at landing.

The characteristic sensory changes in fish post mortem vary considerably depending on fish species and storage method. A general description has been provided by the EEC in the guidelines for quality assessment of fish as shown in Table 5.2. The suggested scale is numbered from 0 to 3, where 3 is the best quality.

The West European Fish Technologists' Association has compiled a multilingual glossary of odours and flavours which also can be very useful when looking for descriptive words for sensory evaluation of freshness of fish (Howgate et al., 1992 (Appendix C).

Changes in eating quality

If quality criteria of chilled fish during storing are needed, sensory assessment of the cooked fish can be conducted. Some of the attributes for cooked fish and shellfish are mentioned in Table 5.2. A characteristic pattern of the deterioration of fish stored in ice can be found and divided into the following four phases:

- **Phase 1** The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic. In cod, haddock, whiting and flounder, the sweet taste is maximized 2-3 days after catching.
- **Phase 2** There is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours. The texture is still pleasant.
- **Phase 3** There is sign of spoilage and a range of volatile, unpleasant-smelling substances is produced depending on the fish species and type of spoilage (aerobic, anaerobic). One of the volatile compounds may be trimethylamine (TMA) derived from the bacterial reduction of trimethyl-aminoxide (TMAO). TMA has a very characteristic "fishy" smell. At the beginning of the phase the off-flavour may be slightly sour, fruity and slightly bitter, especially in fatty fish. During the later stages sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smells develop. The texture becomes either soft and watery or tough and dry.
- **Phase 4** The fish can be characterized as spoiled and putrid.

Table 5.2 Freshness ratings: Council Regulation (EEC) No. 103/76 OJ No. L20 (28 January 1976) (EEC, 1976)

Criteria	
	Marks

Part of fish inspected	3	2	1	0
Appearance				
Skin	Bright, iridescent pigmentation, no discoloration Aqueous, transparent, mucus	Pigmentation bright but not lustrous Slightly cloudy mucus	Pigmentation in the process of becoming discoloured and dull Milky mucus	[1]Dull pigmentation Opaque mucus
Eye	Convex (bulging) Transparent cornea Black, bright pupil	Convex and slightly sunken Slightly opalescent cornea Black, dull pupil	Flat Opalescent cornea Opaque pupil	[1]Concave in the centre Milky cornea Grey pupil
Gills	Bright colour No mucus	Less coloured Slight traces of clear mucus	Becoming discoloured Opaque mucus	[1]Yellowish Milky mucus
Flesh (cut from abdomen)	Bluish, translucent, smooth, shining No change in original colour	Velvety, waxy, dull Colour slightly changed	Slightly opaque	[1]Opaque
Colour (along vertebral column)	Uncoloured	Slightly pink	Pink	[1]Red
Organs	Kidneys and residues of other organs should be bright red, as should the blood inside the aorta	Kidneys and residues of other organs should be dull red; blood becoming discoloured	Kidneys and residues of other organs and blood should be pale red	Kidneys and residues of other organs and should be brownish in colour
Condition				

Flesh	Firm and elastic Smooth surface	Less elastic	Slightly soft (flaccid), less elastic Waxy (velvety) and dull surface	[1]Soft (flaccid) Scales easily detached from skin, surface rather wrinkled, inclining to mealy
Vertebral column	Breaks instead of coming away	Sticks	Sticks slightly	[1]Does not stick
Peritoneum	Sticks completely to flesh	Sticks	Sticks slightly	[1]Does not stick
		Smell		
Gills, skin abdominal cavity	Seaweed	No smell of seaweed or any bad smell	Slightly sour	[1]Sour

[1] Or in a more advanced state of decay.

A numbered scale may be used for the sensory evaluation of cooked fish as shown in Figure 5.1. The scale is numbered from 0 to 10, 10 indicating absolute freshness, 8 good quality and 6 a neutral tasteless fish. The rejection level is 4. Using the scale in this way the graph becomes S-shaped indicating a fast degradation of the fish during the first phase, a slower rate in phase 2 and 3 and finally a high rate when the fish is spoiled.

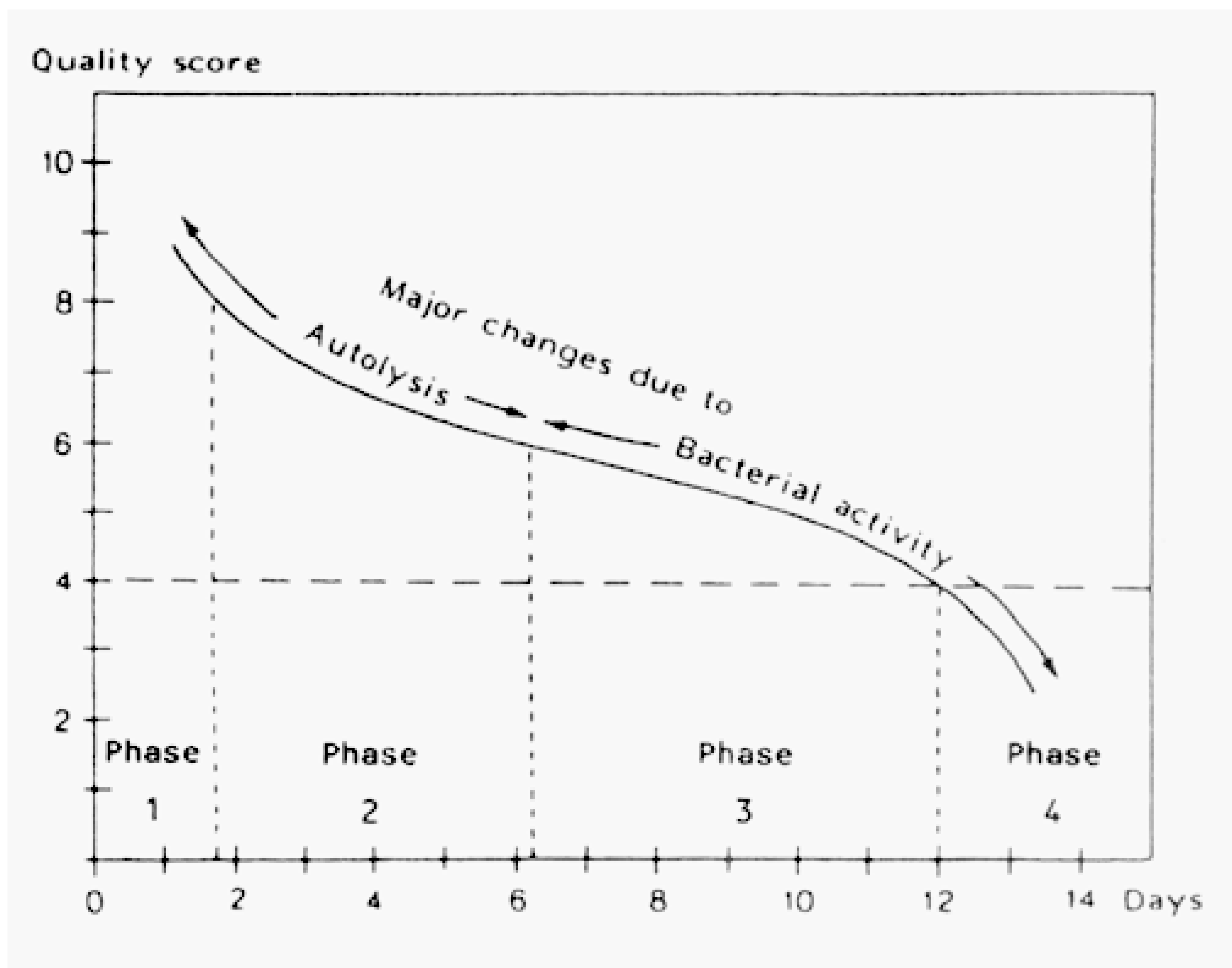


Figure 5.1 Changes in the eating quality of iced (0°C) cod (Huss, 1976)

Other scales can well be used and can change the shape of the graph. It is, however, important to understand the kind of results desired from the sensory analysis in order to ask the right questions to the sensory assessors.

5.2 Autolytic Changes

Autolysis means "self-digestion". It has been known for many years that there are at least two types of fish spoilage: bacterial and enzymatic. Uchyama and Ehira (1974) showed that for cod and yellowtail tuna, enzymatic changes related to fish freshness preceded and were unrelated to changes in the microbiological quality. In some species (squid, herring), the enzymatic changes precede and therefore predominate the spoilage of chilled fish. In others, autolysis contributes to varying degrees to the overall quality loss in addition to microbially-mediated processes.

Production of energy in post mortem muscle

At the point of death, the supply of oxygen to the muscle tissue is interrupted because the blood is no longer pumped by the heart and is not circulated through the gills where, in the living fish, it becomes enriched with oxygen. Since no oxygen is available for normal respiration, the production of energy from ingested nutrients is greatly restricted.

Figure 5.2 illustrates the normal pathway for the production of muscle energy in most living teleost fish (bony finfish). Glycogen (stored carbohydrate) or fat is oxidized or "burned" by the tissue enzymes in a series of reactions which ultimately produce carbon dioxide (CO_2), water and the energy-rich organic compound adenosine triphosphate (ATP). This type of respiration takes place in two stages: an anaerobic and an aerobic stage. The latter depends on the continued presence of oxygen (O_2) which is only available from the circulatory system. Most crustaceans are capable of respiring outside the aquatic environment by absorption of atmospheric oxygen for limited periods.

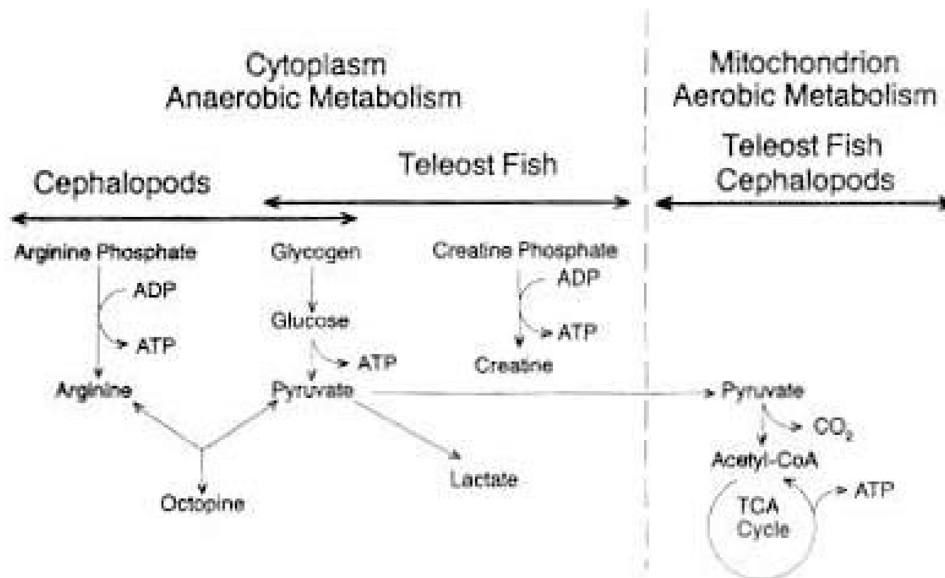


Figure 5.2 Aerobic and anaerobic breakdown of glycogen in fish muscle

Figure 5.2 also illustrates that, under anaerobic conditions, ATP may be synthesized by two other important pathways from creatine phosphate or from arginine phosphate. The former source of energy is restricted to vertebrate muscle (teleost fish) while the latter is characteristic of some invertebrates such as the cephalopods (squid and octopus). In either case, ATP production ceases when the creatine or arginine phosphates are depleted. It is interesting to note that octopine is the end-product from the anaerobic metabolism of cephalopods and is not acidic (unlike lactate), thus any changes in post mortem pH in such animals are not related to the production of lactic acid from glycogen.

For most teleost fish, glycolysis is the only possible pathway for the production of energy once the heart stops beating. This more inefficient process has principally lactic and pyruvic acids as its end-products. In addition, ATP is produced in glycolysis, but only 2 moles for each mole of glucose oxidized as compared to 36 moles ATP produced for each mole of glucose if the glycolytic end products are oxidized aerobically in the mitochondrion in the living animal. Thus, after death, the anaerobic muscle cannot maintain its normal level of ATP, and when the intracellular level declines from 7-10 µmoles/g to £ 1.0 µmoles/g tissue, the muscle enters rigor mortis. Post mortem glycolysis results in the accumulation of lactic acid which in turn lowers the pH of the muscle. In cod, the pH drops from 6.8 to an ultimate pH of 6.1-6.5. In some species of fish, the final pH may be lower: in large mackerel, the ultimate rigor pH may be as low as

5.8-6.0 and as low as 5.4-5.6 in tuna and halibut, however such low pH levels are unusual in marine teleosts. These pHs are seldom as low as those observed for post mortem mammalian muscle. For example, beef muscle often drops to pH levels of 5.1 in rigor mortis. The amount of lactic acid produced is related to the amount of stored carbohydrate (glycogen) in the living tissue. In general, fish muscle contains a relatively low level of glycogen compared to mammals, thus far less lactic acid is generated after death. Also, the nutritional status of the fish and the amount of stress and exercise encountered before death will have a dramatic effect on the levels of stored glycogen and consequently on the ultimate post mortem pH. As a rule, well-rested, well-fed fish contain more glycogen than exhausted fish. In a recent study of Japanese loach (Chiba et al., 1991), it was shown that only minutes of pre-capture stress resulted in a decrease of 0.50 pH units in 3 hours as compared to non-struggling fish whose pH dropped only 0.10 units in the same time period. In addition, the same authors showed that bleeding of fish significantly reduced the post mortem production of lactic acid.

The post mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water-holding capacity. Muscle tissue in the state of rigor mortis loses its moisture when cooked and is particularly unsuitable for further processing which involves heating, since heat denaturation enhances the water loss. Loss of water has a detrimental effect on the texture of fish muscle and it has been shown by Love (1975) that there is an inverse relationship between muscle toughness and pH, unacceptable levels of toughness (and water-loss on cooking) occurring at lower pH levels (Figure 5.3).

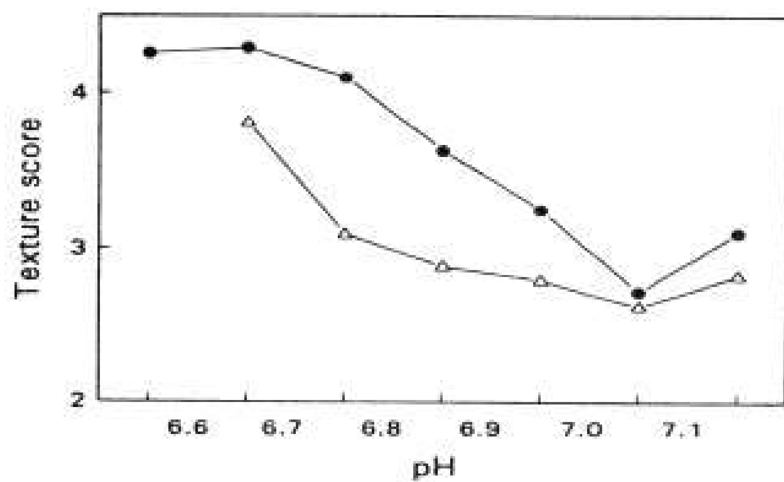


Figure 5.3.Relationship between cod muscle texture and pH, adapted from Love (1975). Black spots refer to fish caught from St. Kilda, Atlantic Ocean, whereas triangles refer to fish caught on Fells Bank, Davis Strait

Autolysis and nucleotide catabolism

As mentioned earlier, rigor mortis sets in when the muscle ATP level drops to £ 1.0 µmoles/g. ATP is not only a source of high energy which is required for muscle contraction in the living animal, but also acts as a muscle plasticizer. Muscle contraction

per se is controlled by calcium and an enzyme, ATP-ase which is found in every muscle cell. When intracellular Ca^{+2} levels are 1 μM, Ca^{+2} - activated ATP-ase reduces the amount of free muscle ATP which results in the interaction between the major contractile proteins, actin and myosin. This ultimately results in the shortening of the muscle, making it stiff and inextensible. A fish in rigor mortis cannot normally be filleted or processed because the carcass is too stiff to be manipulated and is often contorted, making machine-handling impossible (see also section 3.2 on bleeding and section 5.1 on sensory changes).

The **resolution** of rigor is a process still not completely understood but always results in the subsequent softening (relaxation) of the muscle tissue and is thought to be related to the activation of one or more of the naturally-occurring muscle enzymes, digesting away certain components of the rigor mortis complex. The softening of the muscle during resolution of rigor (and eventually spoilage processes) is coincidental with the autolytic changes. Among the changes, one of the first to be recognized was the degradation of ATP-related compounds in a more-or-less predictable manner after death. Figure 5.4 illustrates the degradation of ATP to form adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx). The degradation of ATP catabolites proceeds in the same manner with most fish but the speed of each individual reaction (from one catabolite to another) greatly varies from one species to another and often progresses coincidentally with the perceived level of spoilage as determined by trained analysts. Saito et al. (1959) were the first to observe this pattern and to develop a formula for fish freshness based on these autolytic changes:

$$K\% = \frac{[Ino] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [Ino] + [Hx]} \times 100$$

where [ATP], [ADP], [AMP], [IMP], [Ino] and [Hx] represent the relative concentrations of these compounds in fish muscle measured at various times during chilled storage.

The K or "freshness" index gives a relative freshness rating based primarily on the autolytic changes which take place during post mortem storage of the muscle. Thus, the higher the K value, the lower the freshness level. Unfortunately, some fish species such as Atlantic cod reach a maximum K value well in advance of the shelf life as determined by trained judges, and K is therefore not considered reliable as a freshness index for all marine finfish. Also, the degradation of nucleotide catabolites is only coincidental with perceived changes in freshness and not necessarily related to the cause of freshness deterioration since only Hx is considered to have a direct effect on the perceived bitter off-flavour of spoiled fish (Hughes and Jones, 1966). It is now widely accepted that IMP is responsible for the desirable fresh fish flavour which is only present in top quality seafood. None of the nucleotide catabolites are considered to be related to the perceived changes in texture during the autolytic process except of course ATP whose loss is associated with *rigor mortis.*

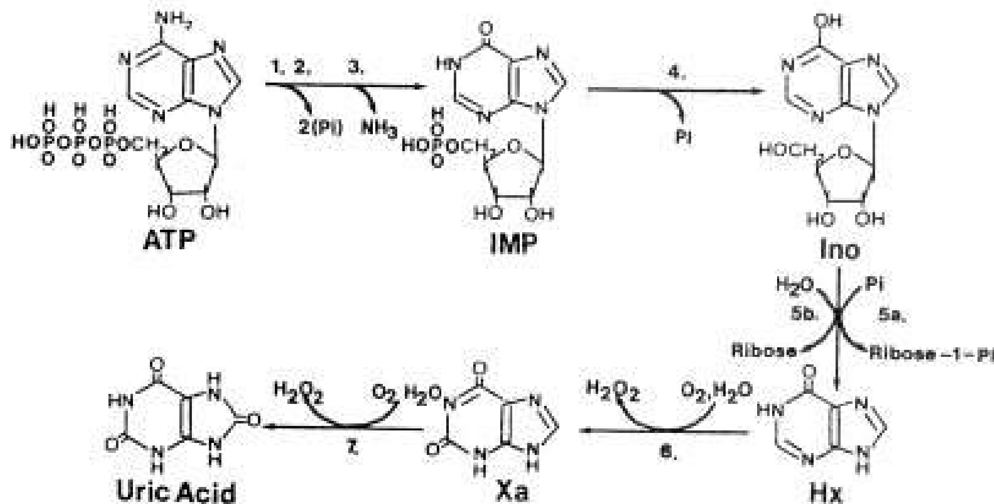


Figure 5.4 *Postmortem* ATP degradation in fish muscle. Enzymes include: 1. ATP-ase; 2. myokinase; 3. AMP deaminase; 4.IMP phosphohydrolase; 5a. nucleoside phosphorylase; 5b. inosine nucleosidase; 6,7. xanthine oxidase. Source: Gill (1992)

Surette *et al.* (1988) followed the autolysis of sterile and non-sterile cod as indicated by the ATP catabolites. The rates of formation and breakdown of IMP were the same in both sterile and non- sterile samples of cod tissue (Figures 5.5a and 5.5b), indicating that the catabolic pathway for the degradation of ATP through to inosine is entirely due to autolytic enzymes.

The conversion of Iino to Hex was accelerated by about 2 days for the non-sterile samples, suggesting that bacterial nucleoside phosphorylase (enzyme 5.a in Figure 5.4) plays a major role in the *postmortem* production of Hx in refrigerated cod (see also section 5.3). It is interesting to note that Surette *et al.* (1988) were not able to recover nucleoside phosphorylase from freshly killed cod, but Surette *et al.* (1990) later went on to isolate and purify this enzyme from a *Proteus* bacterium recovered from spoiled cod fillets. As mentioned earlier, large variations can be expected in the patterns of nucleotide degradation from one species to another. The variations in Hx among various types of fish are shown in Figure 5.6. It is clear therefore that Hx determination would likely not be useful for such species as swordfish and redfish.

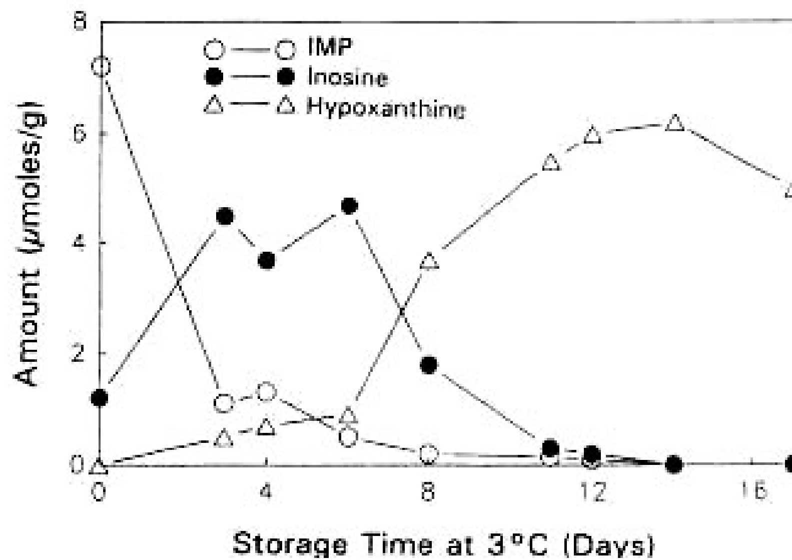


Figure 5.5a Changes in IMP, Ino and Hx in sterile cod fillets at 3°C adapted from Gill (1990)

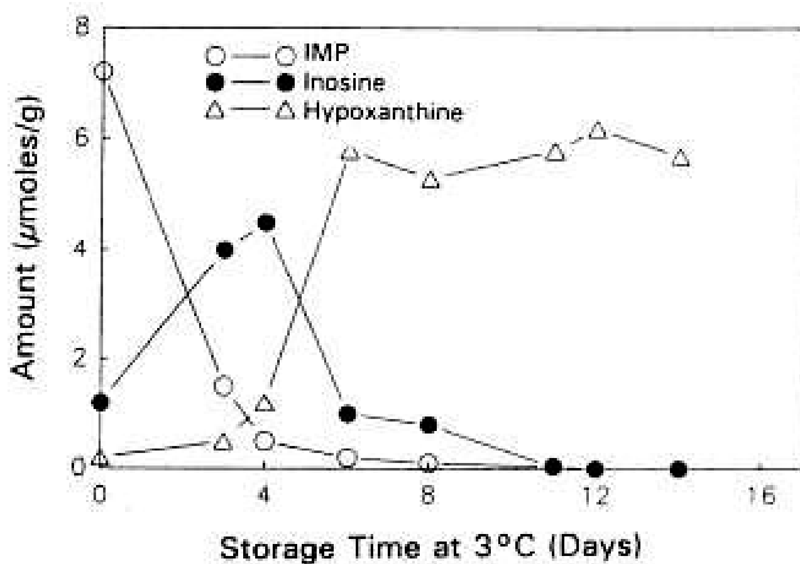


Figure 5.5b Changes in IMP, Ino and Hx in non-sterile cod fillets at 3°C adapted from Gill (1990)

There is little doubt that physical handling accelerates the autolytic changes in chilled fish. Surette et al. (1988) reported that the breakdown rate of the nucleotide catabolites was greater in sterile fillets than in non-sterile gutted whole cod. This is perhaps not surprising since many of the autolytic enzymes have been shown to be compartmentalized in discrete membrane-bound packages which become broken when subjected to physical abuse and result in the intimate mixing of enzyme and substrate. Crushing of the fish by ice or other fish can seriously affect the edibility and filleting yields even for fish which have a relatively low bacterial load, demonstrating the importance of autolytic processes. Iced fish should never be stored in boxes deeper than 30 cm and it is equally important to be sure that fish boxes are not permitted to "nest" one on top of the other if autolysis is to be minimized. Systems for conveying fish and for

discharge from the vessels must be designed so as to avoid physical damage to the delicate tissues.

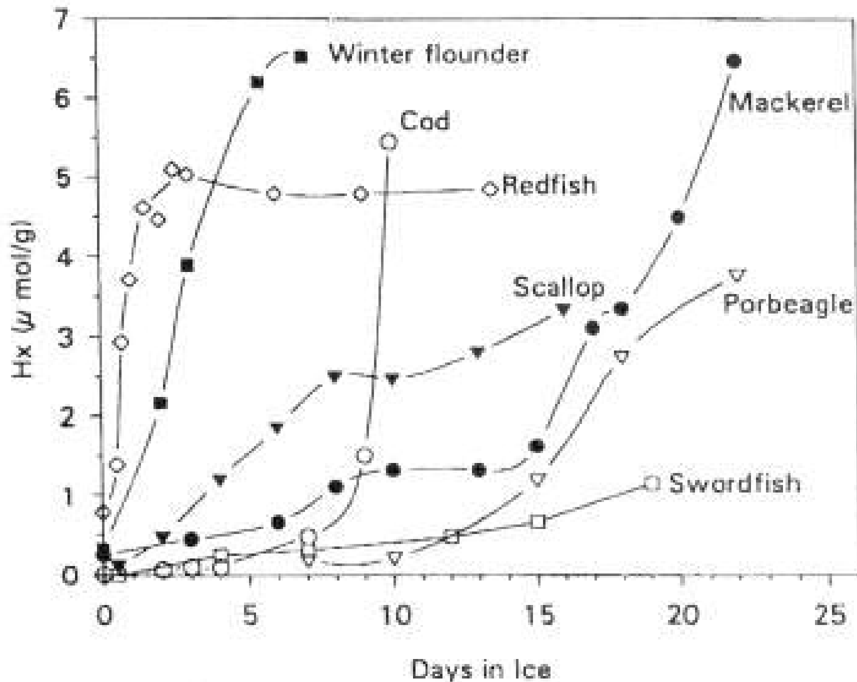


Figure 5.6 Variation in the rate of Hx accumulation of several species during storage in ice. Adapted from Fraser *et al.* (1967)

Several rapid methods have been developed for the determination of individual nucleotide catabolites or combinations including the freshness index. Two recent reviews should be consulted (Gill, 1990, 1992).

Autolytic changes involving proteolytic enzymes

Many proteases have been isolated from fish muscle and the effects of proteolytic breakdown are often related to extensive softening of the tissue. Perhaps one of the most notable examples of autolytic proteolysis is the incidence of belly-bursting in pelagic (fatty fish) species such as herring and capelin. This type of tissue softening is most predominant in summer months when pelagics are feeding heavily, particularly on "red feed" consisting of copepods and euphausiids. The low molecular weight peptides and free amino-acids produced by the autolysis of proteins not only lower the commercial acceptability of pelagics, but in bulk-stored capelin, autolysis has been shown to accelerate the growth of spoilage bacteria by providing a superior growth environment for such organisms (Aksnes and Brekken, 1988). The induction of bacterial spoilage in capelin by autolysis also resulted in the decarboxylation of amino-acids, producing biogenic amines and lowered the nutritive value of the fish significantly. This is particularly important since autolysis and bacterial growth greatly lower the commercial value of pelagics used for the production of fishmeal.

Similarly, bulk-stored herring used for fishmeal has been found to contain carboxy-peptidases A and B, chymotrypsin, and trypsin; and preliminary studies have shown that

proteolysis can be inhibited by the addition of potato extracts which not only slowed the proteolysis but resulted in lower microbial growth and preservation of the nutritional value of the meal (Aksnes, 1989).

More recently, Botta *et al.* (1992) found that autolysis of the visceral cavity (belly-bursting) of herring was related more to physical handling practices than to biological factors such as fish size, amount of red feed in the gut or roe content. In particular, it was shown that for herring, freezing/thawing, thawing time at 15°C and time of iced storage, had a far greater influence on belly- bursting than biological factors.

Cathepsins

Although several proteolytic enzymes have been discovered in the fish tissues, it has perhaps been the cathepsins which have been described most often. The cathepsins are "acid" proteases usually found packaged in tiny, submicroscopic organelles called lysozomes. In living tissue, lysozomal proteases are believed to be responsible for protein breakdown at sites of injury. Thus cathepsins are for the most part inactive in living tissue but become released into the cell juices upon physical abuse or upon freezing and thawing of *post mortem* muscle.

Cathepsins D and L are believed to play a major role in the autolytic degradation of fish tissue since most of the other cathepsins have a relatively narrow pH range of activity far too low to be of physiological significance. Reddi *et al.* (1972) demonstrated that an enzyme believed to be cathepsin D from winter flounder was active over a pH range of 3-8 with a maximum near pH 4.0, although no attempt was made to confirm the identity of the enzyme using synthetic substrates or specific inhibitors. Nevertheless, the enzyme was far less active in the presence of ATP, suggesting that such an enzyme would only be active in *post mortem* fish muscle. Also, the enzyme activity was inhibited strongly by the presence of salt (Figure 5.7) with virtually no activity remaining after a 25-hour incubation in the presence of 5% sodium chloride. It is therefore unlikely that Reddi's enzyme was active in salted fish products.

Cathepsin L has been implicated in the softening of salmon muscle during spawning migration. It is likely that this enzyme contributes more to autolysis of fish muscle than cathepsin D since it is far more active at neutral pH, and has been shown to digest both myofibrillar proteins (actomyosin) as well as connective tissue. Yamashita and Konogaya (1990) produced strong evidence implicating cathepsin L rather than other cathepsins in the softening of salmon during spawning. They demonstrated that electrophoresis of purified myofibrils treated with cathepsin L resulted in patterns which were almost identical to patterns of proteins recovered from muscle from spawning fish. Furthermore, the cathepsin L autolytic activity correlated well with the texture of the muscle as measured instrumentally. The linear correlation between cathepsin L activity and breaking strength of the muscle was excellent; r = 0.86 and -0.95 for fresh and frozen/thawed tissue, respectively. It is interesting that, in all cases, the autolytic ability as measured by cathepsin L activity was higher in frozen/thawed tissue than in fresh tissue. Freezing and thawing often break down cell membranes allowing autolytic membrane-bound enzymes to react with their natural substrates. The enzyme and its

naturally occurring inhibitor were further studied by the same authors (Yamashita and Konogaya, 1992). Cathepsin L has also been associated with the production of a jelly-like softening of flounder (Toyohara *et l.,* 1993 a) and the uncontrollable softening of Pacific hake muscle which has been parasitized by Myxosporidia (Toyohara *et al.,* 1993 b).

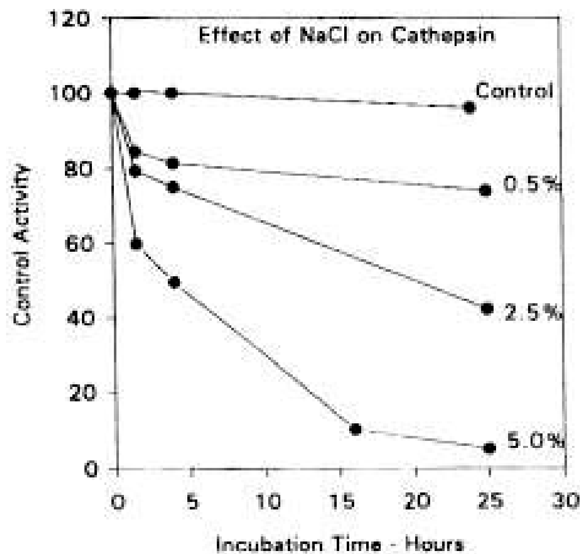


Figure 5.7 Effect of NaCl on the catheptic activity. Adapted from Reddi *et al.* (1972)

The tissues of such infected fish have little commercial value, but at present it is not known if it is the parasite or the host which secretes the proteolytic enzymes which autolyze the muscle.

In addition to their detrimental effect on texture, catheptic enzymes induce intentional autolytic changes in fermented fish products. For example, cathepsins are believed to be responsible for major textural changes during the fermentation of salted preserved Japanese squid and Crucian carp (Makinodan *et al.,* 1991, 1993).

Calpains

A second group of intracellular proteases called "calpains" or "calcium activated factor" (CAF) has recently been associated with fish muscle autolysis and is found in meats, finfish and crustaceans. Tenderness is probably the most important quality characteristic of red meat. It has been known for nearly a century that *post mortem* aging of red meat results in the tenderization process. Calpains have been found primarily responsible for the *post mortem* autolysis of meat through digestion of the z- line proteins of the myofibril. Although toughness is seldom a problem with unfrozen fish muscle, softening through autolysis is a serious problem limiting the commercial value. The calpains are intra-cellular endopeptidases requiring cysteine and calcium; μ-calpain requiring 5-50 μM Ca^{2+} , m-calpain requiring 150-1000 μM Ca^{2+} . Most calpains are active at physiological pH, making it reasonable to suspect their importance in fish-softening during chilled storage.

Studies have shown that in crustacean muscle, calpains are associated with moltinduced textural changes to the muscle and carry out non-specific generalized digestion of the myofibrillar proteins. However, vertebrate muscle calpains have been shown to be very specific, digesting primarily tropinin- T, desmin, titin and nebulin, attacking neither vertebrate actin or myosin (Koohmaraie, 1992). In contrast, fish calpains digest myosin (specifically the myosin heavy chain) to form an initial fragment with approximate molecular weight of 150 000 Da (Muramoto *et al.,* 1989). The same authors demonstrated that fish calpains were far more active at low temperatures than were mammalian calpains and that the rates of cleavage were species-specific, being most active against myosins with lowest heat stabilities. Thus, fish species adapted to colder environmental temperatures are more susceptible to calpain autolysis than those from tropical waters. Although calpain has been identified in several fish species including carp (Toyohara *et al.,* 1985), tilapia and shrimp (Wang *et al.,* 1993), as well as tuna, croaker, red seabream and trout (Muramoto *et al.,* 1989) to name a few, little work has to date demonstrated a "cause and effect" relationship between calpain activity and instrumental measurements of texture.

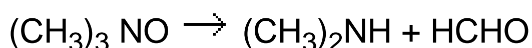
Collagenases

To this point, all of the *post mortem* autolytic changes described have involved changes within the muscle cell *per se.* However, the flesh of teleost fish is divided into blocks of muscle cells separated into "flakes" or myotomes by connective tissue called myocommata (Figure 3.3). Each muscle cell or fibre is surrounded with connective tissue which attaches to the myocommata at the ends of the cells by means of fine collagenous fibrils. During chilled storage, these fibrils deteriorate (Bremner and Hallett, 1985). More recently, it was shown that instrumental measurements of texture of chilled trout muscle decreased as the amount of type V collagen was solubilized, presumably due to the action of autolytic collagenase enzymes (Sato *et al.,* 1991). It is these enzymes which presumably cause "gaping" or breakdown of the myotome during long-term storage on ice or short term storage at high temperature. For Atlantic cod, it has been shown that upon reaching 17°C, gaping is inevitable presumably because of degradation of the connective tissue and rapid shortening of the muscle due to high temperature *rigor.*

The relatively short shelf life of chilled prawns due to softening of the tissue has also been shown to be due to the presence of collagenase enzymes (Nip *et al.,* 1985). The source of the collagenase enzymes in prawn is thought to be the hepatopancreas (digestive organ).

Autolytic changes during frozen storage

The reduction of trimethylamine oxide (TMAO), an osmoregulatory compound in many marine teleost fish, is usually due to bacterial action (section 5.3) but in some species an enzyme is present in the muscle tissue which is able to break down TMAO into dimethylamine (DMA) and formaldehyde (FA):



$$(CH_3)_3\,NO \rightarrow (CH_3)_2NH + HCHO$$

It is important to note that the amount of formaldehyde produced is equivalent to the dimethylamine formed but is of far greater commercial significance. Formaldehyde induces cross- linking of the muscle proteins making the muscle tough and readily lose its water holding capacity. The enzyme responsible for formal dehyde-induced toughening is called TMAO-ase or TMAO demethylase and is most commonly found in the gadoid fishes (cod family). Most of the TMAO demethylase enzymes reported to date weremembrane-bound and become most active when the tissue membranes are disrupted by freezing or artificially by detergent solubilization. Dark (red) muscle has a higher rate of activity than white muscle whereas other tissues such as kidney, spleen and gall bladder are extremely rich in the enzyme. Thus, it is important that minced fish is completely free of organ tissue such as kidney from gadoid species if toughening in frozen storage is to be avoided. It is often difficult to ensure that the kidney is removed prior to mechanical deboning since this particular organ runs the full length of the backbone and is adherent to it. The TMAO-ase enzyme has been isolated from the microsomal fraction in hake muscle (Parkin and Hultin, 1986) and the lysosomal membrane in kidney tissue (Gill *et al.,* 1992). It has been shown that the toughening of frozen hake muscle is correlated to the amount of formaldehyde produced, and that the rate of FA production is greatest at high frozen-storage temperatures (Gill *et al., 1979).* In addition, it has been shown that the amount of FA-induced toughening is enhanced by physical abuse to the catch prior to freezing and by temperature fluctuations during frozen storage. The most practical means of preventing the autolytic production of FA is to store fish at temperatures < -30°C to minimize temperature fluctuations in the cold store and to avoid rough handling or the application of physical pressure on the fish prior to freezing. The autolytic changes affecting the edibility of fresh and frozen fish are summarized in Table 5.3. Generally, the most important single factor affecting autolysis is physical disruption of the muscle cells. No attempt has been made here to deal with the alkaline proteases associated with the softening of cooked surimi products. An article by Kinoshita *et al.* (1990) deals with the heat-activated alkaline proteases associated with the softening in surimi-based products.

Table 5.3 Summary of Autolytic Changes in Chilled Fish

Enzyme(s)	Substrate	Changes Encountered	Prevention/Inhibition
glycolytic enzymes	glycogen	production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle high temperature rigor may result in gaping	fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible pre-rigor stress must be avoided

autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	loss of fresh fish flavour, gradual production of bitternes with Hx (later stages)	same as above rough handling or crushing accelerates breakdown
cathepsins	proteins, peptides	softening of tissue making processing difficult or impossible	rough handling during storage and discharge
chymotrypsin, trypsin, carboxy-peptidases	proteins, peptides	autolysis of visceral cavity in pelagics (belly-bursting)	problem increased with freezing/thawing or long- term chill storage
calpain	myofibrillar proteins	softening, molt-induced softening in crustaceans	removal of calcium thus preventing activation?
collagenases	connective tissue	gaping" of fillets softening	connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	formaldehyde-induced toughening of frozen gadoid fish	store fish at temperature <= -30°C physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

5.3 Bacteriological changes

The bacterial flora on live fish

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously and Liston (1980) states a normal range of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g (Shewan, 1962).

The bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1977). Fish caught in very cold, clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e., 10^7 cfu/cm^2 are found on fish from polluted warm waters. Many different bacterial species can be found on the fish surfaces. The bacteria on temperate water fish are all classified according to their growth temperature range as either psychrotrophs or psychrophiles. Psychrotrophs (cold-tolerant) are bacteria capable of growth at 0°C but with optimum around 25°C. Psychrophiles (cold-loving) are

bacteria with maximum growth temperature around 20°C and optimum temperature at 15°C (Morita, 1975). In warmer waters, higher numbers of mesophiles can be isolated. The microflora on temperate water fish is dominated by psychrotrophic Gram-negative rodshaped bacteria belonging to the genera *Pseudomonas, Moraxella, Acinetobacter, Shewanella* and *Flavobacterium.* Members of the *Vibrionaceae (Vibrio* and *Photobacterium)* and the *Aeromonadaceae (Aeromonas* spp.) are also common aquatic bacteria and typical of the fish flora (Table 5.4). Gram-positive organisms as *Bacillus, Micrococcus, Clostridium, Lactobacillus* and coryneforms can also be found in varying proportions, but in general, Gram-negative bacteria dominate the microflora. Shewan (1977) concluded that Gram-positive *Bacillus* and Micrococcus dominate on fish from tropical waters. However, this conclusion has later been challenged by several studies which have found that the microflora on tropical fish species is very similar to the flora on temperate species (Acuff *et al.,*1984; Gram *et al.,* 1990; Lima dos Santos 1978; Surendran *et al.,* 1989). A microflora consisting of *Pseudomonas, Acinetobacter, Moraxella* and *Vibrio* has been found on newly-caught fish in several Indian studies (Surendran *et al.,* 1989). Several authors conclude, as Liston (1980), that the microflora on tropical fish often carry a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish.

Aeromonas spp. are typical of freshwater fish, whereas a number of bacteria require sodium for growth and are thus typical of marine waters. These include *Vibrio*, *Photobacterium* and *Shewanella*. However, although *Shewanella putrefaciens* is characterized as sodium-requiring, strains of *S. putrefaciens* can also be isolated from freshwater environments (DiChristina and DeLong, 1993; Gram et al., 1990; Spanggaard et al., 1993). Although *S. putrefaciens* has been isolated from tropical freshwaters, it is not important in the spoilage of freshwater fish (Lima dos Santos, 1978; Gram, 1990).

Table 5.4 Bacterial flora on fish caught in clean, unpolluted waters

Gram-negative	**Gram-positive**	**Comments**
Pseudomonas	*Bacillus*	
Moraxella	*Clostridium*	
Acinetobacter	*Micrococcus*	
Shewanella putrefaciens	*Lactobacillus*	
Flavobacterium	Coryneforms	
Cytophaga		
Vibrio *Photobacterium* *Aeromonas*		*Vibrio* and *Photobacterium* are typical of marine waters; *Aeromonas* is typical of freshwater

In polluted waters, high numbers of *Enterobacteriaceae* may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Fujioka *et al.,* 1988).

The taxonomy of S. *putrefaciens* has been rather confused. The organism was originally associated with the *Achromobacter* group but was later placed in the Shewan *Pseudomonas* group IV. Based on percentage of guanine+ cytosine (GC%) it was transferred to the genus *Alteromonas,* but on the basis of 5SRNA homology it was reclassified to a new genus, *Shewanella* (MacDonnell and Colwell, 1985). It has recently been suggested that the genus *Aeromonas* spp. which was a member of the *Vibrionaceae* family be transferred to its own family, the *Aeromonadaceae* (Colwell *et al.,* 1986).

Japanese studies have shown very high numbers of microorganisms in the gastrointestinal tract of fish, and as such numbers are much higher than in the surrounding water, this indicates the presence of a favourable ecological niche for the microorganisms. Similarly, Larsen *et al.* (1978) reported up to 10^7 cfu/g of vibrio-like organisms in the intestinal tract of cod and Westerdahl *et al.* (1991) also isolated high numbers of vibrio-like organisms from the intestines of turbot. *Photobacterium phosphoreum* which can be isolated from the surface can also be isolated in high numbers from the intestinal tract of some fish species (Dalgaard, 1993). On the contrary, some authors believe that the microflora of the gastrointestinal tract is merely a reflection of the environment and the food intake.

Microbial invasion

The flesh of healthy live or newly-caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh (Figure 5.8 a). When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibres. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during iced storage. Ruskol and Bendsen (1992) showed that bacteria can be detected by microscope in the flesh when the number of organisms on the skin surface increases above 10^6 cfu/cm^2 (Figure 5.6 b). This was seen at both iced and ambient temperatures. No difference was found in the invasive patterns of specific spoilage bacteria (e.g., S. *putrefaciens)* and non-spoilage bacteria.

Since only a limited number of organisms actually invade the flesh and microbial growth mainly takes place at the surface, spoilage is probably to a large extent a consequence of bacterial enzymes diffusing into the flesh and nutrients diffusing to the outside.

Fish spoil at very different rates (see also section 6.5), and differences in surface properties of fish have been proposed to explain this. Skins of fish have very different textures. Thus whiting *(Merlangius merlangus)* and cod *(Gadus morhua)* which have a very fragile integument spoil rapidly compared to several flatfish such as plaice that has a very robust dermis and epidermis. Furthermore, the latter group has a very thick slime layer, which includes several antibacterial components, such as antibodies, complement and bacteriolytic enzymes (Murray and Fletcher, 1976; Hjelmland *et al.,* 1983).

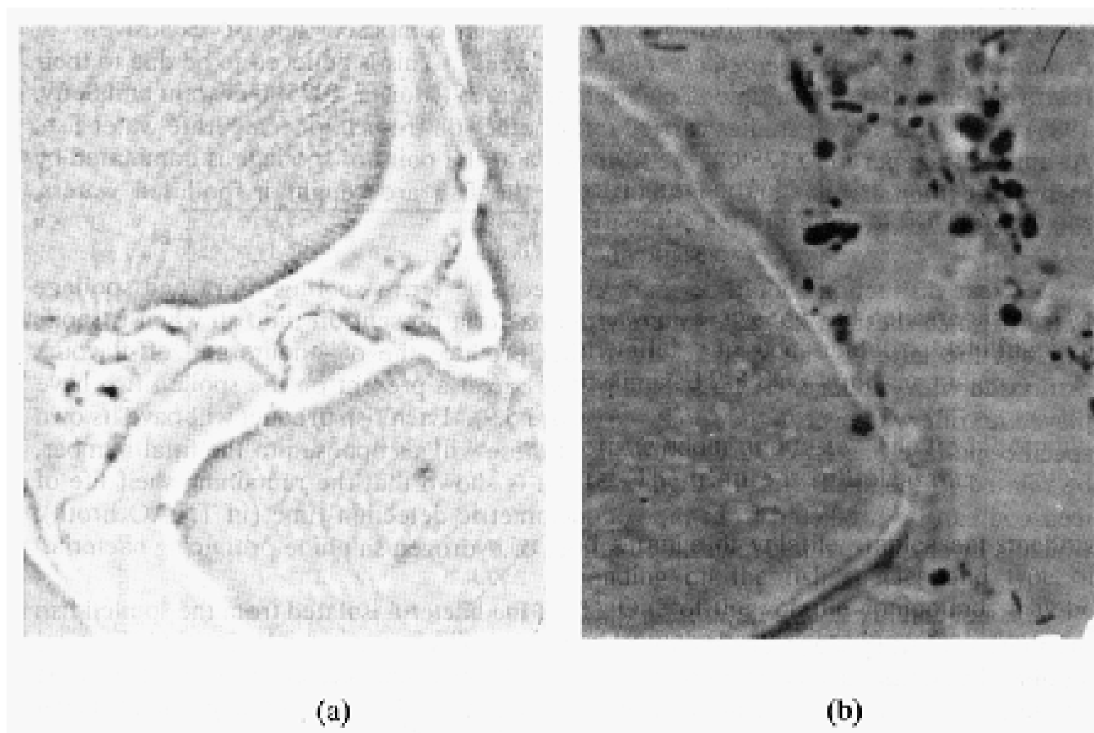


Figure 5.8 Histological section of (a) newly-caught cod and (b) cod fillets stored 12 days in ice. The section has been Giemsa-stained (Ruskol and Bendsen, 1992)

Changes in the microflora during storage and spoilage/Specific spoilage organisms

Bacteria on fish caught in temperate waters will enter the exponential growth phase almost immediately after the fish have died. This is also true when the fish are iced, probably because the microflora is already adapted to the chill temperatures. During ice storage, the bacteria will grow with a doubling time of approximately 1 day and will, after 2-3 weeks, reach numbers of 10^8-10^9 cfu/g flesh or cm^2 skin. During ambient storage, a slightly lower level of 10^7-10^8 cfu/g is reached in 24 hours. The bacteria on fish caught in tropical waters will often pass through a lag-phase of 1-2 weeks if the fish are stored in ice, whereafter exponential growth begins. At spoilage, the bacterial level on tropical fish is similar to the levels found on temperate fish species (Gram, 1990; Gram *et al.,* 1990).

If iced fish are stored under anaerobic conditions or if stored in CO_2 containing atmosphere, the number of the normal psychrotrophic bacteria such as S. *putrefaciens* and *Pseudomonas* is often much lower, i.e., 10^6-10^7 cfu/g than on the aerobically stored fish. However, the level of bacteria of psychrophilic character such as P. *phosphoreum* reaches a level of 10^7-10^8 cfu/g when the fish spoil (Dalgaard *et al.,* 1993).

The composition of the microflora also changes quite dramatically during storage. Thus, under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas* spp. and S. *putrefaciens* after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985) and is true for all studies carried out whether on tropical or temperate-water fish. At ambient temperature (25°C), the microflora at the point of spoilage is dominated by mesophilic

Vibrionaceae and, particularly if the fish are caught in polluted waters, *Enterobacteriaceae.*

A clear distinction should be made between the terms **spoilage flora** and **spoilage bacteria** since the first describes merely the bacteria present on the fish when it spoils whereas the latter is the specific group that produce the off-odours and off-flavours associated with spoilage. A large part of the bacteria present on the spoiled fish have played no role whatever in the spoilage (Figure 5.9). Each fish product will have its own specific spoilage bacteria and the number of these will, as opposed to the total number, be related to the shelf life. In Figure 5.10, it is shown that the remaining shelf life of iced cod can be predicted from the conductometric detection time (in TMAO broth), which is inversely correlated with the number of hydrogen sulphide-producing bacteria.

It is not an easy task to determine which of the bacteria isolated from the spoiled fish are those causing spoilage, and it requires extensive sensory, microbiological and chemical studies. First, the sensory, microbiological and chemical changes during storage must be studied and quantified, including a determination of the level of a given chemical compound that correlates with spoilage (the chemical spoilage indicator). Second, bacteria are isolated at the point of sensory rejection. Pure and mixed cultures of bacteria are screened in sterile fish substrates for their **spoilage potential**, i.e., their ability to produce sensory (off-odours) and chemical changes typical of the spoiling product. Finally, the selected strains are tested to evaluate their **spoilage activity**, i.e., if their growth rate and their qualitative and quantitative production of off-odours are similar to the measurements in the spoiled product (Dalgaard, 1993).

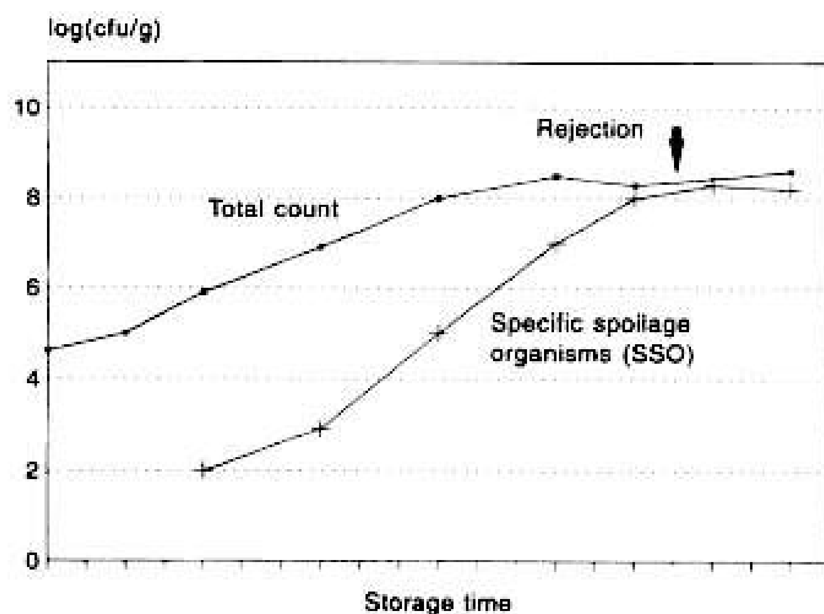


Figure 5.9 Changes in total counts and specific spoilage bacteria during storage (modified after Dalgaard (1993)

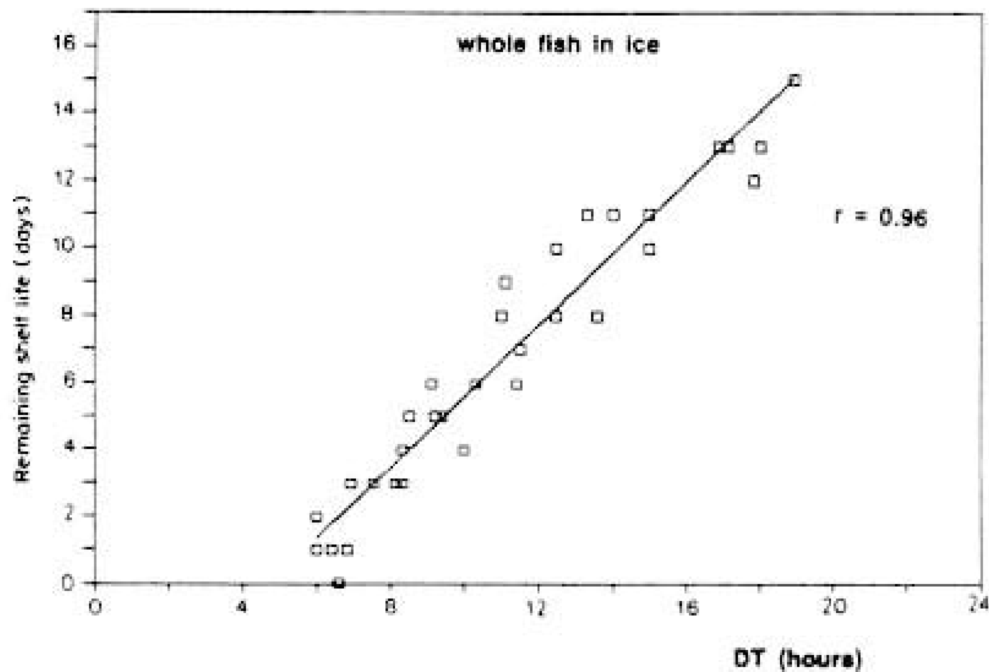


Figure 5.10 Comparison of remaining shelf life of iced cod and detection time in a TMAO broth (Jorgensen et al., 1988)

The latter step is particularly important, as some bacteria may produce the chemical compounds associated with spoilage but are unable to do so in significant amounts, and they are thus not the specific spoilage bacteria. When stored aerobically, levels of 10^8-10^9 cfu/g of specific spoilage bacteria are required to cause spoilage. The spoilage of packed fish is seen at a much lower level of 10^7 cfu P. *phosphoreum* per gramme. This relatively low level is probably due to the very large size (5 µm) of the bacterium resulting in a much higher yield of for example, TMA per cell (Dalgaard, 1993).

Spoilage potential and activity can be assessed in several fish substrates as sterile, raw fish juice (Lerke *et al.,* 1963), heat-sterilized fish juice (Castell and Greenough, 1957; Gram *et al. , 1987*; Dalgaard, 1993) or on sterile muscle blocks (Herbert *et al.,* 1971). The latter is the most complicated but is also that yielding results comparable to the product. If any of the fish juices are chosen, it is important that the growth rate of the spoilage bacteria in the model system is equal to the growth rate in the product.

A qualitative test for the ability of the bacteria to produce H_2S and/or reduce TMAO may also be used when the spoilage flora is screened for potential spoilage bacteria. A medium where the reduction of TMAO to TMA is seen as a redox indicator changes colour, and the formation of H_2S is evident from a black precipitation of FeS which has been developed for this purpose (Gram *et al.,*1987).

Shewanella putrefaciens has been identified as the specific spoilage bacteria of marine temperate- water fish stored aerobically in ice. If the product is vacuum-packed, P. phosphoreum participates in the spoilage and it becomes the specific spoilage bacteria of CO_2 packed fish (see section 6.3). The spoilage flora on iced tropical fish from marine

waters is composed almost exclusively of *Pseudomonas* spp. and *S. putrefaciens*. Some *Pseudomonas* spp. are the specific spoilers of iced stored tropical freshwater fish (Lima dos Santos, 1978; Gram et al., 1990) and are also, together with *S. putrefaciens*, spoilers of marine tropical fish stored in ice (Gillespie and MacRae, 1975; Gram, 1990).

At ambient temperature, motile aeromonads are the specific spoilers of aerobically stored freshwater fish (Gorzyka and Pek Poh Len, 1985; Gram *et al.,* 1990). Barile *et al.* (1985) showed that a large proportion of the flora on ambient-stored mackerel consisted of *S. putrefaciens,* indicating that this bacterium may also take part in the spoilage.

Table 5.5 gives an overview of the specific spoilage bacteria of fresh fish products stored in ice and at ambient temperature.

Table 5.5 Dominating microflora and specific spoilage bacteria at spoilage of fresh, white fish (cod) (from Huss, 1994)

Storage temperature	Packaging atmosphere	Dominating microflora	Specific spoilage organisms (SSO)	References
0°C	Aerobic	Gram-negative psychrotrophic, non-fermentative rods *(Pseudomonas spp., S. putrefaciens, Moraxella, Acinetobacter)*	S. *putrefaciens* *Pseudomonas* [3]	2,3,4,9
0°C	Vacuum	Gram-negative rods; psychrotrophic or with psychrophilic character (S. *putrefaciens, Photobacterium)*	S. *putrefaciens P. phosphoreum*	1,9
0°C	MAP[1]	Gram-negative fermentative rods with psychrophilic character *(Photobacterium)* Gram-negative non-fermentative psychrotrophic rods (1-10% of flora; *Pseudomonas, S. putrefaciens)* Gram-positive rods (LAB 2)	*P. phosphoreum*	1,7
5°C	Aerobic	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10

5°C	Vacuum	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10
5°C	MAP	Gram-negative psychrotrophic rods *(Vibrionaceae)*	*Aeromonas spp.*	6
20-30°C	Aerobic	Gram-negative mesophilic fermentative rods *(Vibrionaceae, Enterobacteriaceae)*	Motile *Aeromonas* spp. *(A. hydrophila)*	2,4,5,8

1) Modified Atmosphere Packaging (CO_2 containing)
2) LAB: Lactic Acid Bacteria
3) Fish caught in tropical waters or freshwaters tend to have a spoilage dominated by *Pseudomonas spp.*

References: 1) Battle *et al.* (1985); 2) Dalgaard *et al.* (1993); 3) Donald and Gibson (1992); 4) Gorczyca and Pek Poh Len (1985); 5) Gram *et al.* (1987); 6) Gram *et al.* (1990); 7) Gram and Dalgaard (pers. comm.); 8) Jorgensen and Huss (1989); 9) Lima dos Santos (1978); 10) van Spreekens (1977)

Biochemical changes induced by bacterial growth during storage and spoilage

Comparison of the chemical compounds developing in naturally spoiling fish and sterile fish has shown that most of the volatile compounds are produced by bacteria (Shewan, 1962) as shown in Figure 5.11. These include trimethylamine, volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecularweight compounds.

The substrates for the production of volatiles are the carbohydrates (e.g., lactate and ribose), nucleotides (e.g., inosine mono-phosphate and inosine) and other NPN molecules. The amino-acids are particularly important substrates for formation of sulphides and ammonia.

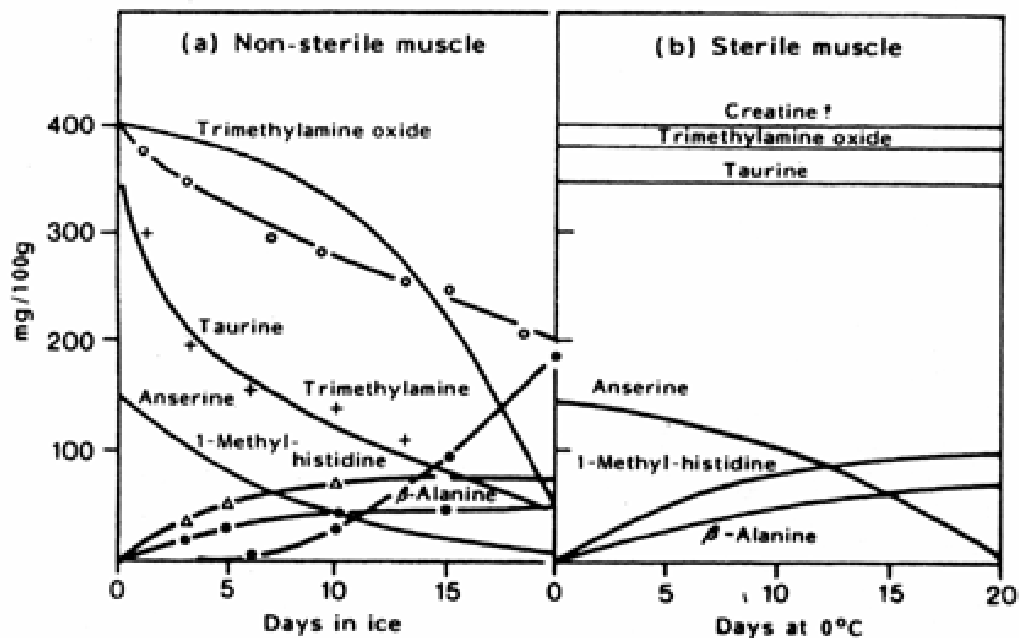


Figure 5.11 Changes in the nitrogenous extractives in a) spoiling and b) autolysing cod muscle (Shewan, 1962)

Microorganisms obtain far more energy from aerobic oxidation than from an anaerobic fermentation; thus the complete oxidation of 1 mole glucose (or other hexose) via Kreb's cycles yields 6 moles of CO_2 and 36 moles of ATP. On the contrary, the fermentation of 1 mole glucose gives only 2 moles of ATP and two moles of lactic acid. The initial aerobic growth on fish is dominated by bacteria using carbohydrates as substrate and oxygen as terminal electron-acceptor with the concurrent production of CO_2 and H_2O.

Reduction of Trimethylarnine Oxide (TMAO)

The growth of oxygen-consuming bacteria results in the formation of anaerobic or microaerophilic niches on the fish. This does, however, not necessarily favour the growth of anaerobic bacteria. Some of the bacteria present on fish are able to carry out a respiration (with the ATP advantage) by using other molecules as electron acceptor. It is typical of many of the specific spoilage bacteria on fish that they can use TMAO as electron acceptorin an anaerobic respiration. The reduced component, TMA, which is one of the dominant components of spoiling fish, has a typical fishy odour. The level of TMA found in fresh fish rejected by sensory panels varies between fish species, but is typically around 10-15 mg TMA-N/100 g in aerobically stored fish and at a level of 30 mg TMA-N/100 g in packed cod (Dalgaard *et al.,* 1993).

The TMAO reduction is mainly associated with the genera of bacteria typical of the marine environment *(Alteromonas, Photobactetium, Vibrio* and S. *putrefaciens),* but is also carried out by *Aeromonas* and intestinal bacteria of the *Enterobacteriaceae.* TMAO reduction has been studied in fermentative, facultative anaerobic bacteria like *E. coli* (Sakaguchi *et al.,* 1980) and *Proteus* spp. (Stenberg *et al.,* 1982) as well as in the non-

fermentative S. *putrefaciens* (Easter *et al,* 1983; Ringo *et al,* 1984). During aerobic growth, S. *putrefaciens* uses the Kreb's cycle to produce the electrons that are later channelled through the respiratory chain. Ringo *et al.* (1984) suggested that during anaerobic respiration S. *putrefaciens* also uses the complete Kreb's cycle (Figure 5.12), whereas it has recently been shown that in the anaerobic respiration in S. *putrefaciens,* only part of the Kreb's cycle is used (Figure 5.13) and electrons are also generated by another metabolic pathway, namely the serine pathway (Scott and Nealson, 1994). S. *putrefaciens* can use a variety of carbon sources as substrate in its TMAO-dependent anaerobic respiration, including formate and lactate. Compounds like acetate and succinate that are used in the oxygen respiration cannot be used when TMAO is terminal electron acceptor (DiChristina and DeLong, 1994) and on the contrary, acetate is a product of the anaerobic TMAO reduction (Ringo *et al.,* 1984; Scott and Nealson, 1994).

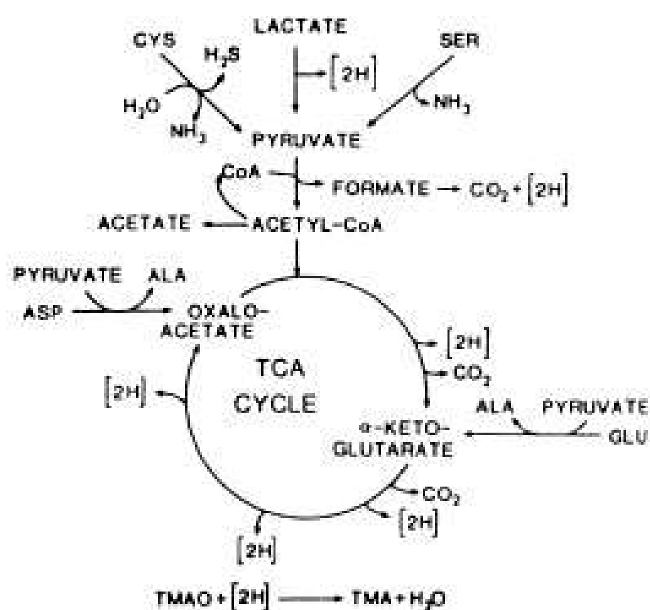


Figure 5.12 Anaerobic reduction of TMAO by *Shewanella putrefaciens* (formerly *Alteromonas)* as suggested by Ringo *et al.* (1984)

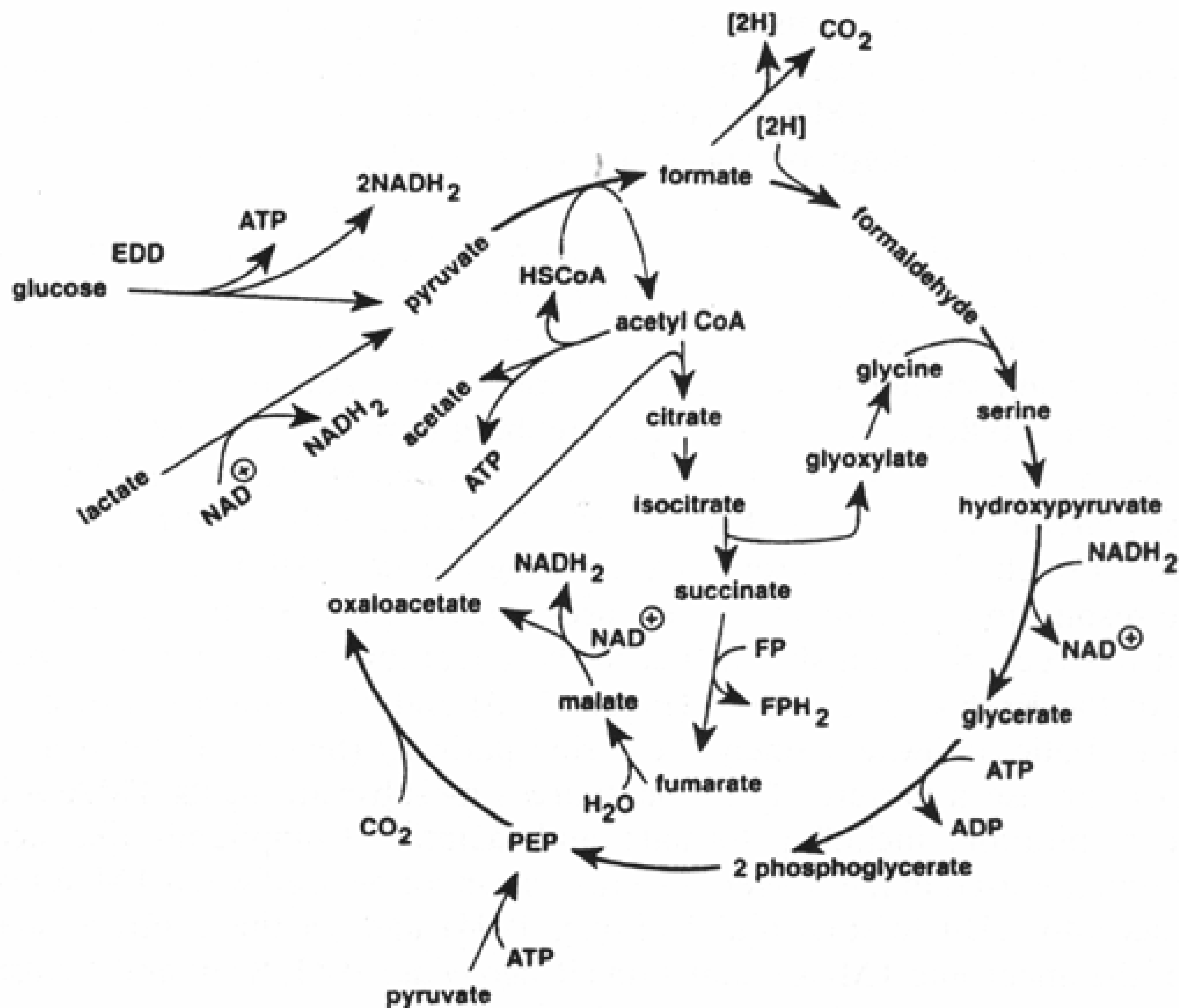


Figure 5.13 Proposed route of carbon during anaerobiosis for S. *putrefaciens* (Scott and Nealson, 1994)

Contrary to this, sugars and lactate are the main substrates generating electrons when *Proteus* spp. reduces TMAO. The reduction is accompanied by a production of acetate as the main product (Kjosbakken and Larsen, 1974).

TMAO is, as mentioned in section 4.4, a typical component of marine fish, and it has recently been reported that also some tropical freshwater fish contain high amounts of TMAO (Anthoni *et al.,* 1990). However, TMA is not necessarily a characteristic component during spoilage of such fish because spoilage is due to *Pseudomonas spp.* (Gram *et al.,* 1990).

The development of TMA is in many fish species paralleled by a production of hypoxanthine. Hypoxanthine can, as described in section 5.2. be formed by the autolytic decomposition of nucleotides, but it can also be formed by bacteria; and the rate of bacterial formation is higher than the autolytic. Both Jorgensen *et al.* (1988) and

Dalgaard (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod (Figure 5.14). Several of the spoilage bacteria produce hypoxanthine from inosine or inosine mono-phosphate, including *Pseudomonas* spp. (Surette *et al.,* 1988) S. *putrefaciens* (van Spreekens, 1977; Jorgensen and Huss, 1989; Gram, 1989) and P. *phosphoreum* (van Spreekens, 1977).

In cod and other gadoid fishes, TMA constitutes most of the so-called total volatile bases, TVB (also called total volatile nitrogen, TVN) until spoilage. However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB levels still rise due to formation of NH3 and other volatile amines. A little ammonia is also formed in the first weeks of iced storage due to autolysis. In some fish that do not contain TMAO or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVB is seen during storage, probably resulting from the deamination of amino-acids.

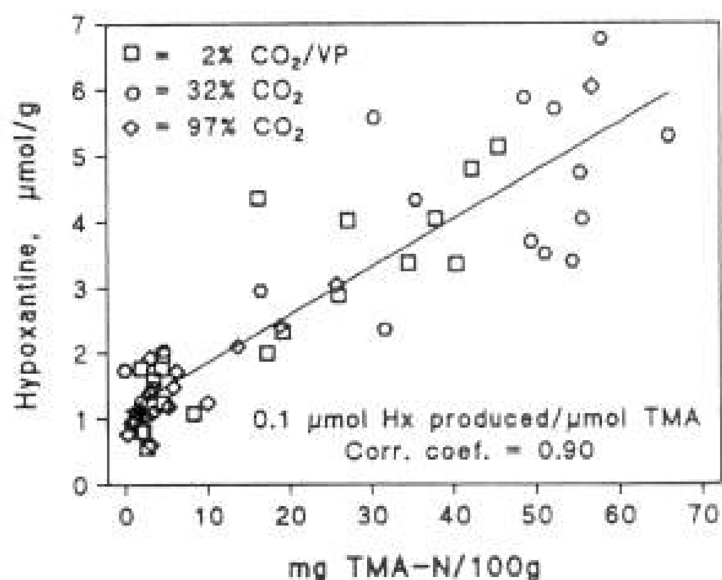


Figure 5.14 Relationship between contents of TMA and Hx during storage of packed cod in ice (Dalgaard *et al.,* 1993)

Volatile sulphur-compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. S. *putrefaciens* and some *Vibrionaceae* produce H_2S from the sulphur containing amino-acid 1-cysteine (Stenstroem and Molin, 1990; Gram *et al.,* 1987). On the contrary, neither *Pseudomonas* nor P. *phosphoreum* produce significant amounts of H_2S. Thus, hydrogen sulphide, which is typical of spoiling iced cod stored aerobically, is not produced in spoiling CO_2 packed fish (Dalgaard *et al.,* 1993). Methylmercaptan (CH_3SH) and dimethylsulphide ($(CH_3)_2S$) are both formed from the other sulphur-containing amino-acid, methionine. Taurine, which is also sulphur-containing, occurs as free amino-acid in very high concentrations in fish muscle. It disappears from the fish flesh during storage (Figure 5.11) but this is because of leakage rather than because of bacterial attack (Herbert and Shewan, 1975). The formation of compounds in naturally-spoiling cod as compared to sterile muscle is shown in Figure 5.15.

The volatile sulphur-compounds are very foul-smelling and can be detected even at ppb levels, so even minimal quantities have a considerable effect on quality. Ringo *et al. (1984)* have shown that cysteine is used as substrate in the Kreb's cycle when electrons are transferred to TMAO, and the formation of H_2S and TMA is thus to some extent a linked reaction (Figure 5.12).

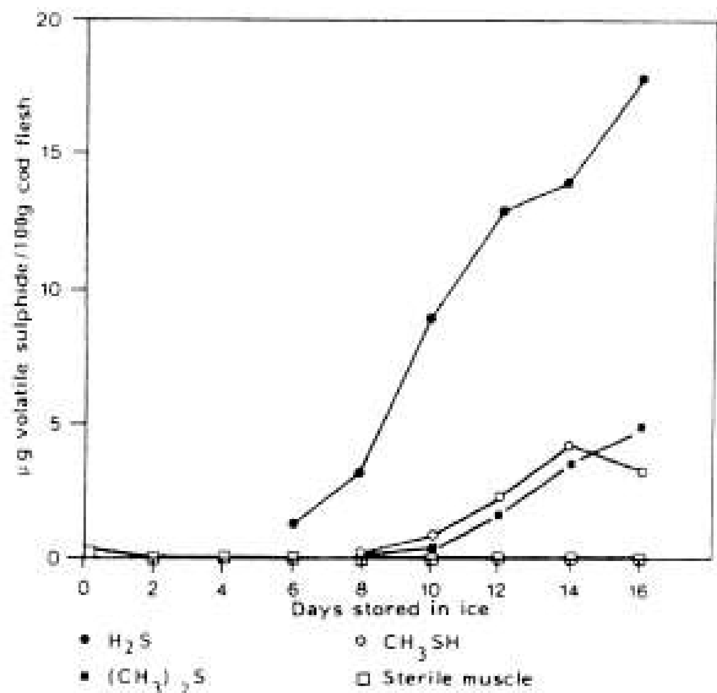


Figure 5.15 Production of HA CH_3SH and $(CH_3)_2S$ in naturally spoiling cod fillets and sterile muscle blocks (Herbert and Shewan, 1976)

Contrary to the iced spoilage by S. *putrefaciens* and the ambient spoilage by *Vibrionaceae* which is dominated by H_2S and TMA, the spoilage caused by *Pseudomonas* spp. is characterized by absence of these compounds (Gram *et al.,* 1989, Gram *et al.,* 1990). Fruity, rotten, sulphydryl odours and flavours are typical of the Pseudomonas spoilage of iced fish. *Pseudomonas* spp. produce a number of volatile aldehydes, ketones, esters and sulphides (Edwards *et al.,* 1987; Miller *et al.,* 1973 a, 1973 b). However, it is not known which specific compounds are responsible for the typical off-odours (Table 5.6). The fruity off-odours produced by *Pseudomonas fragi* originate from monoaminomonocarboxylic amino-acids.

Table 5.6 Typical spoilage compounds during spoilage of fresh fish stored aerobically or packed in ice or at ambient temperature

Specific spoilage organism	Typical spoilage compounds
Shewanella putrefaciens	*TMA, H_2S, CH_3SH, $(CH_3)_2S$, Hx*
Photobacterium phosphoreum	TMA, Hx
Pseudomonas spp.	ketones, aldehydes, esters, non-H_2S sulphides

Vibrionaceae	TMA, H_2S
anaerobic spoilers	NH_3, acetic, butyric and propionic acid

As mentioned above, TVB will continue to rise even after TMA has reached its maximum. This latter rise is due to proteolysis commencing when several of the free amino-acids have been used. Lerke *et al.* (1967) separated fish juice into a protein and a non-protein fraction and inoculated spoilage bacteria in each fraction and in the whole juice. The non-protein fraction of a fish juice spoiled as the whole juice whereas only faint off-odours were detected in the protein fraction of the juice. Although some authors have used the number of proteolytic bacteria as indicators of spoilage, it must be concluded that the turnover of the protein fraction is not of major importance in spoilage of fresh fish.

Some of the compounds typically formed by bacteria during spoilage of fish are shown in Table 5.7 together with the substrate used for the formation.

Table 5.7 Substrate and off-odour/off-flavour compounds produced by bacteria during spoilage of fish

Substrate	Compounds produced by bacterial action
TMAO	TMA
cysteine	H_2S
methionine	CH_3SH, $(CH_3)_2S$
carbohydrates and lactate	acetate, CO_2, H_2O
inosine, IMP	hypoxanthine
amino-acid s (glycine, serine, leucine)	esters, ketones, aldehydes
amino-acids, urea	NH_3

The formation of TMA is accompanied by a formation of ammonia during anaerobic storage of herring and mackerel (Haaland and Njaa, 1988). Prolonged anaerobic storage of fish results in vigorous production of NH_3 owing to further degradation of the amino-acids, and in the accumulation of lower fatty acids as acetic, butyric and propionic acid. The very strong NH_3-producers were found to be obligate anaerobes belonging to the family Bacteroidaceae genus *Fusobacterium* (Kjosbakken and Larsen, 1974; Storroe *et al.,* 1975, 1977). These organisms grow only in the spoiled fish extract and have little or no proteolytic activity relying on already hydrolysed proteins.

During iced storage of fresh fatty fish, changes in the lipid fraction is caused almost exclusively by chemical action, e.g., oxidation, whereas bacterial attack on the lipid fraction contributes little to the spoilage profile. During storage of lightly preserved fish, lipid hydrolysis caused by bacteria may be part of the spoilage profile.

5.4 Lipid oxidation and hydrolysis

The two distinct reactions in fish lipids of importance for quality deterioration are:

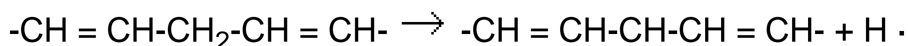
- oxidation
- hydrolysis

They result in production of a range of substances among which some have unpleasant (rancid) taste and smell. Some may also contribute to texture changes by binding covalently to fish muscle proteins. The various reactions are either *nonenzymatic* or catalyzed by *microbial* enzymes or by *intracellular* or *digestive* enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature.

Fatty fish are, of course, particularly susceptible to lipid degradation which can create severe quality problems even on storage at subzero temperatures.

Oxidation

The large amount of polyunsaturated fatty acid moieties found in fish lipids (see section 4.2) makes them highly susceptible to oxidation by an autocatalytic mechanism (Figure 5.16). The process is initiated as described below by abstraction of a hydrogen atom from the central carbon of the *pentadiene structure* found in most fatty acid acyl chains containing more than one double bond:



$$-CH = CH-CH_2-CH = CH- \rightarrow -CH = CH-CH-CH = CH- + H \cdot$$

Contrary to the native molecule, the lipid radical (L) reacts very quickly with atmospheric oxygen making a peroxy-radical (LOO) which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical L. This propagation continues until one of the radicals is removed by reaction with another radical or with an *antioxidant* (AH) whose resulting radical (A) is much less reactive. The hydroperoxides produced in relatively large amounts during propagation are tasteless, and it is therefore perhaps not surprising that the widely used "peroxide value" (section 8.2) usually correlates rather poorly to sensorial properties.

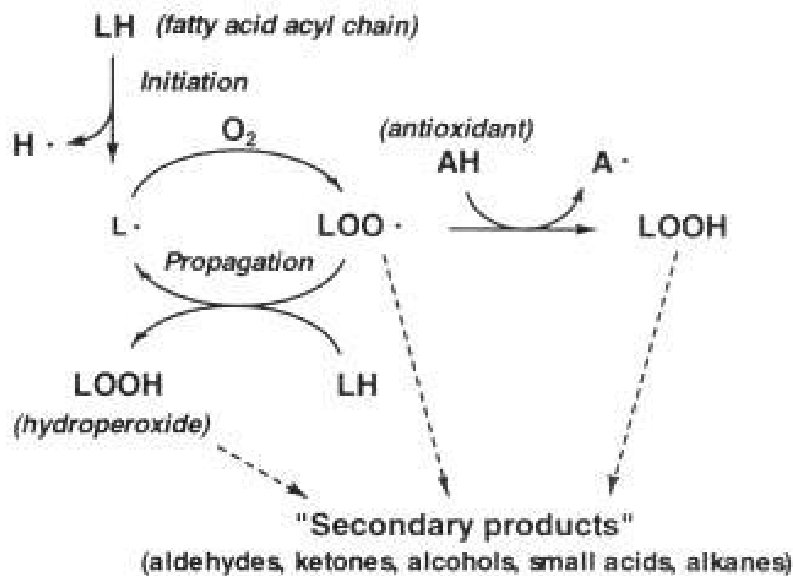


Figure 5.16 Autoxidation of polyunsaturated lipid

The hydroperoxides are readily broken down, catalyzed by heavy metal ions, to secondary autoxidation products of shorter carbon chain-length. These secondary products - mostly aldehydes, ketones, alcohols, small carboxylic acids and alkanes - give rise to a very broad odour spectrum and in some cases to a yellowish discoloration. Several of the aldehydes can be determined as "thiobarbituric acid-reactive substances" (section 8.2).

Metal ions are very important in the first step of lipid autoxidation - the initiation process - in catalyzing the formation of reactive oxygen species as for example the hydroxyl radical (OH). This radical immediately reacts with lipids or other molecules at the site where it is generated. The high reactivity may explain that free fatty acids have been found to be more susceptible to oxidation than the corresponding bound ones, because the amount of iron in the aqueous phase is probably greater than the amount bound to the surface of cellular membranes and lipid droplets.

Fatty acid hydroperoxides may also be formed enzymatically, catalyzed by *lipoxygenase* which is present in variable amounts in different fish tissues. A relatively high activity has been found in the gills and under the skin of many species. The enzyme is unstable and is probably important for lipid oxidation only in fresh fish. Cooking or freezing/thawing rather effectively destroys the enzyme activity.

The living cells possess several protection mechanisms directed against lipid oxidation products. An enzyme, glutathione peroxidase, exists which reduces hydroperoxides in the cellular membranes to the corresponding hydroxy-compounds. This reaction demands supply of reduced glutathione and will therefore cease post mortem when the cell is depleted of that substance. The membranes also contain the phenolic compound a-tocopherol (Vitamin E) which is considered the most important natural antioxidant. Tocopherol can donate a hydrogen atom to the radicals L- or LOO- functioning as the molecule AH in Figure 5.16. It is generally assumed, that the resulting tocopheryl radical

reacts with ascorbic acid (Vitamin C) at the lipid/water interface regenerating the tocopherol molecule. Other compounds, for example the carotenoids, may also function as antioxidants. Wood smoke contains phenols which may penetrate the fish surface during smoking and thereby provide some protection against lipid oxidation.

Hydrolysis

During storage, a considerable amount of free fatty acids (FFA) appears (Figure 5.17). The phenomenon is more profound in ungutted than in gutted fish probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase (TL in Figure 5.18) originating from the digestive tract or excreted by certain microorganisms. Cellular lipases may also play a minor role.

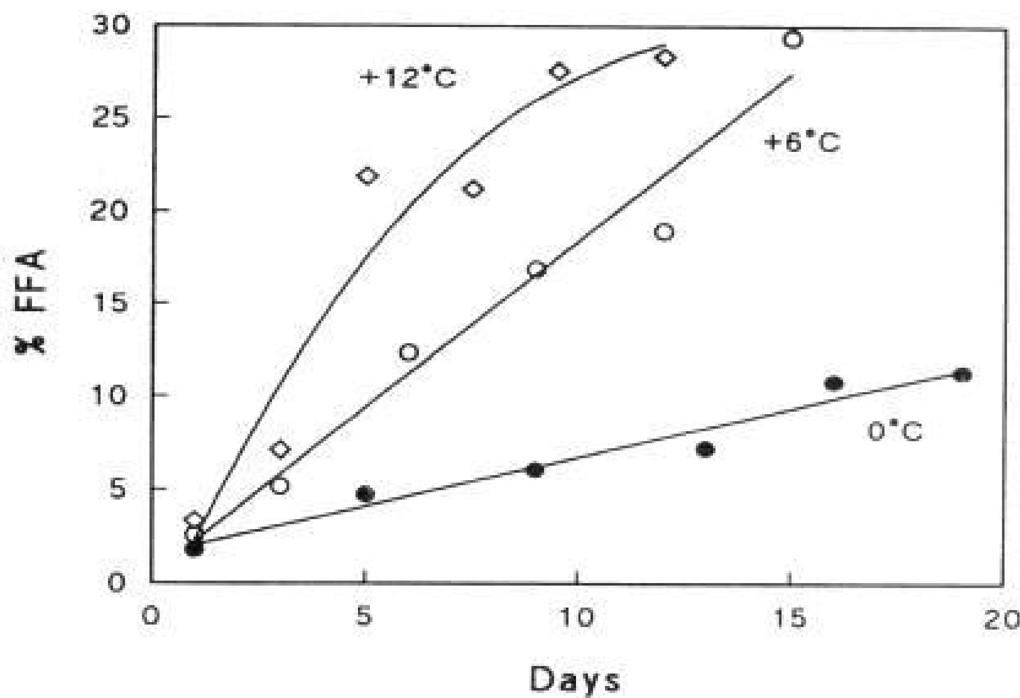


Figure 5.17 The development of free fatty acids in herring stored at different temperatures (Technological Laboratory, Danish Ministry of Fisheries, Annual Report, 1971)

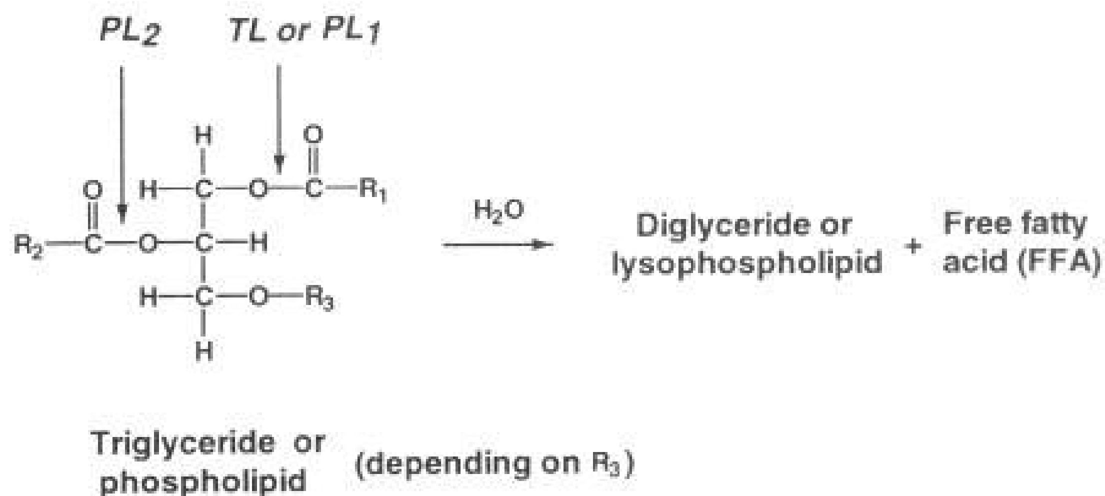
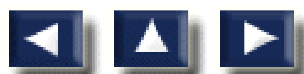


Figure 5.18 Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: PL_1 & PL_2 phospholipases;TL, triglyceride lipase

In lean fish, for example Atlantic cod, production of free fatty acids also occurs, even at low temperatures. The enzymes responsible are believed to be cellular phospholipases - in particular phospholipase A_2 (PL2 in Figure 5.18) - although a correlation between activity of these enzymes and the rate of appearance of FFA has as yet not been firmly established. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely of the polyunsaturated type, and hydrolysis therefore often leads to increased oxidation as well. Furthermore, the fatty acids themselves may cause a "soapy" off-flavour.



6. QUALITY CHANGES AND SHELF LIFE OF CHILLED FISH

6.1. The effect of storage temperature
6.2. The effect of hygiene during handling
6.3. The effect of anaerobic conditions and carbon dioxide
6.4. The effect of gutting
6.5. The effect of fish species, fishing ground and season

6.1 The effect of storage temperature

Chill storage (0-25°C)

It is well known that both enzymatic and microbiological activity are greatly influenced by temperature. However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity (Figure 6.1).

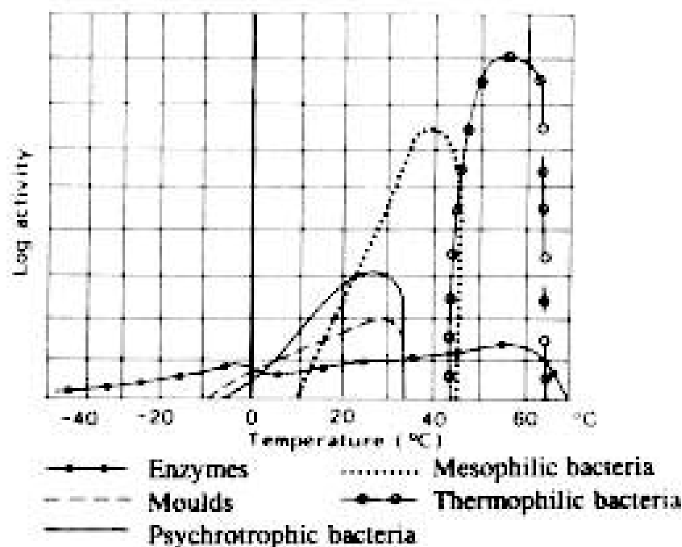


Figure 6.1 Relative enzyme activity and growth rate of bacteria in relation to temperature (Andersen et *al.,* 1965)

Many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0°C Figure 6.2 shows the effect of temperature on the growth rate of the fish spoilage bacterium *Shewanella Putrefaciens.* At 0°C the growth rate is less than one-tenth of the rate at the optimum growth temperature.

Microbial activity is responsible for spoilage of most fresh fish products. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures. In industrialized countries it is common practice to store fresh fish in ice (at 0°C) and the shelf life at different storage temperatures (at t°C) has been expressed by the relative rate of spoilage (RRS), defined as shown in Equation 6.a (Nixon, 1971).

$$\text{Relative rate of spoilage at } t\,^{\circ}C = \frac{\text{keeping time at } 0^{\circ}C}{\text{keeping time at } t\,^{\circ}C} \qquad 6.a$$

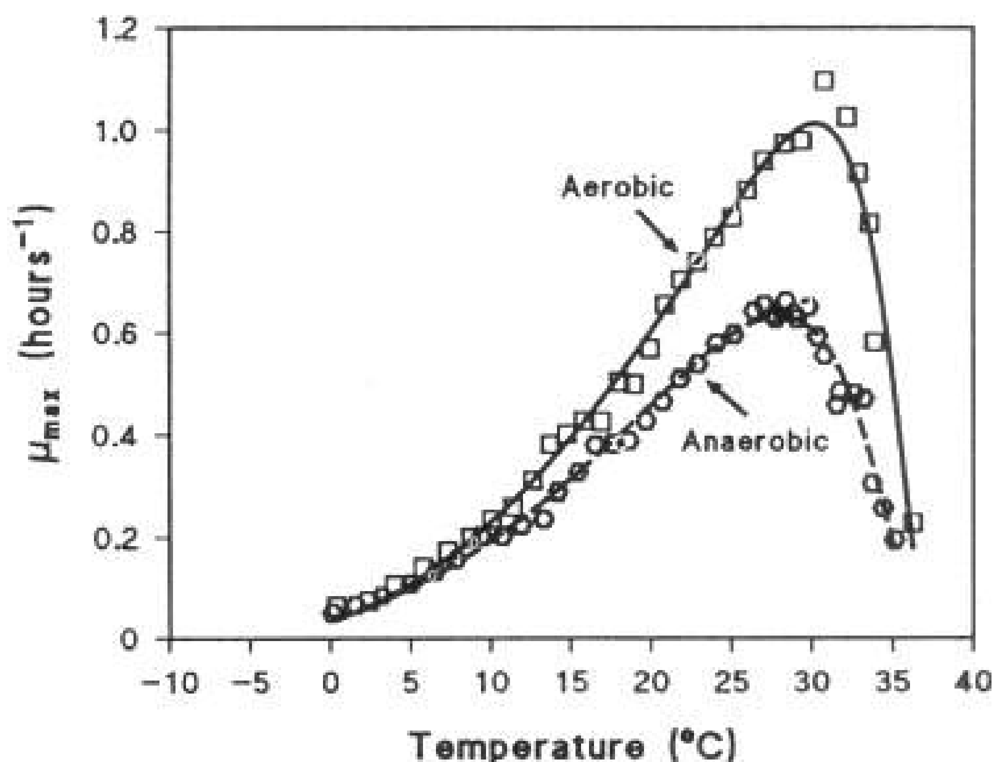


Figure 6.2 Effect of temperature on the maximum specific growth rate (μmax) of *Shewanella putrefaciens* grown in a complex medium containing TMAO (Dalgaard, 1993)

While broad differences are observed in shelf lives of the various seafood products, the effect of temperature on RRS is similar for fresh fish in general. Table 6.1 shows an example with different seafood products.

Table 6.1 Shelf lives in days and relative rates of spoilage (RRS) of seafood products stored at different temperatures

	0°C		**5°C**		**10°C**	
	shelf life	**RRS**	**shelf life**	**RRS**	**shelf life**	**RRS**
Crab claw[a]	10.1	1	5.5	1.8	2.6	3.9
Salmon[b]	11.8	1	8.0	1.5	3.0	3.9
Sea bream[c]	32.0	1	-	-	8.0	4.0

Packed cod[d)]	14	1	6.0	2.3	3.0	4.7

a) Cann et al. (1985); b) Cann et al. (1984); c) Olley and Quarmby (1981); d) Cann et al. (1983)

The relationship between shelf life and temperature has been thoroughly studied by Australian researchers (Olley and Ratkowsky, 1973 a, 1973 b). Based on data from the literature they found that the relationship between temperature and RRS could be expressed as an S-shaped general spoilage curve (Figure 6.3). Particularly at low temperatures (e.g., < 10°C this curve is similar to, and confirms the results of Spencer and Baines (1964). These authors, 10 years earlier, found a straight line relationship between RRS and the storage temperatures of cod from the North Sea (Figure 6.3).

The effect of temperature on the rate of chemical reactions is often described by the Arrhenius Equation. This Equation, however, has been shown not to be accurate when used for the effect of a wide range of temperatures, on growth of microorganisms and on spoilage of foods (Olley and Ratkowsky, 1973 b; Ratkowsky *et* al., 1982). Ratkowsky *et al.* (1982) suggested the 2-parameter square root model (Equation 6.b) for the effect of sub-optimal temperature on growth of microorganisms

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad \text{6b}$$

T is the absolute temperature (Kelvin) and T_{min} in a parameter expressing the theoretical minimum temperature of growth. The square root of the microbial growth rates plotted against the temperature form a straight line from which T_{min} is determined. Several psychrotrophic bacteria isolated from fish products have T_{min} values of about 263 Kelvin (-10°C) (Ratkowsky *et al.,* 1982; Ratkowsky *et al.,* 1983). Based on this T_{min} value, a spoilage model has been developed. It has been assumed that the relative microbial growth rate would be similar to the relative rate of spoilage. The relative rate concept (Equation 6.a) was then combined with the simple square root model (Equation 6.b) to give a temperature spoilage model (Equation 6.c). As just described, this model was derived from growth of psychrotrophic model has been shown to give good estimates of the effect of temperature on bacteria (T_{min} = -10°C) but the RRS of chilled fresh fish as shown in Figure studies (Storey, 1985; Gibson, 1985). 6.1 and also confirmed in other

$$\sqrt{Relative\ rate\ of\ spoilage} = \frac{b(t°C - (-10°C)}{b(0°C - (-10°C))} = 0.1 * t°C + 1 \qquad 6.c$$

If the shelf life of a fish product is known at a given temperature, the shelf life at other storage temperatures can be calculated from the spoilage models. The effect of temperature, shown in Table 6.2, as calculated from Equation 6.c for products with different shelf lives when stored at 0°C.

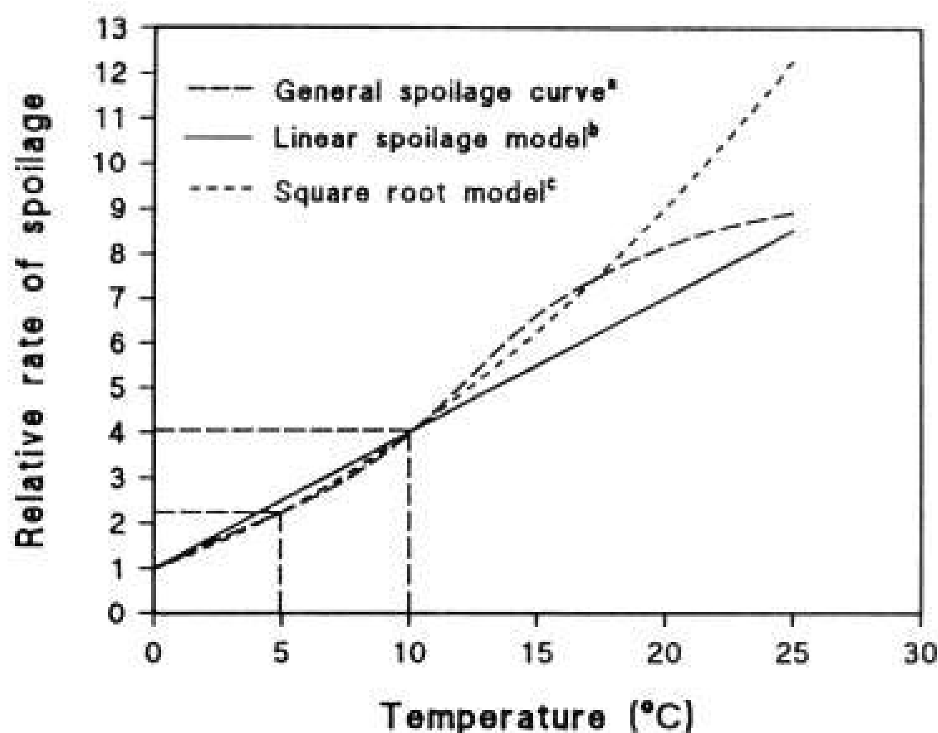


Figure 6.3 Effect of temperature on the relative rate of spoilage of fresh fish products. a) the general spoilage curve (Olley and Ratkowsky, 1973 a); b) the linear spoilage model suggested by Spencer and Baines (1964); c) the square root spoilage model derived from growth for psychrotrophic bacteria (Equation 6. c)

The effect of time/temperature storage conditions on product shelf life has been shown to be cumulative (Charm et al, 1972). This allows spoilage models to be used for prediction of the effect of variable temperatures on product keepability. An electronic time/temperature function integrator for shelf life prediction was developed, based on Equation 6.c. The instrument predicts RRS accurately, but a high price has limited its practical application (Owen and Nesbitt, 1984; Storey, 1985).

Table 6.2 Predicted shelf lives of fish products stored at different temperatures

Shelf life in days of product stored in ice (0° C)	**Shelf life at chill temperatures (days)**		
	5° C	**10°C**	**15°C**
6	2.7	1.5	1
10	4.4	2.5	1.6
14	6.2	3.5	2.2
18	8	4.5	2.9

The temperature history of a product, e.g., through a distribution system, can be determined by a temperature logger. Using a spoilage model and simple PC software, the effect of a given storage temperature profile can then be predicted. McMeekin *et al.* (1993) reviewed the literature on application of temperature loggers and on predictive temperature models. A product temperature profile also allows growth of pathogenic microorganisms to be estimated from safety models. Computers and temperature loggers are today available at reasonable prices and it is most likely that spoilage and safety models will be used frequently in the future.

The microflora responsible for spoilage of fresh fish changes with changes in storage temperature. At low temperatures (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas spp.* and *Pseudomonas s*pp. cause spoilage (Table 5.5). However, at high storage temperatures (15-30°C) different species of Vibrionaceae, Enterobacteriaceae and Gram-positive organisms are responsible for spoilage (Gram *et al.,* 1987; Gram *et al.,* 1990; Liston, 1992). Equation 6.c does not take into account the change in spoilage microflora. Nevertheless, reasonable estimates of RRS are obtained for whole fresh fish, for packed fresh fish and for superchilled fresh fish products (Figure 6.3; Gibson and Ogden, 1987; Dalgaard and Huss, 1994). For tropical fish, however, the average relative rate of spoilage of a large number of species stored at 20°-30°C was approximately 25 times higher than at 0°C The RRS of tropical fish is thus more than twice as high as estimated from the temperature models shown in Figure 6.3. Tropical fish are likely to be exposed to high temperatures and a new tropical spoilage model, covering the range of temperatures from 0°-30°C, was

recently developed (Equation 6.d; Dalgaard and Huss, 1994). Figure 6.4 shows that the natural logarithm of RRS of tropical fish is linearly related to the storage temperature. This figure also shows the differences between the new tropical model and previous spoilage models developed for fish from temperate waters.

*Ln (relative rate of spoilage for tropical fish) = 0. 12 * t °C*
6.d

Temperature models based on the relative rate concept do not take into account the initial product quality. Inaccurate shelf life predictions, therefore, may be obtained for products with variable initial quality. Spencer and Baines (1964), however, suggested that both the effect of the initial product quality and the effect of storage temperature could be predicted. At a constant storage temperature measurements of quality will change linearily from an initial to a final level reached when the product is no longer acceptable (Equation 6.e). Shelf life at a given temperature and a given initial quality is determined (Equation 6.e) and then the shelf life at other temperatures can be determined from a temperature spoilage model.

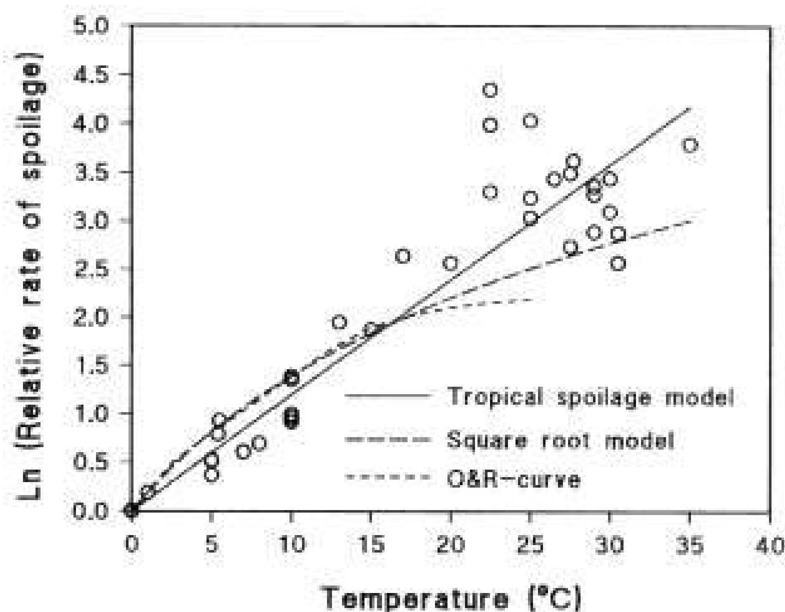


Figure 6.4 Natural logarithm of the relative rateof spoilage of tropical fish species plotted against storage temperatures (Dalgaard and Huss, 1994)

$$\text{Shelf life} = \frac{\text{final} - \text{initial level of a quality indicator}}{\text{rate of spoilage at the actual storage conditions}} \qquad 6.e$$

Much later, the demerit point system, also known as the quality index method, was developed and has proved most useful for obtaining a straight line

relationship between quality scores and storage time (see section 8.1). Bremner et al. (1987) suggested that the rate of change in quality scores, determined by the demerit point system, couldbe quantitatively described at different temperatures by Equation 6.c. Gibson (1985) related microbiological conductance detection times (DT), determined with the Malthus Growth Analyzer, to shelf life of cod. At storage temperatures from 0° to 10°C the daily rate of change in DT values was well predicted by Equation 6.c, and shelf lives were predicted at different temperatures from initial and final DT values and from the temperature spoilage model.

Many aspects of fresh fish spoilage remain to be studied; e.g., the activity of the microorganisms responsible for spoilage at different storage temperatures. Despite this lack of understanding, the relative rate concept has made it possible to quantify and mathematically describe the effect of temperature on the rate of spoilage of various types of fish products. These temperature spoilage models allow time/temperature function integration to be used for evaluation of production, distribution and storage conditions, and when combined with methods for determination of initial product quality, shelf life of various fish products can be predicted.

Apart from the actual storage temperature, the delay before chilling is of great importance. Thus, it can be observed that if white-fleshed, lean fish enter rigor mortis at temperatures above + 17°C, the muscle tissue may be ruptured through severe muscle contractions and weakening of the connective tissue (Love, 1973). The flakes in the fillets separate from each other and this "gaping" ruins the appearance. The fish also become difficult to fillet (Table 6.3) and the water- binding capacity decreases.

Table 6.3 Fillet yield of gutted cod (Hansen, 1981)

	Percentage fillet yield	
	Iced 1 hour after catch	**Iced 6 ½ hour after catch**
Yield of fillets	48.4	46.5
Yield after trimming	43.3	40.4

Rapid chilling is also crucial for the quality of fatty fish. Several experiments have shown that herring and garfish *(Belone belone)* have a significantly reduced

storage life if they are exposed to sun and wind for 4-6 hours before chilling. The reason for the observed rapid quality loss is oxidation of the lipids, resulting in rancid off-flavours. It should be noted, however, that high temperatures are only partly responsible for the speed of the oxidation processes. Direct sunlight combined with wind may have been more important in this experiment as it is difficult to stop autocatalytic oxidation processes once they have been initiated (see section 5.1).

Superchilling (0°C to -4°C)

Storage of fish at temperatures between 0°C and -4°C is called superchilling or partial freezing. The shelf life of various fish and shellfish can be extended by storage at subzero temperatures. The square root spoilage model (Equation 6.c) gives a reasonable description of RRS of superchilled products (Figure 6.5). The shelf life predicted by the square root model at -1°C, -2°C and -3°C for a product that keeps 14 days in ice is 17, 22 and 29 days, respectively.

Superchilling extends the shelf life of fish products. The technique can be used, for example where productive fishing grounds are so far from ports and consumers that normal icing is insufficient for good quality products to be landed and sold. The application of superchilling to replace transport of live fish has also been studied in Japan (Aleman *et al.,* 1982).

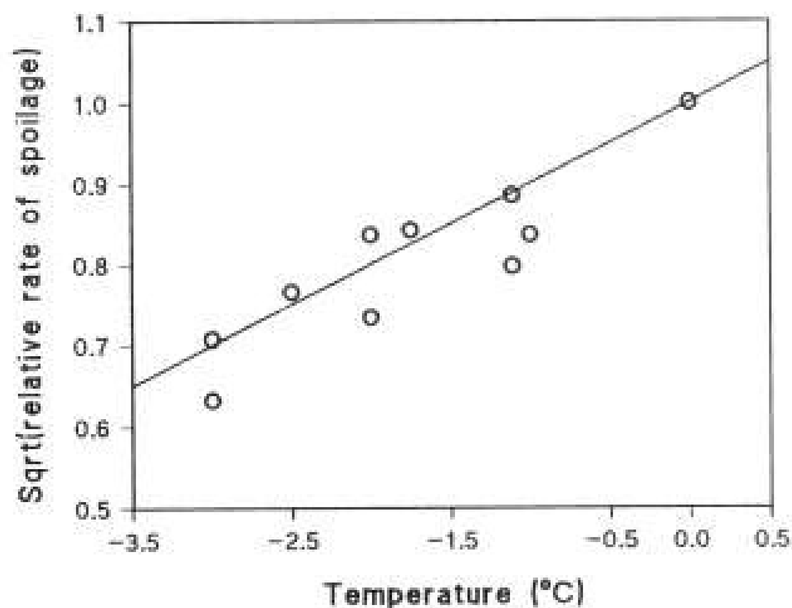


Figure 6.5 Square root plot of the relative rate of spoilage of superchilled cod, shrimp and mullet. The solid line shows relative rates of spoilage predicted by Equation 6.c (Dalgaard and Huss, 1994)

The technology needed to use superchilling at sea as well as for storage on-land

is available today. The "Frigido-system", developed in Portugal in the 1960s, uses heat exchanges in the fish holds. Sub- zero temperatures were kept constant (±0.5°C) and the fish:ice ratio was reduced from the normal 1:1 to 3:1. Sub-zero storage temperatures in fishing vessels can also be obtained in refrigerated sea water (RSW) where the freezing point of water is reduced by NaCl or other freezing point depressors. Compared to ice storage, the RSW systems chill fish more rapidly, reduce the exposure to oxygen, reduce the pressures that often occur when fish are iced and also give significant labour-saving (Nelson and Barnett, 1973). Promising results have been obtained with superchilling, but both technical problems and problems in relation to product quality have been observed. Unloading of fish is difficult when heat exchanges are used in fishing vessels and RSW increases the corrosion of the vessels (Partmann, 1965; Barnett *et* al., 1971). Also, superchilling extends product shelf life, but a negative effect on freshness/prime quality has been observed for some fish species. Merritt (1965) found that cod stored at -2°C for 10 days had an appearance and texture inferior to fish stored at 0°C in ice. The drip of the superchilled fish was increased and at -3°C the texture of whole cod made them unsuitable for filleting. RSW storage gives several fish species a salty taste due to the take-up of sea water (Barnett *et al.,* 1971; Shaw and Botta, 1975; Reppond and Collins, 1983; Reppond *et al.,* 1985). This negative effect of RSW, however, has not been found in all studies (Lemon and Regier, 1977; Olsen *et al.,* 1993). As opposed to cod and several other fish species, the prime quality of superchilled shrimp from Pakistan was increased from 8 days in ice to 16 days in NaCl-ice at -3°C (Fatima *et al.,* 1988). Also, both freshness (measured by a K-value of 20%) and shelf life of cultured carp *(Cyrinus carpio),* cultured rainbow trout *(Salmo gairdnerii)* and mackerel *(Scomber japonicus)* have been improved by superchilling at -3°C as compared to storage at 0°C (Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

The percentage of frozen water in superchilled fish is highly temperature-dependent (-1°C = 19%; -2°C = 55%; -3°C = 70%; -4°C = 76%) (Ronsivalli and Baker, 1981). It has been suggested that negative effects of superchilling on drip loss, appearance, and texture of cod and haddock are due to formation of large ice crystals, protein denaturation and increased enzymatic activity in the partially frozen fish (Love and Elerian, 1964). Simpson and Haard (1987), however, found only very little difference in biochemical and chemical deterioration of cod *(Gadus morhua)* stored at 0°C and at - 3°C In Japanese studies with seabass, carp, rainbow trout and mackerel, it has been shown that the drip loss as well as several biochemical and chemical deteriorative reactions were reduced in superchilled fish, compared to ice storage (Uchiyama and Kato, 1974; Kato *et al.,* 1974; Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

Superchilling has been used industrially with a few fish species such as tuna and salmon. The negative effects on sensory quality found for some other species

may have limited the practical application of the technique. Nevertheless, it seems that shelf life of at least some seafood products is improved considerably by superchilling. Consequently, for selected products, superchilling may well be more suitable than other technologies.

6.2 The effect of hygiene during handling

Onboard handling

Much emphasis has been placed on hygienic handling of the fish from the moment of catching in order to ensure good quality and long storage life. The importance of hygiene during handling onboard has been tested in a series of experiments where various hygienic measures were employed (Huss *et al.,* 1974). The quality and storage life of completely aseptically treated fish (aseptic handling) were compared with fish iced in clean plastic boxes with clean ice (clean handling) and with fish treated badly, i.e., iced in old, dirty wooden boxes (normal handling). As expected, a considerable difference is found in the bacterial contamination of the three batches (Figure 6.6). However, a similar difference in the organoleptic quality is not detected. During the first week of storage no difference whatsoever is found. Only during the second week does the initial contamination level become important and the heavily contaminated fish have a reduction in storage life of a few days compared with the other samples. These results are not surprising if it is kept in mind that bacterial activity is normally only important in the later stages of the storage period as illustrated in Figure 5.1.

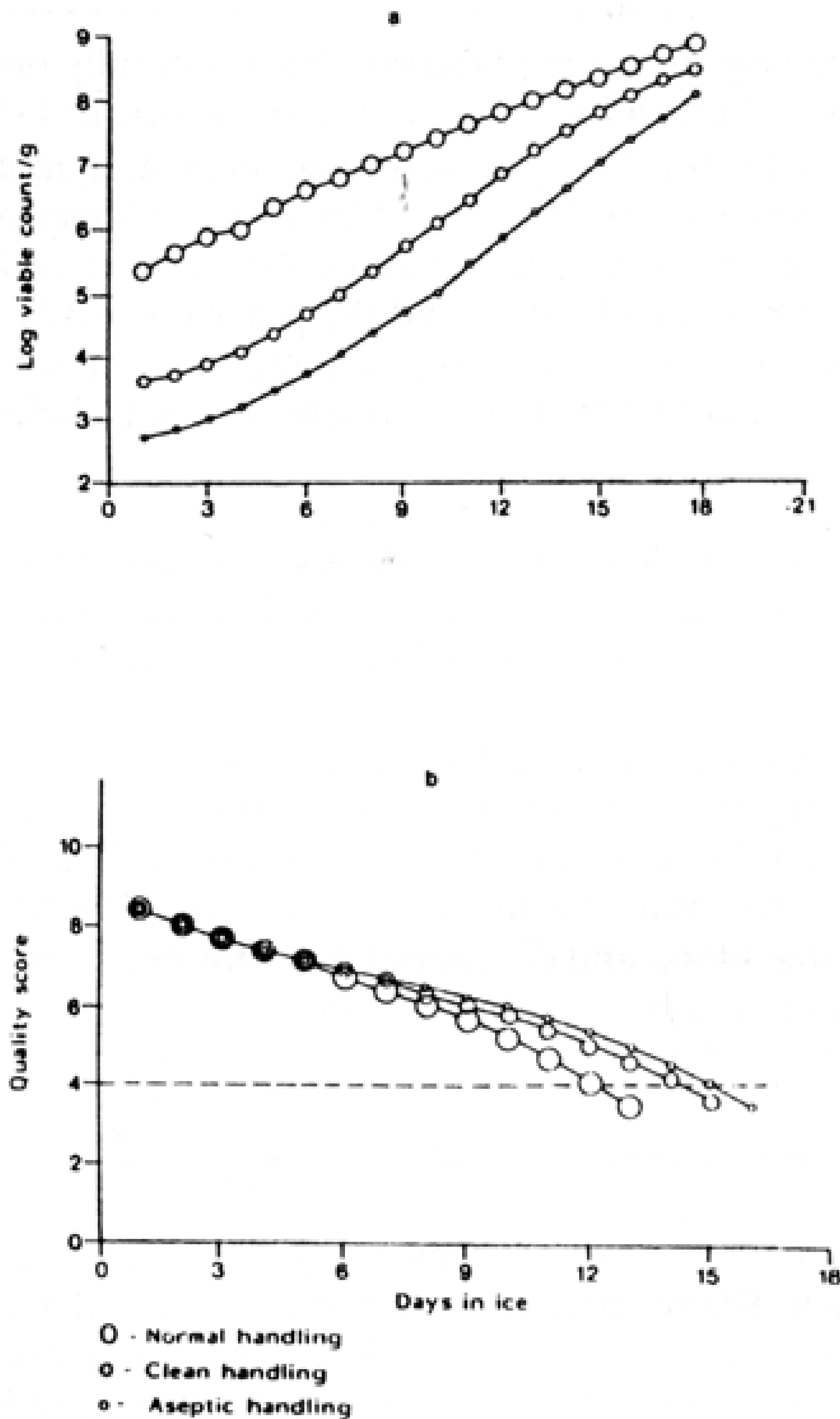


Figure 6.6 Bacterial growth (a) and organoleptic quality (b) of plaice stored at 0° C with initial high, medium and low bacterial counts (after Huss *et al.,* 1974)

On the basis of these data it seems sensible to advocate reasonably hygienic handling procedures including use of clean fish boxes. Very strict hygienic measures do not seem to have great importance. In comparison with the impact of quick and effective chilling, the importance of hygiene is minor.

The above-named observations have influenced the discussion about the design of fish boxes. Normally, fish are iced in boxes stacked on top of each other. In this connection it has been argued that fish boxes should have a construction that prevents the ice melt-water from one box draining into the box underneath it. In a system like this, some bacterial contamination of fish in the bottom boxes would be avoided, as melt-water usually contains a large number of bacteria. However, practical experience as well as experiments (Peters *et al.,* 1974) have shown that this type of contamination is unimportant, and it may be concluded that fish boxes allowing the drainage of melt-water from upper into lower boxes are advantageous because the chilling becomes more effective.

Inhibition or reduction of the naturally occurring microflora

In spite of the relatively minor importance of the naturally occurring microflora in the quality of the fish, much effort has been put into reduction or inhibition of this microflora. Many of these methods are only of academic interest. Among these are (at least until now) attempts to prolong the storage life by using radioactive irradiation. Doses of 100 000 - 200 000 rad are sufficient to reduce the number of bacteria and prolong storage life (Hansen, 1968; Connell, 1975), but the process is costly and, to many people, unacceptable in connection with human food. Another method which has been rejected because of concern about public health is treatment with antibiotics incorporated in the ice.

A method that has been used with some success over recent years is treatment with CO_2, which can be applied either in containers with chilled seawater or as part of a modified atmosphere during distribution or in retail packages (see section 6.3).

It should also be mentioned that washing with chlorinated water has been tried as a means of decontaminating fish. However, the amount of chlorine necessary to prolong the storage life creates off- flavours in the fish meat (Huss, 1971). The newly-caught fish should be washed in clean seawater without any additives. The purpose of the washing is mainly to remove visible blood and dirt, and it does not cause any significant reduction in the number of bacteria and has no effect on storage life.

6.3 The effect of anaerobic conditions and carbon dioxide

High CO_2 concentrations can reduce microbial growth and may therefore extend the shelf life of food products, where spoilage is caused by microbial activity (Killeffer, 1930; Coyne, 1933). Technological aspects of modified atmosphere packaging (MAP) have since been studied. Today, materials and techniques for

storage of bulk or retail packed foods are available.

This section discusses the effect of anaerobic conditions and modified atmospheres on the shelf life of fish products. The safety aspects are reviewed in Farber (1991) and Reddy et al. (1992).

Effect on microbial spoilage

Vacuum packaging (VP) and MAP, with high CO_2 levels (25% - 100%), extends the shelf life of meat products by several weeks or months (Table 6.4). In contrast, the shelf life of fresh fish is not affected by VP and only a small increase in shelf life can be obtained by MAP (Table 6.4).

Table 6.4 Effect of packaging on the shelf life of chilled fish and meat products

Type of product	Storage temp.	Shelf life (weeks)		
		Air	VP[a]	MAP[b]
Meat (beef, pork, poultry)	1.0 - 4.4°C	1 - 3	1 - 12	3 - 21
Lean fish (cod, pollock, rockfish, trevally)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Fatty fish (herring, salmon, trout)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Shellfish (crabs, scampi, scallops)	0.0 - 4.0°C	½ - 2	-	½ - 3
Warmwater fish (sheepshead, swordfish, tilapia)	2.0 - 4.0°C	½ - 2	-	2 - 4

a) VP: Vacuum packed
b) MAP: Modified atmosphere packed (High CO_2 concentrations (25 - 100%)

Differences in spoilage microflora. and in pH are mainly responsible for the observed differences in the shelf life of fish and meat products. Spoilage of meat under aerobic conditions is caused by strict aerobic Gram-negative organisms, primarily *Pseudomonas spp.* These organisms are strongly inhibited by

anaerobic conditions and by CO_2. Consequently, they do not play any role in the spoilage of packed meat. Instead the microflora, of VP and MAP meat products changes to be dominated by Gram-positive organisms (Lactic Acid Bacteria), which are much more resistant to CO_2 (Molin, 1983; Dainty and Mackey, 1992). Fish stored under aerobic conditions are also spoiled by Gram negative-organisms, primarily *Shewanella putrefaciens* (see section 5.3).

The spoilage flora on some packed fish products was found to be dominated by Grampositive microorganisms and in this way the microflora, was similar to the flora on packed meats; see Stammen *et al.* (1990) for a review. For packed cod, however, the Gram-negative organism *Photobacterium phosphoreum* has been identified as the organism responsible for spoilage. The growth rate of this organism is increased under anaerobic conditions (Figure 6.7) and this may explain the importance of the organism in VP cod.

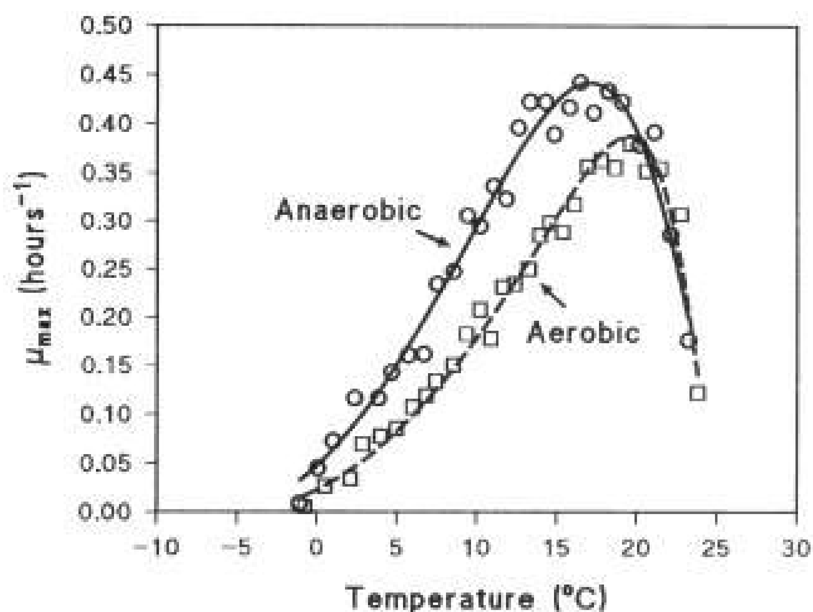


Figure 6.7 Effect of oxygen and temperature on the maximum specific growth rate (max of *Photobacterium phosphoreum* grown in a complex medium containing TMAO (Dalgaard, 1993)

In CO_2-packed fish, the growth of *Shewanella putrefaciens* and of many other microorganisms found on live fish is strongly inhibited. In contrast P. *phosphoreum* was shown to be highly resistent to CO_2 (Figure 6.8). It was also shown that the limited effect of CO_2 on growth of this bacteria correspond very well with the limited effect of CO_2 on the shelf life of packed fresh cod. P. *phosphoreum* reduces TMAO to TMA while very little H_2S is produced during growth in fish substrates. Spoiled VP and MAP cod is characterized by high levels of TMA, but little or no development of the putrid or H_2S odours typical for

some aerobically stored spoiled fish. The growth characteristics of P. *phosphoreum* and the metabolic activity of the organism thus explain both the short shelf life and the spoilage pattern of packed cod (Dalgaard, 1994 a).

The shelf life of VP and MAP cod is similar to various other sea food products (Table 6.4). P. *phosphoreum* is widespread in the marine environment and it seems likely that this organism or other highly CO_2 resistent microorganisms are responsible for spoilage of packed sea food products (Baumann and Baumann, 1981; van Spreekens, 1974; Dalgaard *et al.,* 1993).

The best effect of MAP storage on shelf life has been obtained with fish from warm waters. The shelf life of these products, however, is still relatively short compared to meat products (Table 6.4).

Very low bacterial level (10^5-10^6 cfu/g) has been found at the time of sensory rejection of some packed fish products. In these cases non-microbial reactions may have been responsible for spoilage.

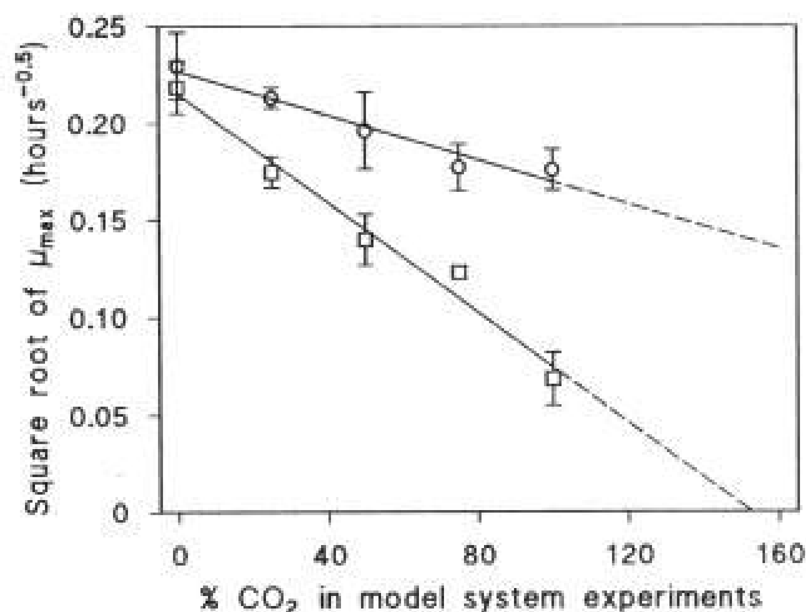


Figure 6.8 Effect of CO_2 on the maximum specific growth rate (μmax) of *Photobacterium phosphoreum* (circles) and of *Shewanella putrefaciens* (squares). Experiments were carried out at 0°C (Dalgaard, 1994 b)

Effect of non-microbial spoilage reactions

CO_2 is dissolved in the water phase of the flesh of MAP fish and a decrease in pH of about 0.2- 0.3 units is observed, depending on the CO_2 concentration in the surrounding gaseous atmosphere. The water-holding capacity of muscle

proteins is decreased by decreased pH and an increased drip loss is expected for fish stored in high CO_2 concentrations. Increased drip has been found for cod fillets, red hake, salmon, and shrimps (Fey and Regenstein, 1982; Layrisse and Matches, 1984; Dalgaard *et al.,* 1993) but not for herring, red snapper, trevally, Dungeness crab, and rockfish (Cann *et al.,* 1983; Gerdes *et al.* 1991; Parking and Brown, 1983 and Parkin *et al.,* 1981).

Coyne (1933) and many later studies have found the textural quality of fish stored in 100% CO_2 to be reduced. However, up to 60% CO_2 has no negative effect on the texture of cod. The colour of the belly flaps, of cornea, and of the skin may be altered for whole fish stored in high CO_2 concentrations (Haard, 1992). Packaging may also stimulate the formation of metmyoglobulin in red-fleshed fish and thereby result in a darkening of fish muscles. Although oxygen-containing modified atmospheres have been used, the development of rancid off-odours in fatty fish species has not been registered as a problem (Haard, 1992).

Carbon dioxide used in combination with refrigerated seawater systems

Storage of fish in refrigerated seawater (RSW) was discussed in section 6. 1. Only the effect of addition of CO_2 to RSW will be considered in this section. Table 6.5 shows the effect of RSW and RSW + CO_2 on the shelf life of various fish products, as compared to storage in ice.

Table 6.5 Shelf life of various fish products stored in Refrigerated Seawater (RSW) and in RSW with added CO_2

Type of product	Storage temp. in RSW	Shelf life (days)			References
		Ice (0° C)	RSW	RSW $+CO_2$	
Pacific cod	-1.1°C	6-9	-	9-12	Reppond and Collins (1983)
Pink shrimp	-1.1°C	-	4-5	6	Barnett et al. (1978)
Herring	-1.0°C	-	8-8.5	10	Hansen et al. (1970)
Walleye Pollock	-1.0°C	6-8	4-6	6-8	Reppond et al. (1979, 1985)

Rockfish	-0.6°C	-	7-10	17	Barnett et al. (1971)
Chum Salmon	-0.6°C	-	7-11	18	Barnett et al. (1971)
Silver Hake	0-1°C	4-5	4-5	5	Hiltz et al. (1976)
Capelin	+0.2 - -1.5°C	6	2	2	Shaw and Botta (1975)

An evident shelf life-extending effect of CO_2 is only seen with some species. Several negative effects of adding CO_2 to RSW-systems have been observed. The fish colour and texture were negatively influenced, and CO_2 dissolved in the flesh made mackerel unsuitable for canning (Longard and Regier, 1974; Lemon and Regier, 1977).

CO_2 acidifies the seawater, and a lowered pH inhibits the enzymatic reactions that otherwise lead to black spots in shrimps and prawns. The shelf life of pink shrimps can be more than doubled by storage in RSW + CO_2, where, compared to ice storage, colour, texture, flavour, and odour were improved (Nelson and Barnett, 1973). RSW+CO_2 stored prawns, however, may be unacceptably tough and have a "soft shell" appearance (Ruello, 1974).

Sea water acidified by CO_2 is highly corrosive. Therefore, inert materials are needed in RSW+CO_2 systems, e.g., for heat exchange. These materials are available, but their cost must be taken into account when the application of RSW + CO_2 systems is evaluated (Nelson and Barnett, 1973).

Future application of carbon dioxide for shelf life extension

For most MAP seafoods, the production of TMA is delayed by only a few days compared to aerobic or anaerobic storage. This indicates that fish products in general are contaminated with a highly CO_2 resistent microflora of TMAO reducing organisms. Very high CO_2 concentrations can inhibit microbial growth but high levels of CO_2 have a negative effects on other aspects of the fish quality. MAP has found little practical application with fish products as compared to meat products. The main reasons for this are probably that:

- MAP used with retail packs is an expensive technique
- the prime fish quality is not improved
- only small shelf life extensions are obtained

- MAP cannot replace good chilling or good hygienic production conditions
- toxin production of *Clostridium botulinum* is increased for bacteria growing under anaerobic conditions, and this may be of importance for the safety of packed fish (Huss *et al.,* 1980; Reddy *et al.,* 1992).

Packaging, however, can be used simply because packed products are more convenient to handle, e.g., in supermarkets. According to the EEC Council Directive of *22 July* 1991 (91/493/EEC), VP and MAP fish products are considered as fresh products. Consequently, CO_2 can be used for preservation of fresh fish products, when a shelf life extension of only a few days is found to be sufficient.

The negative effect Of CO_2 on fish colour is primarily a problem for whole fish and the negative effect of CO_2 on texture and drip loss is only observed with high CO_2 concentrations. A pronounced effect on growth of *S. putrefaciens* and on many other bacteria is obtained with even moderate CO_2 concentrations (40-80%). It is therefore likely that, in the future, MAP will be used in combination with preservation techniques that has been developed specifically to inhibit growth of CO_2 resistent TMAO reducing marine spoilage bacteria such as P. *phosphoreum.*

The effect of MAP also seems to depend on fish species and further studies are needed to determine if MAP can give interesting shelf life extensions for other fish species, e.g., those from warm waters. Finally, high CO_2 concentrations could be used for fish intended for fishmeal as the negative effects of CO_2 on colour and texture in this case are less important.

6.4 The effect of gutting

It is a common experience that the quality and storage life of many fish decrease if they have not been gutted. During feeding periods the fish contain many bacteria in the digestive system and strong digestive enzymes are produced. The latter will be able to cause a violent autolysis *post mortem,* which may give rise to strong off-flavour especially in the belly area, or even cause belly-burst. On the other hand, gutting means exposing the belly area and cut surfaces to the air thereby rendering them more susceptible to oxidation and discoloration. Thus, many factors such as the age of the fish, the species, amount of lipid, catching ground and method, etc., should be taken into consideration before deciding whether or not gutting is advantageous.

Fatty species

In most cases,small- and medium-sized fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is partly that a large number of small fish are caught at the same time and partly because of problems with discoloration and the acceleration of rancidity.

However, problems may arise with ungutted fish during periods of heavy feeding due to belly- burst. The reactions leading to belly-burst are complex and not fully understood. It is known that the strength of the connective tissue is decreased during these periods and that post mortem pH is normally lower in well-fed fish, this also weakens the connective tissue (Figure 6.9). Furthermore, it seems that the type of feed ingested may play an important role in the belly-burst phenomenon.

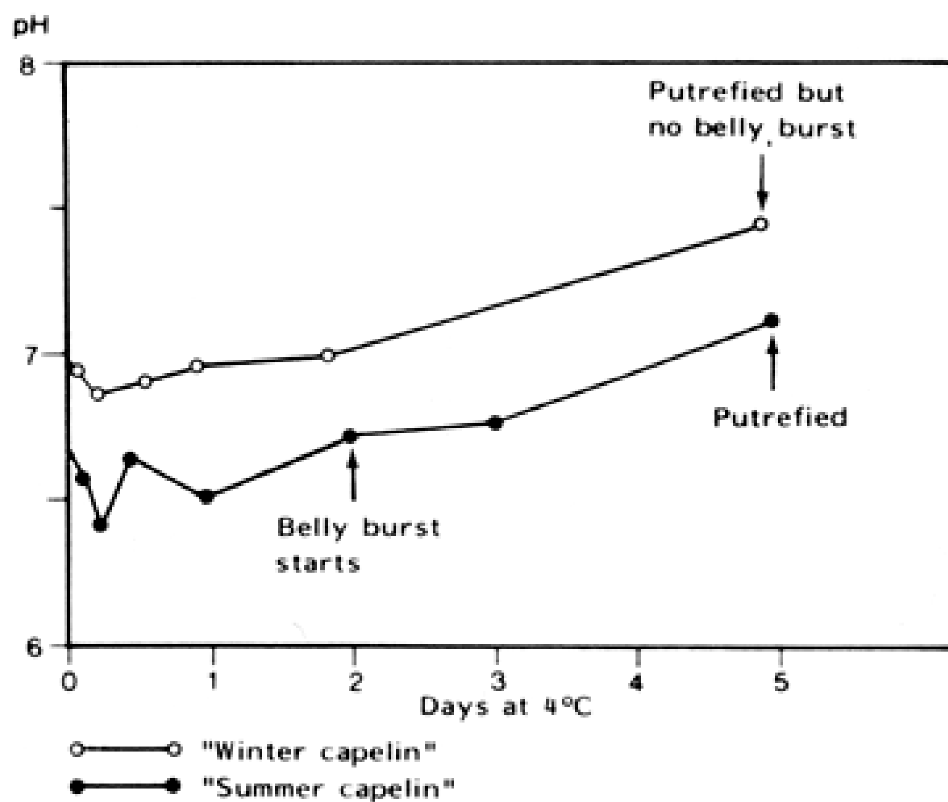


Figure 6.9 pH in winter capelin (o) and summer capelin (·) during storage at +4° C (Gildberg, 1978)

Lean species

In most North European countries, the gutting of lean species is compulsory. It is based on the assumption that the quality of these species suffers if they are not gutted. In the case of cod, it has been shown that omission causes a considerable quality loss and a reduction in the storage life of five or six days. After only two days from catch, discoloration of the belly area is visible and the

raw fillet acquires an offensive cabbagey odour. As seen in Figure 6.10, these odours are removed to some extent by boiling.

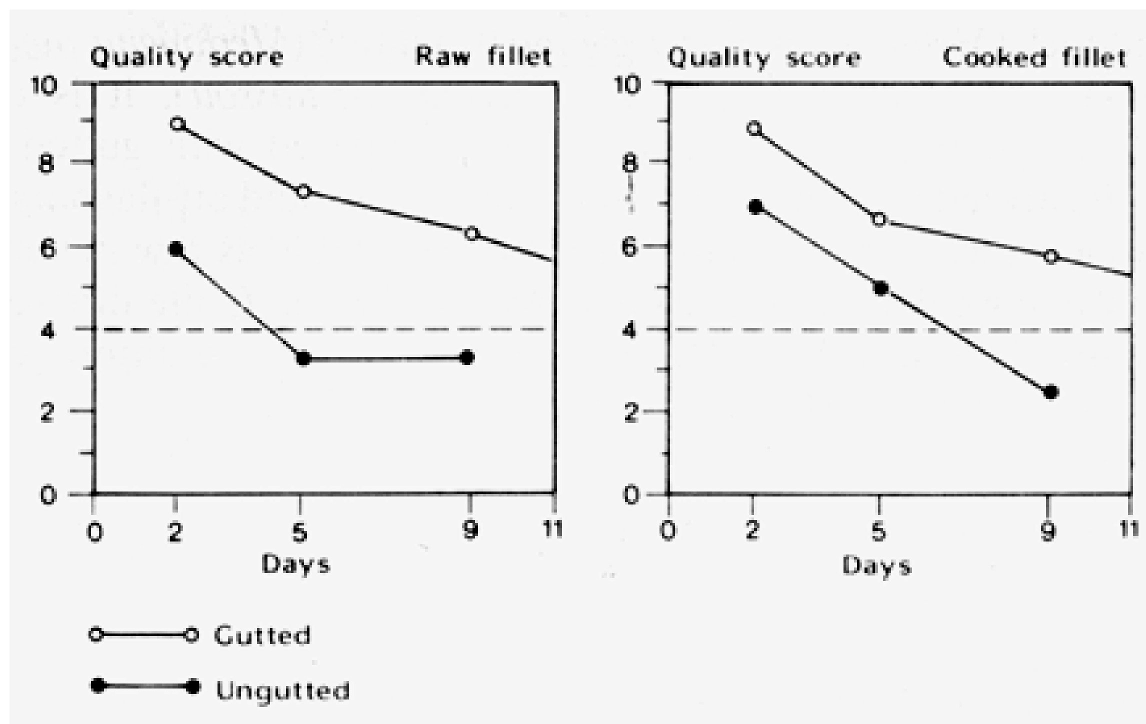


Figure 6.10 Organoleptic quality of raw and boiled fillet, respectively from gutted (o) and ungutted (·) iced cod (Huss, 1976)

These volatile, foul-smelling compounds are mostly found in the gut and surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself (Figure 6.11). These chemical parameters are, therefore, not useful for distinguishing between gutted and ungutted fish (Huss and Asenjo, 1976).

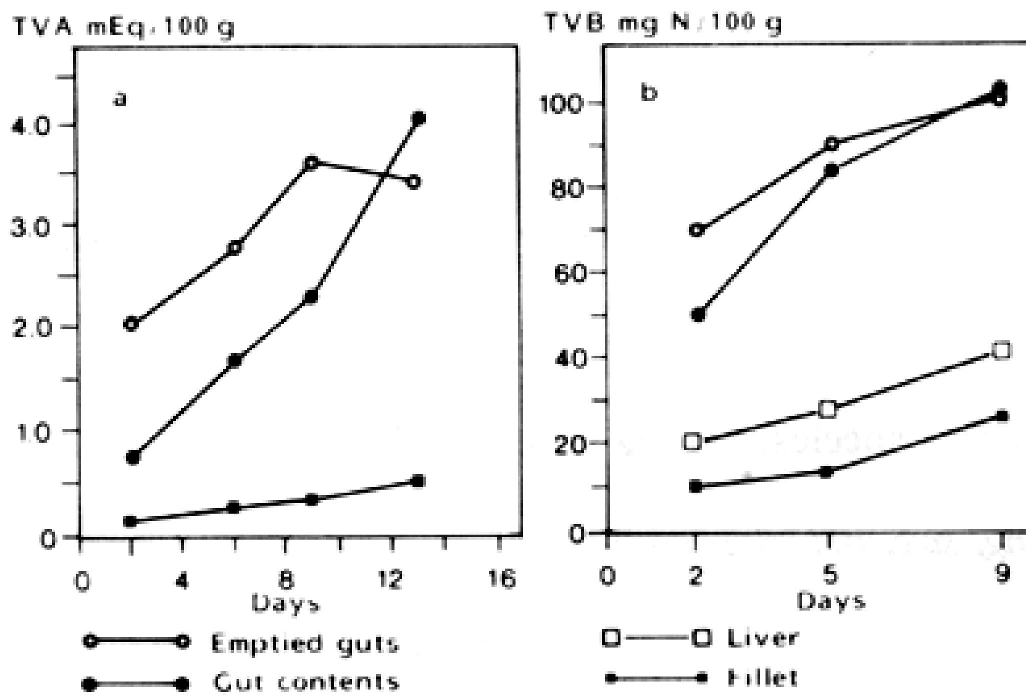


Figure 6.11 Development of (a) volatile acids in iced, ungutted saithe *(Polacchius* virens) and (b) volatile bases in iced, ungutted cod *(Gadus morhua)* *(Huss* and Asenjo, 1976)

Similar experiments with other cod-like species show a more differentiated picture. In the case of haddock *(Melanogrammus aeglefinus),* whiting *(Merlangius merlangus,* saithe *(Pollachius virens)* and blue whiting *(Micromesistius poutassou),* it is observed that ungutted fish stored at 0°C suffer a quality loss compared with gutted fish, but the degree varies as illustrated in Figure 6.12. Some off-odours and off-flavours are detected, but ungutted haddock, whiting and saithe are still acceptable as raw material for frozen fillets after nearly one week on ice (Huss and Asenjo, 1976). Quite different results are obtained with South American hake *(Merluccius gayi),* where no difference is observed between gutted and ungutted fish (Huss and Asenjo, 1977 b).

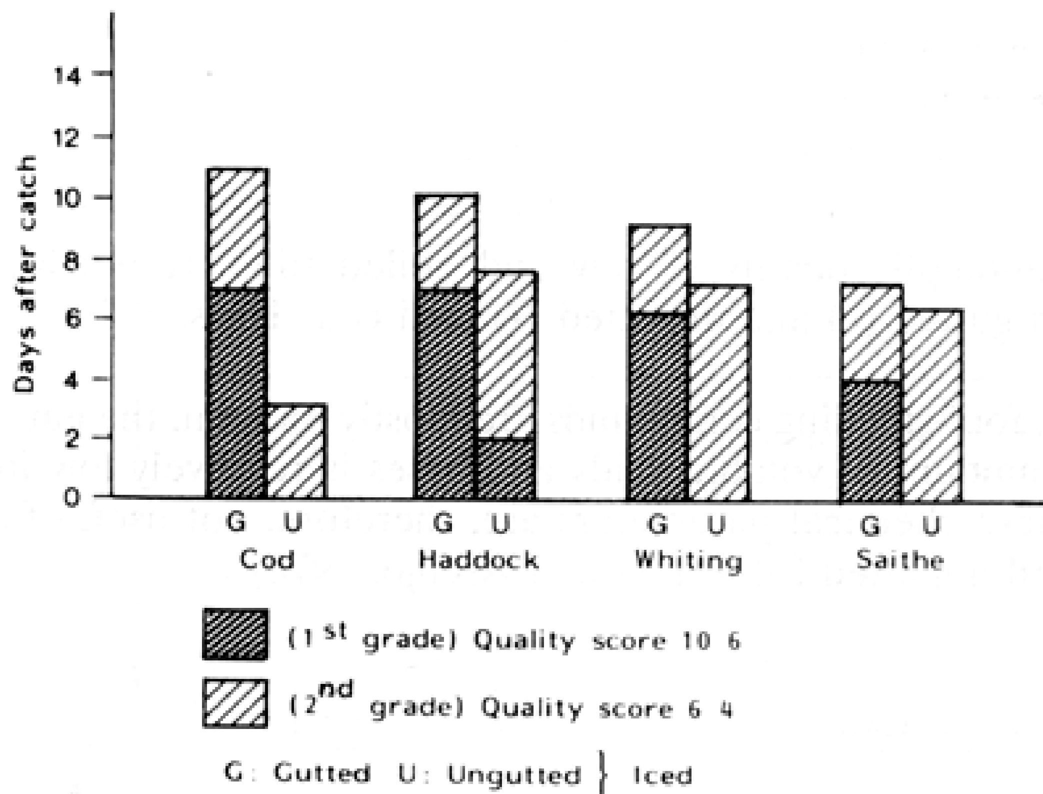


Figure 6.12 Quality and storage life of gutted and ungutted lean fish stored in ice (Huss and Asenjo, 1976)

6.5 The effect of fish species, fishing ground and season

Influence of handling, size, pH, skin properties

The spoilage rate and shelf life of fish is affected by many parameters and, as stated in section 5, fish spoil at different rates. In general it can be stated that larger fish spoil more slowly than small fish, flat fish keep better than round fish, lean fish keep longer than fatty fish under aerobic storage and bony fish are edible longer than cartilaginous fish (Table 6.6). Several factors probably contribute to these differences and whereas some are clear, many are still on the level of hypotheses.

Table 6.6 Intrinsic factors affecting spoilage rate of fish species stored in ice

Factors affecting spoilage rate	Relative spoilage rate	
	fast	slow

size	small fish	larger fish
post mortem pH	high pH	low pH
fat content	fatty species	lean species
skin properties	thin skin	thick skin

Rough handling will, as outlined in section 5.2, result in a faster spoilage rate. This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. The surface/volume ratio of larger fish is lower than that of smaller fish, and, as bacteria are found on the outside, this is probably the reason for the longer shelf life of the former. This is true within a species but may not be universally so.

Post mortem pH varies between species but is, as described in section 5.2, higher than in warm- blooded animals. The long rigor period and the corresponding low pH (5.4-5.6) of the very large flatfish, halibut (*Hippoglossus hipoglossus*), has been offered as an explanation for its relatively long iced storage life (Table 6.7). However, mackerel will often also experience a low pH and this seems to have little effect on shelf life. As can be seen from Table 6.7, fatty fish are in general rejected sensorically long before lean fish. This is mainly due to the appearance of oxidative rancidity.

The skin of the fatty pelagic fish is often very thin, and this may contribute to the faster spoilage rate. This allows enzymes and bacteria to penetrate more quickly. On the contrary, the thick skin of flatfish and the antibacterial compounds found in the slime of these fish may also contribute to the keepability of flatfish. As described earlier, the slime of flat fish contains bacteriolytic enzymes, antibodies and various other antibacterial substances (Hjelmland et al., 1983; Murray and Fletcher, 1976). Although large differences exist in the content of TMAO, this does not seem to affect the shelf life of aerobically-stored fish but rather the chemical spoilage profile of the species.

Table 6.7 Shelf life of various fish species from temperate and tropical waters. Prepared from data published by Lima dos Santos (1981); Poulter *et al.*(1981); and Gram (1989)

Species	Fish type	Shelf life (days in ice)	
		temperate	tropical
Marine species		2-24	6-35
cod, haddock	lean	9-15	
whiting	lean	7-9	
hake	lean	7-15	
bream	lean/low fat		10-31
croaker	lean		8-22
snapper	lean		10-28
grouper	lean		6-28
catfish	lean		16-19
pandora	lean		8-21
jobfish	lean		16-35
spadefish	lean/low fat		21-26
batfish	lean		21-24
sole, plaice,	flat	7-21	21
flounder	flat	7-18	
halibut	flat	21-24	

mackerel[1]	high/ low fat	4-19	14-18
summer herring	high fat	2-6	
winter herring	low fat	7-12	
sardine	high fat	3-8	9-16
Freshwater species		9-17	6-40
catfish	lean	12-13	15-27
trout	low fat	9-11	16-24
perch	lean/ low fat	8-17	13-32
tilapia	lean		10-27
mullet	lean		12-26
carp	lean/ low fat		16-21
lungfish	lean/ low fat		11-25
Haplochromis	lean		6
shad	medium fat		25
corvina	medium fat		30
bagré	medium fat		25
chincuna	fatty		40

pacu	fatty		40

1) fat content and shelf life subject to seasonal variation.

In general, the slower spoilage of some fish species has been attributed to a slower bacterial growth, and Liston (1980) stated that "different spoilage rates seem to be related at least partly to the rate of increase of bacteria on them".

Influence of water temperature on iced shelf life

Of all the factors affecting shelf life, most interest has focused on the possible difference in iced shelf life between fish caught in warm, tropical waters and fish caught in cold, temperate waters. In the mid- and late sixties it was reported that some tropical fish kept 20-30 days when stored in ice (Disney *et al.,* 1969). This is far longer than for most temperate species and several studies have been conducted assessing the shelf life of tropical species. Comparison of the data is, as pointed out by Lima dos Santos (1981), difficult as no clear definition has been given on a "tropical" fish species and as experiments have been carried out using different sensory and bacteriological analyses.

Several authors have concluded that fish taken from warm waters keep better than fish from temperate waters (Curran and Disney, *1979;* Shewan, 1977) whereas Lima dos Santos (1981) concluded that also some temperate water fish species keep extremely well and that the longer shelf lives in general are found in fresh water fish species compared to marine species. However, he also noted that shelf life of more than 3 weeks, which is often observed for fish caught in tropical waters (Table 6.7),never occurs when fish from temperate waters are stored in ice. The iced shelf life of marine fish from temperate waters varies from 2 to 21 days which does not differ significantly from the shelf life of temperate freshwater fish ranging from *9* to 20 days. Contrary to this, fish caught in tropical marine waters keep for 12-35 days when stored in ice and tropical freshwater fish from *6* to 40 days. Although very wide variations occur, tropical fish species often have prolonged shelf lives when stored in ice as shown in Table 6.6. When comparisons are made, data on fatty fish like herring and mackerel should probably be omitted as spoilage is mainly due to oxidation.

Several hypotheses have been launched trying to explain the often prolonged iced spoilage of tropical fish. Some authors have noted an absence in development of TMA and TVN during storage and suggested that the spoilage of tropical fish is not caused by bacteria (Nair *et al.,* 1971). The lack of development of TMA and TVN may be explained by a spoilage dominated by *Pseudomonas* spp.; however, qualitative bacteriological analyses must be carried out to confirm

or reject this suggestion. Low bacterial counts have been claimed in some studies, but often inappropriate media have been used for the examination and too high incubation temperatures (30°C) have not allowed the psychrotrophic spoilage bacteria to grow on the agar plates.

Reviewing the existing literature on storage trials of tropical fish species leads to the conclusion that the overall sensory, chemical and bacteriological changes occurring during spoilage of tropical fish species are similar to those described for temperate species.

Psychrotrophic bacteria belonging to *Pseudomonas spp*. and *Shewanella putrefaciens* dominate the spoilage flora of iced stored fish. Differences exist, as described in section 5.3, in the spoilage profile depending on the dominating bacterial species. *Shewanella* spoilage is characterized by TMA and sulphides (H_2S) whereas the *Pseudomonas* spoilage is characterized by absence of these compounds and occurrence of sweet, rotten sulphydryl odours. As this is not typical of temperate, marine fish species which have been widely studied, this may explain the hypothesis that bacteria are not involved in the spoilage process of tropical fish.

Despite the different odour profiles, the level at which the offensive off-odours are detected sensorially is more or less the same. In model systems (sterile fish juice) 10^8-10^9 cfu/ml of both types of bacteria is the level at which spoilage is evident.

As outlined in section 5.3, the relatively high post*mortem* pH is one of the reasons for the relatively short shelf life of fresh fish as compared to, for instance, chill stored beef. It has been suggested that tropical fish species, such as the halibut from temperate waters, reach a very low pH, and that this explains the longer shelf life. However, pH values of 6-7 have been found in the studies of tropical fish species where pH has been measured (Gram, 1989). As the differences in skin properties are believed to contribute to the longer shelf life of flatfish, it has been suggested that this factor explained the extended shelf lives. It is indeed true that fish from warm waters often have very thick skin, but no systematic investigation has been carried out on the skin properties.

As spoilage of fish is caused by bacterial action, most hypotheses dealing with the long iced shelf life of tropical fish species have centred around differences in bacterial flora. Shewan (1977) attributed the long iced shelf lives to the lower number of psychrotrophs on tropical fish. However, in 1977 only a very limited number of studies of the bacterial flora on tropical fish were published. During the last 10- 15 years several investigations have concluded that Gram-negative rod-shaped bacteria (e.g., *Pseudomonas, Moraxella* and *Acinetobacter)* dominate on many fish caught in tropical waters (Gram, 1989; Surendram *et al.,* 1989; Acuff *et*

al., 1984). Similarly, Sieburth (1967) concluded that the composition of the bacterial flora in Narragansett Bay did not change during a 2-year survey even though the water temperature fluctuated with 23°C on a year-round basis. Gram (1989) showed that 40-90% of the bacteria found on Nile perch were able to grow at 7°C. The number of psychrotrophic bacteria is within one log unit of the total count, and the level of psychrotrophic organisms is not *per se low* enough to account for the extended iced storage lives of tropical fish; Jorgensen *et al.* (1989) showed that a two log difference in number of spoilage bacteria only resulted in a difference of 3 days in the shelf life of iced cod.

As described in section 5, the bacterial flora on temperate water fish species resume growth immediately after the fish have been caught and rarely is a lag phase seen. Contrary to this, Gram (1989) concluded that a bacterial lag phase of 1-2 weeks is seen when tropical fish are stored in ice. Also, the subsequent growth of psychrotrophic bacteria is often slower on iced tropical than on iced temperate water fish. This is in agreement with Liston (1980) who attributed differences in shelf life to differences in bacterial growth rates. Although a large part of the bacteria on tropical fish are capable of growth at chill temperatures, they will (as this has never been necessary) require a period of adaptation (i.e., the lag phase and slow growth phase). Gram (1989) illustrated this by investigating the growth rate at 0°C of fish spoilage bacteria that had either been pre-cultured at 20°C or at 5°C. For some strains, the same bacterial strain would grow more quickly at 0°C if pre-cultured at 5°C than if pre-cultured at 20°C (Table 6.8). Preculturing was done with several sub-culture steps at each temperature. Similarly, Sieburth (1967) showed that although the taxonomic composition of the bacterial flora in Narrangansett Bay did not change with fluctuating temperature, the growth profile of the bacteria fluctuated following the water temperature. However, the adaptation hypothesis does not explain why some tropical fish spoil at rates comparable to temperate water fish.

Table 6.8 Generation times at 0°C for fish spoilage bacteria pre-cultured at high (20°C) or low (5°C) temperatures

Species	**Origin**	**Pre-culture temperature (°C)**	**Subsequent generation time (hours) at 0°C**
Aeromonas spp.	spoiled chilled trout	5 20	11 20
Pseudomonas spp.	iced cod (Denmark)	5 20	9 14

	spoiled iced sardine (Senegal)	5 20	12 14
Shewanella spp.	iced cod (Denmark)	5 20	8 17
	iced sole (Senegal)	5 20	9 17

It can be concluded that many factors affect shelf life of fish and that differences in the physiology of the bacterial flora are likely to be of major importance.

Off flavours related to fishing ground

Occasionally fish with off-flavours are caught, and in certain localities this is a fairly common phenomenon. Several of these off-flavours can be attributed to their feeding on different compounds or organisms. The planktonic mollusc, *Spiratella helicina,* gives rise to an off-flavour described as "mineral oil" or "petrol". It is caused by dimthyl-B-propiothetin which is converted to dimethylsulphide in the fish (Connell, 1975). The larvae of *Mytilus* spp. cause a bitter taste in herring. A very well known off-flavour is the muddy-earthy taint in many freshwater fish. The flavour is mainly caused by two compounds: geosmin (1a, 10ß-dimethyl-9a-decalol) and 2-methylisoborneol, which also are part of the chemical profile of wine with cork flavour. Geosmin, the odour of which is detectable in concentrations of 0.01-0.1 µg/l, is produced by several bacterial taxa, notably the actinomycetes *Streptomyces* and *Actinomyces.*

An iodine-like flavour is found in some fish and shrimp species in the marine environment. This is caused by volatile bromophenolic compounds; and it has been suggested that the compounds are formed by marine algae, sponges and Bryozoa and become distributed through the food chain (Anthoni *et al.,* 1990).

Oil taint may be found in the fish flesh in areas of the world where off-shore exploitation of oil is intensive or in areas where large oil spills occur. The fraction of the crude oil that is soluble in water is responsible for the off-flavours. This is caused by the accumulation of various hydrocarbon compounds, where particularly the aromatic compounds are strong flavourants (Martinsen *et al.,* 1992).

Figure 6.13 The situation on a South American hake trawler. The fishermen have spent considerable time and effort gutting the fish, where rapid chilling of whole, ungutted fish would have been more beneficial to quality

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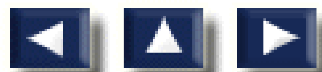


7. IMPROVED FRESH FISH HANDLING METHODS

7.1. Basics of fresh fish handling and use of ice
7.2. Fish handling in artisanal fisheries
7.3. Improved catch handling in industrial fisheries

7.1 Basics of fresh fish handling and use of ice

Throughout history, man has preferred to consume fresh fish rather than other types of fish products. However, fish spoil very quickly and man has had to develop methods to preserve fish very early in history.

Keeping and transporting live fish

The first obvious way of avoiding spoilage and loss of quality is to keep caught fish alive until consumption. Handling of live fish for trade and consumption has been practised in China with carp probably for more than three thousand years. Today, keeping fish alive for consumption is a common fish-handling practice both in developed and developing countries and at both artisanal and industrial level.

In the case of live fish handling, fish are first conditioned in a container with clean water, while the damaged, sick and dead fish are removed. Fish are put to starve and, if possible, water temperature is reduced in order to reduce metabolic rates and make fish less active. Low metabolic rates decrease the fouling of water with ammonia, nitrite and carbon dioxide that are toxic to fish and impair their ability to extract oxygen from water. Such toxic substances will tend to increase mortality rates. Less active fish allow for an increase in the packing density of fish in the container.

A large number of fish species are usually kept alive in holding basins, floating cages, wells and fish yards. Holding basins, normally associated with fish culture companies, can be equipped with oxygen control, water filtering and circulation and temperature control. However, more simple methods are also used in practice, for instance large palm woven baskets acting as floating cages in rivers (China), or simple fish yards constructed in a backwater of a river or rivulet for large "surubi" (Platystoma spp.), "pacu" (Colossoma spp.) and "pirarucu" (Arapalma gigas) in the Amazonian and Parana basins in South America.

Methods of transporting live fish range from very sophisticated systems installed on trucks that regulate temperature, filter and recycle water and add oxygen (Schoemaker, 1991), to very simple artisanal systems of transporting fish in plastic bags with an oxygen supersaturated

atmosphere (Berka, 1986). There are trucks that can transport up to 50 t of live salmon; however, there is also the possibility of transporting a few kilo-grammes of live fish relatively easily in a plastic bag.

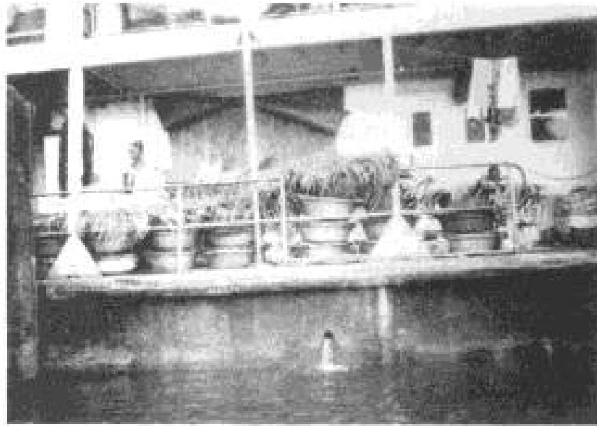
By now a large number of species, inter alia, salmon, trout, carp, eel, seabream, flounder, turbot, catfish, Clarias, tilapias, mussels, oysters, cockles, shrimp, crab and lobster are kept alive and transported, very often from one country to another.

There are wide differences in the behaviour and resistance of the various species. Therefore the method of keeping and transporting live fish should be tailored according to the particular species and the length of time it needs to be kept outside its natural habitat before slaughtering. For instance, the lungfish (Protopterus spp.) can be transported and kept alive out of water for long periods, merely by keeping its skin moist.

Some species of fish, noticeably freshwater fish, are more resistant than others to changes in oxygen in solution and the presence of toxic substances. This is probably due to the fact that their biology is adapted to the wide yearly variations in water composition presented by some rivers (cycles of matter in suspension and dissolved oxygen). In these cases, live fish are kept and transported just by changing the water from time to time in the transport containers (See Figures 7.1 (a) and (b)). This method is widely used in the Amazonian, Parana and Orinoco basins in South America; in Asia (particularly in the People's Republic of China, where also more sophisticated methods are used) and in Africa (N'Goma, 1993).

In the case presented in Figure 7.1 (a), aluminium containers with live freshwater fish are stored in the aisles of a public transport vessel. Containers are covered with palm leaves and water hyacinth to prevent the fish from jumping out of the containers and to reduce evaporation. The water in the containers is changed from time to time and an almost continuous visual control is kept on fish. Dead fish are immediately put to smoke-drying (African style) in drum smokers, also transported in the vessels or transporting barges.

In the case presented in Figure 7. 1(b), carp is kept in a metal container drawn by a bicycle. This is a rather common practice in China, and other Asian countries; for instance in Bangkok, live catfish is sold daily by street vendors.



(a) (b)

Figure 7.1 (a) Transport of live freshwater fish in Congo (Cuvette Congolaise) (N'Goma, 1993); (b) street vendor of live fish in China today (Suzhou, 1993, photo H. Lupin)

The most recent development is the keeping and transporting of fish in a state of hibernation. In this method, the body temperature of live fish is reduced drastically in order to reduce fish metabolism and to eliminate fish movement completely. The method greatly reduces death rates and increases package density, but careful temperature control should be exercised to maintain the hibernation temperature. There is an appropriate hibernation temperature for each species. Although the method is already utilized for instance to transport live "kuruma" shrimp *(Penaeus japonicus)* and lobster in pre-chilled wet sawdust, it should be considered an experimental technique for most of the species.

Although keeping and transporting live fish is becoming more and more important, it is not a viable solution for most of the bulk fish captures in the world.

Chilling fish with ice

Historical evidence proves that the Ancient Chinese utilized natural ice to preserve fish more than three thousand years ago. Natural ice mixed with seaweed was also used by the Ancient Romans to keep fish fresh. However, it was the development of mechanical refrigeration which made ice readily available for use in fish preservation.

In developed countries, particularly in USA and some European countries, the tradition of chilling fish with ice dates back more than a century. The practical advantages of utilizing ice in fresh fish handling are therefore well established. However, it is worthwhile for young generations of fish technologists and newcomers to the field, to review them, paying attention to the main points of this technique.

Ice is utilized in fish preservation for one or more of the following reasons:

(i) ***Temperature reduction.*** By reducing temperature to about 0°C the growth of spoilage and pathogenic micro-organisms (see section 6) is reduced, thus reducing the spoilage rate and

reducing or eliminating some safety risks.

Temperature reduction also reduces the rate of enzymatic reactions, in particular those linked to early *post mortem* changes extending, if properly applied, the *rigor mortis* period.

Fish temperature reduction is by far the most important effect of ice utilization. Therefore, the quicker the ice chills the better. Although cold-shock reactions have been reported in a few tropical species when iced, leading to a loss of yield of fillets (Curran *et* al., 1986), the advantage of quick chilling usually outweighs other considerations. The development of ad hoc fish handling methods is of course not ruled out in the case of species that could present cold-shock behaviour.

(ii) ***Melting ice keeps fish moist.*** This action mainly prevents surface dehydration and reduces weight losses. Melting water also increases the heat transport between fish and ice surfaces (water conducts heat better than air): the quickest practical chilling rate is obtained in a slurry of water and ice (e.g., the CSW system).

If, for some reason, ice is not utilized immediately after catching the fish, it is worthwhile keeping the fish moist. Evaporative cooling usually reduces the surface temperature of fish below the optimum growth temperature of common spoilage and pathogenic bacteria; although it does not prevent spoiling.

Ice should also be utilized in relation with chilling rooms to keep fish moist. It is advisable to keep chilling room temperature slightly above 0°C (e.g., 3-4°C).

However, water has a leaching effect and may drain away colour pigments from fish skin and gills. Ice melting water can also leach micronutrients in the case of fillets and extract relatively large amounts of soluble substances in some species (e.g., squid).

Depending on the species, severity of leaching and market requirements, an ad *hoc* handling procedure may be justified. In general, it has been found that drainage of ice meltwater is advisable in boxes and containers and that permanence of fish in chilled sea water (CSW) and refrigerated seawater (RSW) should be carefully assessed if leaching and other effects (e.g., uptake of salt from the seawater, whitening of fish eyes and gills) are to be avoided.

During the past there was much discussion about allowing drainage from one fish box to another, and consequent reduction or increase of bacterial load by washing with drainage water. Today, apart from the fact that in many cases box design allows for external drainage of each box in a stack, it is recognized that these aspects have less importance when compared with the need for quick reduction in temperature.

(iii) ***Advantageous physical properties.*** Ice has some advantages when compared with other cooling methods, including refrigeration by air. The properties can be listed as follows:

(a) *Ice has a large cooling capacity.* The latent heat of fusion of ice is about 80 kcal/kg. This means that a comparatively small amount of ice will be needed to cool 1 kg of fish.

For example, for I kg of lean fish at 25°C, about 0.25 kg of melted ice will be needed to reduce its temperature to 0°C (see Equation 7.c). The reason why more ice is needed in practice is mainly because ice melting should compensate for thermal losses.

The correct understanding of this ice characteristic is the main reason for the introduction of insulated fish containers in fish handling, particularly in tropical climates. The rationale is: ice keeps fish and the insulated container keeps ice. The possibility to handle fish with reduced amounts of ice improves the efficiency and economics of fresh fish handling (more volume available for fish in containers, trucks and cold storage rooms, less weight to transport and handle, reduction in ice consumption, less water consumed and less water drained).

(b) *Ice melting is a self-contained temperature control system.* Ice melting is a change in the physical state of ice (from solid to liquid), and in current conditions it occurs at a constant temperature (0°C).

This is a very fortunate property without which it would be impossible to put fresh fish of uniform quality on the market. Ice that melts around a fish has this property on all contact points. In the case of mechanical refrigeration systems (e.g., air and RSW) a mechanical or electronic control system (properly tuned) is needed; nevertheless, controlled temperature will be always an average temperature.

Depending on the volume, design and control scheme of mechanical refrigeration systems, different temperature gradients may appear in chill storage rooms and RSW holds, with fish slow freezing in one comer and maybe above 4°C in another comer. Although the need for proper records and control of temperature of chill storage rooms has been emphasized recently in connection with the application of HACCP (Hazard Analysis Critical Control Point) to fresh fish handling, it is clear that the only system that can assure accurate temperature control at the local level (e.g., in any box within a chill storage room) is ice melting.

Ice made of sea water melts at a lower temperature than fresh water ice, depending on the salt content. Theoretically with 3.5 % of salt content (the average salt content of seawater) seawater ice will melt at about - 2.1°C However, as ice made out of seawater is physically unstable (ice will tend to separate from salt), brine will leach out during storage lowering the overall temperature (and this is the reason why sea water ice always seems wet). In these conditions, fish may become partially frozen in storage conditions and there may be some intake of salt by the fish muscle. Therefore, it cannot be said that ice made out of seawater has a proper self-controlled temperature system.

There is a narrow range of temperature below 0°C before fish muscle starts to freeze. The freezing point of fish muscle depends on the concentration of different solutes in the tissue fluids: for cod and haddock, it is in the range of -0.8 to - 1 °C, for halibut -1 to -1.2°C, and for herring about -1.4°C (Sikorski, 1990).

The process of keeping fish below 0°C and above the freezing point is called superchilling, and it allows achievement of dramatic increases in overall keeping times. In principle it could be obtained using seawater ice or mixtures of seawater and freshwater ice, or ice made out of a 2% brine and/or mechanical refrigeration. However, in large volumes it is very difficult to control temperature so precisely and temperature gradients, partial freezing of fish in some pockets and hence lack of uniformity in quality are unavoidable (see section 6. 1).

(iv) ***Convenience***. Ice has a number of practical properties that makes its use advantageous. They are:

(a) It is *a portable cooling method.* It can be easily stored, transported and used. Depending on

the type of ice, it can be distributed uniformly around fish.

(b) *Raw material to produce ice is widely available.* Although clean, pure water is becoming increasingly difficult to find, it is still possible to consider it a widely available raw material. When there is no assurance that freshwater to produce ice will be up to the standard of drinking water, it should be properly treated, e.g., chlorination.

Clean seawater can also be utilized to produce ice. Ice from seawater is usually produced where freshwater is expensive or in short supply. However, it should be remembered that harbour waters are hardly suitable for this purpose.

(c) Ice can be a relatively cheap method of preserving fish. This is particularly true if ice is properly produced (avoiding wastage of energy at ice plant level), stored (to avoid losses) and utilized properly (not wasted).

(d) *Ice is a safe food-grade substance. If* produced properly and utilizing drinking water, ice is a safe food substance and does not entail any harm either to consumers or those handling it. Ice should be handled as food.

(v) **Extended shelf life.** The overall reason for icing fish is to extend fresh fish shelf life in a relative simple way as compared to storage of un-iced fish at ambient temperatures above 0°C (see Chapter 6). However, extension of shelf life is not an end in itself, it is a means for producing safe fresh fish of acceptable quality.

Most landed fish can be considered a commodity, that is, an article of trade. Unlike other food commodities, it is usually highly perishable and it is thus in the interest of the seller and the buyer to ensure fish safety at least until it is consumed or further processed into a less perishable product. Ice and refrigeration in general, by making possible extension of fish shelf life, convert fresh fish into a true trade commodity, both at local and international level.

Ice is used to make fish safe and of better quality to consumers. It is also used because otherwise the current fish trade at local and international level would be impossible. Shelf life is extended because there is a strong economic reason to do so. Fishermen and fish processors who fail to handle fresh fish appropriately ignore the essence of their business. The inability to recognize fresh fish also as a trade commodity is at the root of misunderstandings and difficulties linked to the improvement of fish handling methods and prevention of post-harvest losses.

Types of ice

Ice can be produced in different shapes; the most commonly utilized in fish utilization are flake, plate, tube and block. Block ice is ground before being utilized to chill fish.

Ice from freshwater, of whatever source, is always ice and small differences in salt content or water hardness do not have any practical influence, even if compared with ice made out of distilled water. The physical characteristics of the different types of ice are given in Table 7.1.

Cooling capacity is expressed by weight of ice (80 kcal/kg); therefore it is clear from Table 7.1 that the same volume of two different types of ice will not have the same cooling capacity. Ice volume per unit of weight can be more than twice that of water, and this is important when ice stowage and volume occupied by ice in a box or container are considered. Ice necessary to cool

fish to 0°C or to compensate for thermal losses is always expressed in kilogrammes.

Under tropical conditions ice starts to melt very quickly. Part of the melted water drains away but part is retained on the ice surface. The larger the ice surface per unit of weight the larger the amount of water retained on the ice surface. Direct calorimetric determinations show that at 27°C the water on the surface of flake ice at steady conditions is around 12-16% of the total weight and in crushed ice, 10-14% (Boeri et al., 1985). To avoid this problem, ice may be subcooled; however, under tropical conditions this effect is quickly lost. Therefore a given weight of wet ice will not have the same cooling capacity as the same weight of dry (or subcooled) ice, and this should be taken into account when making estimations of ice consumption.

Table 7.1 Physical characteristics of ice utilized in chilling fish. Adapted from Myers (1981)

Types	Approximate Dimensions (1)	Specific volume (m^3/t) (2)	Specific weight (t/ m^3)
Flake	10/20 - 2/3 mm	2.2 -2.3	0.45-0.43
Plate	30/50 - 8/15 mm	1.7 - 1.8	0.59-0.55
Tube	50(D)- 10/12 mm	1.6 - 2.0	0.62-0.5
Block	Variable (3)	1.08	0.92
Crushed block	Variable	1.4 - 1.5	0.71 -0.66

Notes:

(1) They depend on the type and adjustment of the ice machine.
(2) Indicative values, it is advisable to determine them in practice for each type of ice plant.
(3) Usually in blocks of 25 or 50 kg each.

There is always the question of which is the "best" ice to chill fish. There is no single answer. In general, flake ice will allow for an easier, more uniform and gentle distribution of ice around fish and in the box or container and will produce very little or no mechanical damage to fish and will chill fish rather more quickly than the other types of ice (see Figure 7.2). On the other hand, flake ice will tend to occupy more volume of the box or container for the same cooling capacity and if wet, its cooling capacity will be reduced more than the other types of ice (since it has a higher area per unit of weight).

With crushed ice there is always the risk of large and sharp pieces of ice that can damage fish physically. However, crushed ice usually contains fines that melt quickly on the fish surface and large pieces of ice that tend to last longer and compensate for thermal losses. Block ice requires less stowage volume for transport, melts slowly, and contains less water at the time it is crushed than flake or plate ice. For these reasons, many artisanal fishermen utilize block ice (e.g., in Colombia, Senegal and the Philippines).

Probably tube ice and crushed ice are more suitable for use in CSW systems if ice is wet (as it normally is under tropical conditions), since they will contain less water on their surfaces.

There are also economic and maintenance aspects that may play a role in deciding for one type of ice or another. The fish technologist should be prepared to analyze the different aspects involved.

Cooling rates

Cooling rates depend mainly on the surface per unit of weight of fish exposed to ice or chilled ice/water slurry. The larger the area per unit of weight the quicker the cooling rate and the shorter the time required to reach a temperature around 0°C at the thermal centre of the fish. This concept is also expressed as "the thicker the fish the lower the cooling rate".

Small species such as shrimp, sardines, anchovies and jack mackerels cool very quickly if properly handled (e.g., in CSW or CW). Large fish (e.g., tuna, bonito, large sharks) could take considerable time to cool. Fish with fat layers and thick skin will take longer to cool than lean fish and fish with thin skin of the same size.

In the case of large fish, it is advisable to gut them and to put ice into the empty belly as well as around it. In large sharks, gutting alone may not be enough to prevent spoilage during chilling, and therefore it is advisable to gut the shark, to skin it and to cut the flesh into sizeable portions (e.g., 2-3 cm thick) and to chill them as soon as possible. Chilled sea water (CSW) has in this case the advantage of extracting some of the urea present in shark muscle (see section 4.4). However, this is an extreme case, since in current situations fillets kept in ice will last less time than gutted fish or whole fish (because of the unavoidable microbial invasion of the flesh) and will lose soluble substances.

Typical curves for cooling fish in ice, using different types of ice and chilled water (CW) are shown in Figure 7.2.

From Figure 7.2 it is clear that the quickest method to chill fish is with chilled water (CW) or chilled sea water (CSW), although the practical difference with flake ice is not great. There are, however, noticeable differences after the quick initial drop in temperature with crushed block ice and tube ice, due to differences in contact areas between fish and ice and flow of melt-water.

Cooling curves may also be affected by the type of container and external temperature. Since ice will melt to cool fish and simultaneously to compensate for thermal losses, temperature gradients may appear in actual boxes and containers. This type of temperature gradient could affect the cooling rate, particularly in boxes at the top or side of the stacks, and more likely with tube and block crushed ice.

Curves such as those shown in Figure 7.2 are useful to determine the critical limit of chilling rates when applying HACCP to fresh fish handling. For instance, in specifying a critical limit for chilling fish "to be at 4.5°C in the thermal centre in no more than 4 hours", in the case of Figure 7.2 it could be achieved only by using flake ice or CW (or CSW).

In most cases the delay in reaching 0°C in the thermal centre of the fish may not have much practical influence because the surface temperature of the fish will be at 0°C. On the other hand, warming-up of the fish is much riskier because the fish surface temperature (which is actually the

riskiest point) will almost immediately be at the external temperature, and therefore ready for spoilage. As large fish will take longer than small fish to warm up and also have less surface area (where spoilage starts) per unit of volume than small fish, they usually take a little longer to spoil than small fish. This circumstance has been widely used (and abused) in practice in the handling of large species (e.g., tuna and Nile perch).

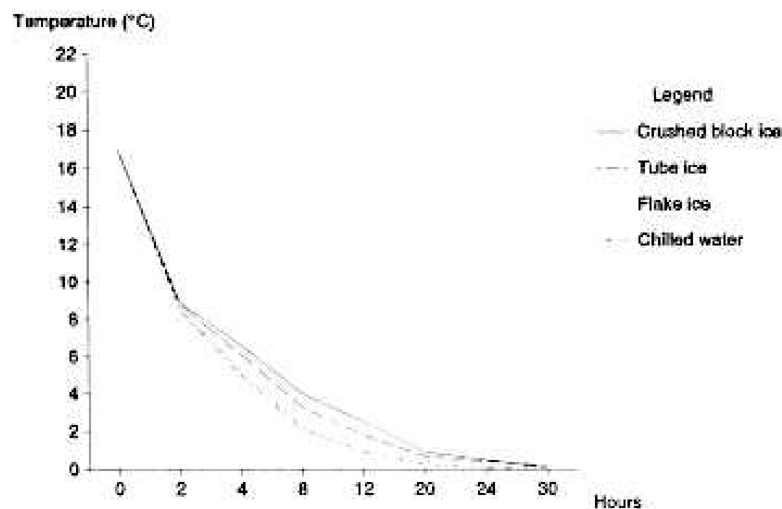


Figure 7.2 Chilling of large yellow croaker *(Pseudosciaena crocea)* with three different types of ice and chilled water (CW). Ice-to-fish ratio 1: 1; the same type of insulated containers (with drainage) was used in a parallel experiment (data obtained at the FAO/DANIDA National Workshop on Advances in Chilling and Processing Technology of Fish, Shanghai, China, June 1986)

Small species will warm up very quickly and definitely more quickly than large species (warming-up the same reason for which they cool faster). Although warming-up studies of fresh fish have received little attention in the past, they are necessary within an HACCP scheme, to determine critical limits (e.g., maximum time fish can be handled without ice in a fish processing line).

With application of HACCP and HACCP-based systems, thermometers including electronic thermometers, should be a standard tool in fish processing plants. Therefore, it is advisable to perform fish cooling and warming-up trials on actual conditions.

Ice consumption

Ice consumption can be assessed as the sum of two components: the ice necessary to cool fish to 0°C and the ice to compensate for thermal losses through the sides of the box or container.

Ice necessary to cool fish to 0°C

The amount of ice theoretically necessary to cool down fish from a temperature Tf to 0°C using ice can easily be calculated from the following energy balance:

$$L \cdot mi = mf \cdot cpf \cdot (Tf - 0) \qquad 7.a$$

where:

L = latent heat of fusion of ice (80 kcal/kg)
mi = mass of ice to be melted (kg)
mf = mass of fish to be cooled (kg)
cpf = specific heat capacity of fish (kcal/kg · °C)

From (7.a) it emerges that:

$$mi = mf \cdot cpf \cdot Tf / L \qquad 7.b$$

The specific heat capacity of lean fish is approximately 0. 8 (kcal/kg · °C). This means that as a first approximation:

$$mi = mf \cdot Tf / 100 \qquad 7.c$$

This is a very convenient formula, easily remembered, to quickly estimate the quantity of ice needed to cool fish to 0°C.

Fatty fish have lower cpf values than lean fish and, in theory, require less ice per kilogramme than lean fish; however, for safety purposes it is advisable to make calculations as if fish were always lean. Refinements in the determination of cpf are possible; however, they do not drastically alter the results.

The theoretical quantity necessary to cool fish to 0°C is relatively small and in practice much more ice is used to keep chilled fish. If we relate the proper fish handling principle of surrounding middle and large sized fish with ice, to the approximate dimensions of ice pieces (see Table 7. 1), it is clear that with some types of ice (tube, crushed block and plate) greater quantities are required for physical considerations alone.

However, the main reason for using more ice is losses. There are losses due to wet ice and ice spilt during fish handling, but by far the most important losses are thermal losses.

Ice necessary to compensate for thermal losses

In principle, the energy balance between the energy taken by the melted ice to compensate heat from outside the box or container could be expressed as follows:

$$L \cdot (dMi/dt) = U \cdot A \cdot (Te - Ti) \qquad 7.\ d$$

where:

Mi = mass of ice melted to compensate for thermal losses (kg)
U = overall heat transfer coefficient (kcal/hour · m^2 · °C)
A = surface area of the container (m^2)
Te = external temperature
Ti = ice temperature (usually taken as 0 °C)
t = time (hours)

Equation (7.d) can be easily integrated (assuming Te = constant) and the result can be expressed as:

$$Mi = Mio - (U \cdot A \cdot Te / L) \cdot t \qquad 7.e$$

It is possible to estimate thermal losses, calculating U and measuring A. However, this type of calculation will seldom give an accurate indication of ice requirements, for a number of practical factors (lack of reliable data on materials and conditions, irregularities in the construction of containers, irregular geometric shape of boxes and containers, influence of lid and drainage, radiation effect, type of stack).

More accurate calculations of ice requirements can be made if meltage tests are used to determine the overall heat transfer coefficient of the box or container, under actual working conditions (Boeri *et al.,* 1985; Lupin, 1986 a).

Ice meltage tests are very easy to conduct and no fish are needed. Containers or boxes should be filled with ice and weighed before commencing the test. At given periods, the melted water is drained (if it has not already drained) and the container is weighed again. The reduction of weight is an indication of the ice lost due to thermal losses. In Figure 7.3 the results of two ice meltage tests obtained under field conditions are presented.

Initially, some ice will be melted to cool down the walls of the box or container; depending on the relative size and weight of the container, wall materials and thickness and entity of the thermal losses this amount may be negligible. If it is not, the container can be cooled down before starting the test, or the ice necessary to cool down the container can be calculated by the difference disregarding the first part of the meltage test. A constant air surrounding temperature would be preferable and it can be achieved during short periods (e.g., the testing of a plastic box in tropical conditions). However, reasonably constant temperatures may be achieved during the intervals between weight loss measurements and an average used in the calculations.

Results as shown by Figure 7.3 can be interpolated empirically by a straight line equation of the form:

$$Mi = Mio - K \cdot t \qquad 7.f$$

Comparing Equations 7.e and 7.f, it is clear that:

$$K = (Uef \cdot Aef \cdot Te / L)$$

where:

Uef = overall effective heat transfer coefficient
Aef = effective surface area

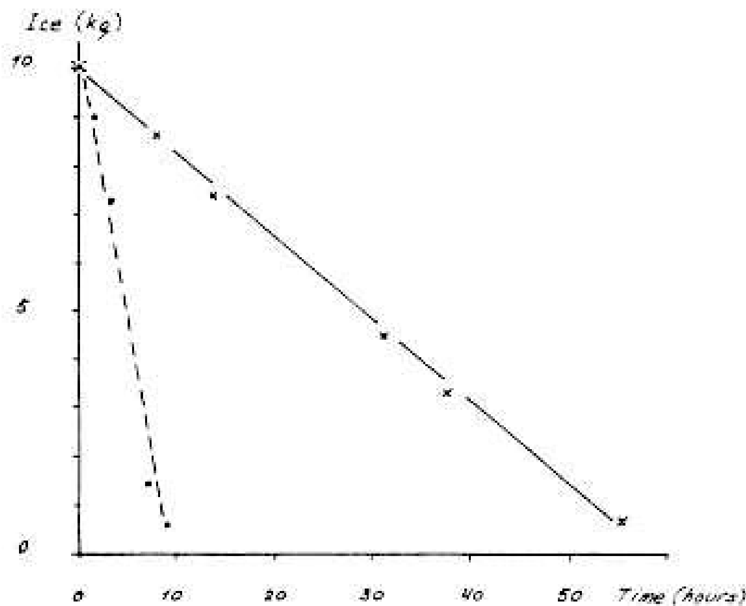


Figure 7.3 Results of ice meltage tests under field conditions. (·) standard plastic box (not insulated) 40 kg total capacity, (x) insulated plastic fish container (Metabox 70, DK). Both kept in the shade, un-stacked, flake ice, average external temperature (Te) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

From Expression 7.g it follows that:

$$K = K' - Te \qquad 7.h$$

and eventually K' could be determined, if experiments can be conducted at different controlled temperatures.

The advantage of meltage tests is that K can be obtained experimentally from the slope of straight lines, as appears in Figure 7.3, either graphically or by numerical regression (now found as sub-routine in common pocket scientific calculators). In the case of the straight lines appearing in Figure 7.3 the correlations found are as follows:

Plastic box:

$$Mi = 10.29 - 1.13 \cdot t, \quad r = -0.995 \qquad 7.i$$
$$K = 1.13 \text{ kg of ice/hour}$$

Insulated container:

$$Mi = 9.86 - 0.17 \cdot t, \quad r = 0.998 \qquad 7.j$$
$$K = 0.17 \text{ kg of ice/hour}$$

where r = correlation coefficient.

From 7.i and 7.j it follows that the ice consumption due to thermal losses in these conditions will be 6.6 times greater in the plastic box than in the insulated container. It is clear that under tropical conditions it will be practically impossible to handle fish in ice properly utilizing only non-insulated boxes, and that insulated containers will be needed, even if additional mechanical refrigeration is used.

The total amount of ice needed will be the result of adding mi (see Equations 7.b and 7.c) to Mi (according to expression 7.f) once t (the time fish should be kept chilled in the box or container in the particular case) has been estimated.

Under tropical conditions it may happen that, depending on the estimated t, total available volume in the box or container might not be enough even for ice to compensate for thermal losses, or the remaining volume for fish could be insufficient to make the chilling operation attractive.

In such cases it might be feasible to introduce one or more re-icing steps, or to resort to additional mechanical refrigeration (see Figure 7.5 to observe the effect of storage in a chill room on ice consumption). In practice, an indication of when re-icing is needed would be given to foremen or people in charge.

An analytical approach to this problem in connection with the estimation of the right ice-to-fish ratio in insulated containers can be found in Lupin (1986 b).

Ice consumption in the shade and in the sun

An important consideration, particularly in tropical countries, is the increased ice consumption in boxes and insulated containers when exposed to the sun. Figure 7.4 gives the results of a experimental meltage test conducted with a box in the shade and the same box (same colour) in the sun.

The plastic box in the shade is the same plastic box of Figure 7.3 (see Equation 7.i). The correlation for the plastic box in the sun is:

$$Mi = 9.62 - 3.126 \cdot t \qquad 7.k$$

This means that for this condition and this type of box, the ice consumption in the sun will be 2.75 times that in the shade (3.126/1.13). This considerable difference is due to the radiation effect. Depending on the surface material, type of material, colour of the surface and solar irradiation, it

will be a surface radiation temperature, that is higher than dry bulb temperature. Direct measurements on plastic surfaces of boxes and containers on field conditions, in tropical countries, have given values of surface radiation temperature up to 70°C.

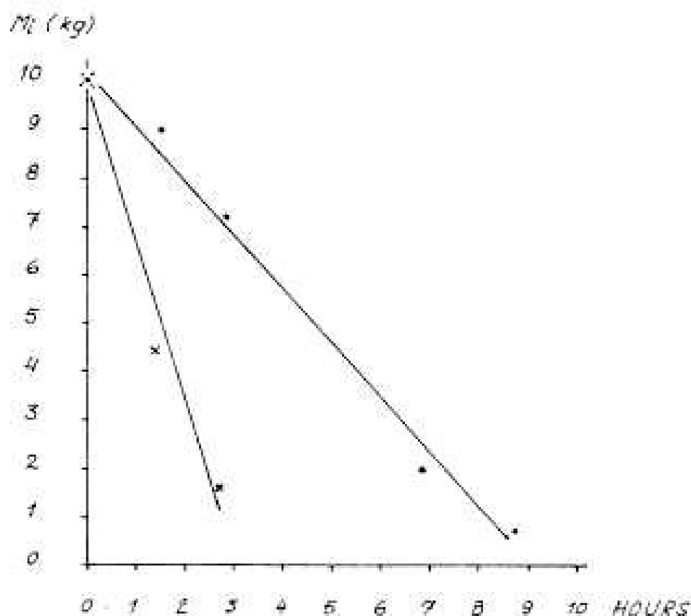


Figure 7.4 Results of ice meltage tests under field conditions. (·) plastic box in the shade, (x) plastic box in the sun. Plastic boxes, 40 kg capacity, red colour, unstacked, flake ice, external average temperature (dry bulb) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

It is clear that there is little practical possibility in tropical countries to handle chilled fish in plastic boxes exposed to the sun. An increase in ice consumption, even if less dramatic than in plastic boxes, can be measured in insulated containers exposed to the sun.

The obvious advice in this case is to keep and handle fish boxes and containers in the shade. This measure can be complemented by covering the boxes or containers with a wet tarpaulin. The wet tarpaulin will reduce the temperature of the air in contact with boxes and containers to the wet bulb temperature (some degrees below the dry bulb temperature, depending on the Equilibrium Relative Humidity - ERH - of the air), and will practically stop noticeable radiation effect (since there are always radiation effects between a body and its background).

Ice consumption in stacks of boxes and containers

In a stack of boxes or containers not all of them will lose ice in the same way. Figure 7.5 gives the results of an ice meltage test conducted on a stack of boxes. Boxes or containers at the top will consume more ice than boxes and containers at the bottom, and those in the middle will consume less than either.

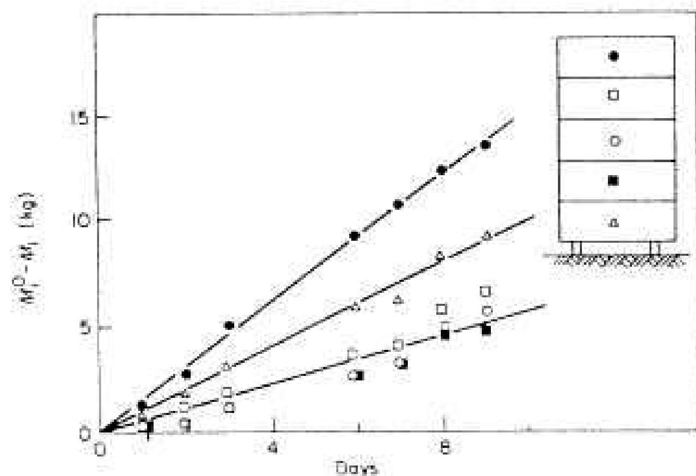


Figure 7.5 Results of ice meltage tests during storage in a stack of plastic boxes. Plastic boxes 35 kg in a chill storage room at 5°C, flake ice (from Boeri et al. (1985)

Jensen and Hansen (1973) and Hansen (1981) presented a system ("Icibox"), mainly for artisanal fisheries. In this system, a stack of plastic boxes were insulated by placing wooden frames, filled with polystyrene, at the top and at the bottom of the stack, and covering the whole with a case made out of canvas or oil skin. A similar system, composed of stacks of styropor boxes, accommodated in a pallet, and covered by an insulated mat of high reflective (Al) surface, is used in practice for shipment of fresh fish by air (e.g., it is utilized to ship fresh fillets of Nile perch from Lake Victoria to Europe).

Results of Figure 7.5 are also of interest to demonstrate the effect of a chill room on fresh fish handling. The use of chill rooms drastically reduces the ice consumption in plastic boxes, avoiding the need of re-icing. In a fish handling system chilling fish with ice, mechanical refrigeration is used to reduce the ice consumption and not to chill fish.

Although analytical models of ice consumption (e.g., Equations 7.a to 7.h) can be applied directly to estimate the ice consumption in simple and repetitive fish handling operations, their main importance is that they can help in arriving at solutions for the proper handling of chilled fish in rational. way (as seen from Figures 7.3, 7.4 and 7.5).

Ice consumption in the sides of boxes and containers

It is necessary to bear in mind that ice will not melt uniformly in the interior of a box or container, but meltage will follow the pattern of temperature gradients between the interior of the box/ container and the ambient. In Figure 7.6, a commercial plastic box with chilled hake shows the lack of ice in the sides due to the temperature gradients at the walls.

Following Figure 7.5, and supposing that a simple box could be devided into five subboxes, it is clear that the bottom and top of boxes and containers should receive more ice to compensate for thermal losses, the top receiving more ice than the bottom. However, in practice more ice should also be put in the sides of boxes and containers.

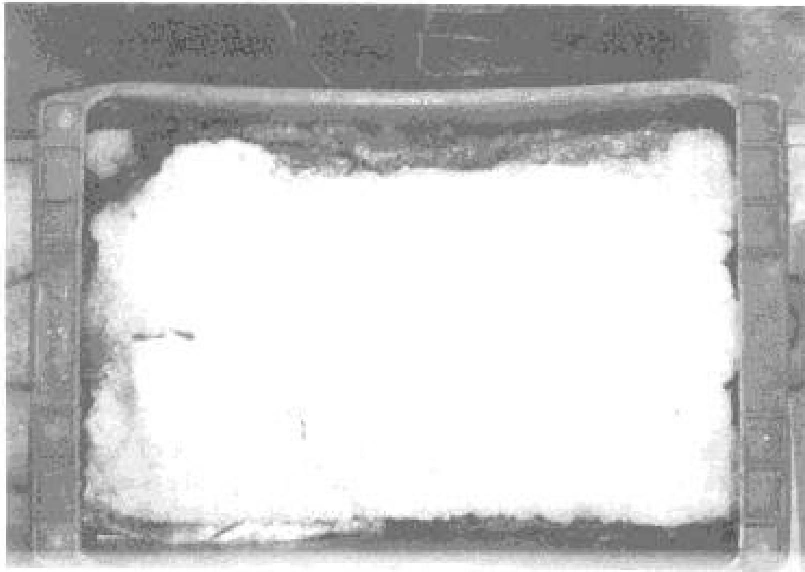


Figure 7.6 Commercial plastic box with chilled hake (M. *hubbsi)* showing the effects of lack of ice in the sides (photo H. Lupin)

The box of Figure 7.6 was initially prepared with enough ice, and it can be seen that ice is still abundant on topof the box. However, after a period of storage in a chill room, ice has melted, mainly on the sides, leaving some fish and parts of fish exposed to the air with a consequent rise in temperature and dehydration. In addition, ice and fish have formed a compact mass that can produce physical damage to exposed fish when the box is moved.

In chilled fish onboard fishing vessels or transported by truck, this problem may not exist if there is a continuous gentle movement which allows for ice melt water from the top to move to the sides. However, in chill rooms or storage rooms (insulated containers) it would be advisable to re-ice if this problem is observed. Under tropical conditions this effect is observed, even with insulated containers, in less than 24 hours of storage.

7.2 Fish handling in artisanal fisheries

Artisanal fisheries, existing both in developed and developing countries, encompass a very wide range of fishing boats from pirogues and canoes (large and small) to small outboard and onboard engine vessels, utilizing also a variety of fishing gears. It is difficult to find a common denominator; however, from a fish handling point of view, artisanal vessels handle relatively small amounts of fish (when compared with industrial vessels) and fishing journeys are usually short (usually less than one day and very often only a few hours).

In general, in tropical fisheries the artisanal fleet land a variety of species, although there are examples of the use of selective fishing gear. In temperate and cold climates artisanal fleets can focus more easily on specific species according to the period of the year; nevertheless, they may land a variety of species to respond to the market demand.

Although very often artisanal fisheries are seen as an unsophisticated practice, closer scrutiny will reveal that in many cases they are passing through a process change. There are many reasons for this process but very often the main driving forces are: urbanization, fish exports and competition with the industrial fleet.

This change in the scenario of artisanal fisheries is essential to understanding the fish handling problems faced by the artisanal and small sector of the fish industry, particularly in developing countries.

When the artisanal fleet was serving small villages, the amount of fish handled was very low; the customers usually bought the fish direct from the landing places, fishermen knew customers and their tastes, and fish was consumed within a few hours (e.g., fish caught at 06.00 h, landed and sold at 10.00 h, cooked and consumed by 13.00 h). In this situation, ice was not used, and gutting was unknown; very often fish arrived at landing places in rigor mortis (depending on fish species and fishing gear), and fish handling was at most reduced to covering the fish from the sun, keeping it moist and keeping off the flies. In Figure 7.7 two cases of landing un-iced fish by artisanal fishermen are shown.

(a)

(b)

Figure 7.7 Landing by artisanal fishermen: (a) un-iced shrimp by artisanal fishermen (El Salvador, September 1987, photo H. Lupin); (b) un-iced fish (Bukova, Tanzania, 1994, photo S. P. Chen)

With urbanization and the request for safer and more quality products (as a result of exports and competition with industrial fish) conditions changed drastically. Large cities also demanded increased fish supplies, and thus middlemen and fish processors had to go to more distant landing places for fish. The amount of fish handled increased, fishing journeys lasted longer and/ or passive fishing gears like gillnets were set to fish for longer times, a chain of middlemen and/ or official fish markets replaced the direct buyer at the beach and, as a result of growing business (fish for income), in some places the catch effort also increased with a consequent increase in the number of fishing boats and an increase in the efficiency of the fishing gears.

In one way or another, each of the new circumstances added hours to the time which passed between catching the fish and eating or processing it (e.g., freezing). This increase in exposure of un- iced fish to ambient temperature (or water temperature for a dead fish in a gillnet), even though brief (e.g., an additional 6-12 hours), dramatically changed the situation regarding fish

spoilage and safety.

In the new situation, fish remained at ambient temperature some 13-19 or more hours. It could be already spoiled, at terminal quality and/or could present public health hazards (e.g., from the development of C. *botulinum* toxin to histamine formation). In addition to the safety and quality aspects, post-harvest losses, non-existent at subsistence level and very low at the village stage, become important. For instance, it is estimated that the post-harvest losses of Nile perch caught artisanally in Uganda amount to 25-30% of the total catch.

The situation described in previous paragraphs, and cases like those shown in Figure 7.7, moved extension services in developing countries and international technical assistance to focus on the problem of introducing improved fish handling methods at the artisanal level. The basic technical solution is the introduction of ice, proper fish handling methods and insulated containers, which is the approach utilized by most of the artisanal fleet in developed countries.

There are several examples where this approach was adopted by fishermen in developing countries and has become a self-sustained technology. Two very interesting cases to analyze are the introduction of insulated containers onboard of "navas", the traditional fishing vessels of Kakinada in Andhra Pradesh, India (Clucas, 1991) and the introduction of insulated fish containers in the pirogue fleet of Senegal (Coackley and Karnicki, 1984). The sketch of an insulated fish container for Senegalese pirogues is shown in Figure 7.8.

The insulated container of Figure 7.8 was designed to fit existing pirogues, according to the type of catch and needs expressed by fishermen. The materials and tools needed to construct the insulated container are available to fishermen in Senegal, even though some of them are imported (e.g., foam sheets and resin).

The example of Senegalese fishermen is now spreading steadily to similar fisheries in Gambia, Guinea-Bissau and Guinea which are adopting the use of insulated containers similar to those of Senegal. However, the process of diffusion and adoption of a technology, even if relatively simple, is not as straightforward as could be supposed. A pirogue with two insulated containers onboard is shown in Figure 7.9.

Once artisanal fishermen become aware of the rationale of insulated containers, they tend to favour large insulated fish containers rather than small ones. The reason is clear from Equations 7.e and 7.g, as for the same volume of fish and ice, large containers will present less external area than the area presented by several small containers. For example, a large cubic insulated fish container can be envisaged of a side measuring x m, and eight cubic insulated containers of sides equal to x/2 m presenting the same total volume as the large one. The eight containers will have an external area twice that of the big container, thus increasing the ice consumption by two, and decreasing the amount of fish that can be transported.

Other reasons are that small containers will cost more than a large one of the same total volume (simply because they need more material); small containers are not always easy to secure safely onboard small boats, and large containers allow for transport of large ice bars that can be crushed at sea (reducing stowage rate). However, large containers are difficult to handle and sometimes canoes and pirogues are very small or narrow and they cannot accommodate large insulated fish containers. This is the case for relatively small insulated fish containers. An example is shown in Figure 7. 10.

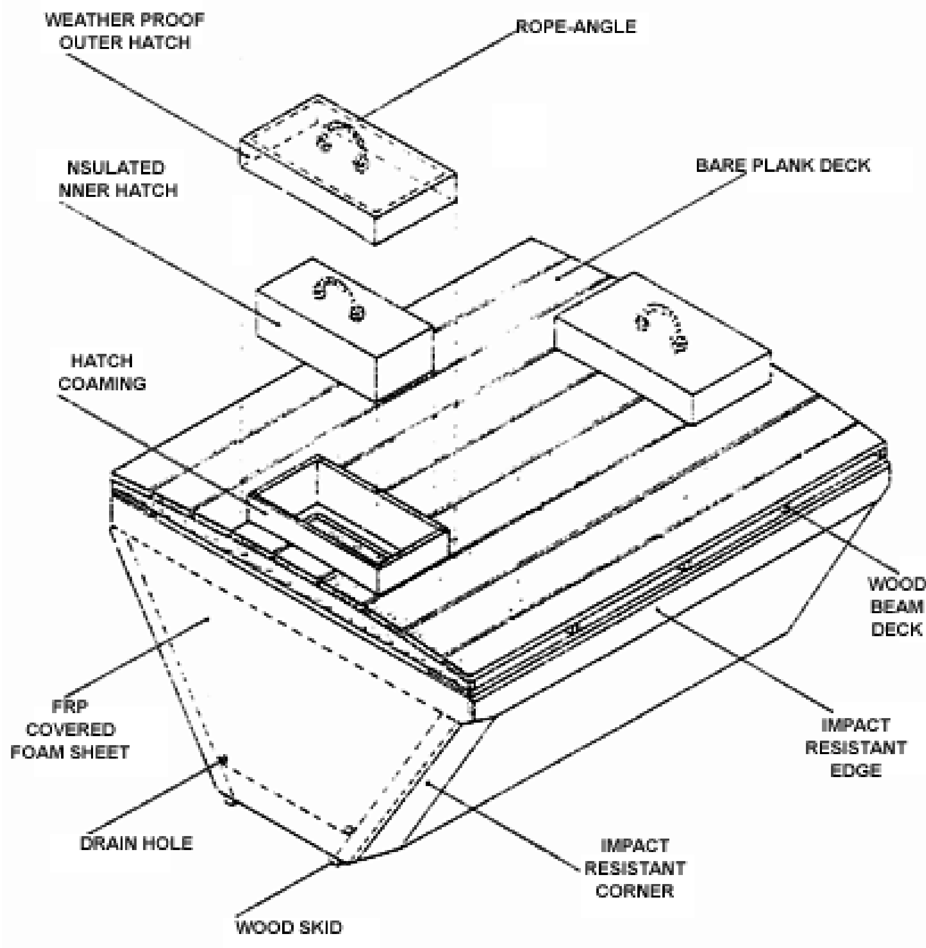


Figure 7.8 Sketch diagram of a two-hatch insulated container for Senegalese pirogues (after Coackley and Karnicki, 1985)

Figure 7.9 A Senegalese pirogue at the beach, carrying two insulated containers (photo B. Diakité, 1992)

Figure 7.10 Small insulated container installed onboard an artisanal fish catamaran (The Philippines, 1982, photo H. Lupin)

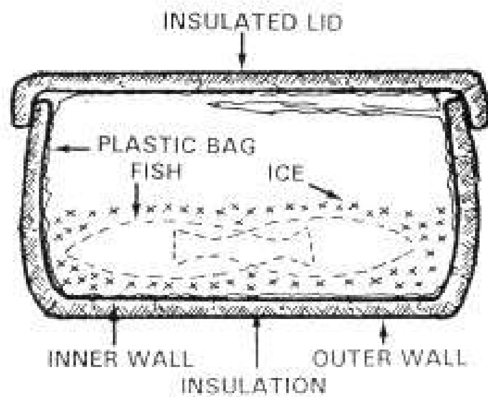
A serious constraint in many artisanal fisheries is the relatively high cost of industrial containers and the difficulty in finding appropriate industrial materials to construct them. For this reason, efforts have been made to develop artisanal containers made from locally available materials (Villadsen et al, 1979; Govindan, 1985; Clucas and Whitehead, 1987; Makene, Mgawe and Mlay, 1989; Wood and Cole, 1989; Johnson and Clucas, 1990; Lupin, 1994).

In some cases, the correct approach could be to add insulation to local fish containers; in other cases it could be necessary to develop a new container. In general, artisanal fish could be cheaper than industrial fish containers, but they will not last as long. An artisanal insulated container developed at Mbegani (Tanzania), based on the local basket container ("tenga") is shown in Figure 7.11.

A key factor in the construction of artisanal insulated containers is the selection of insulation material. There are a number of materials available: inter alia, sawdust, coconut fibre, straw, rice husks, dried grass, old tires and rejected cotton.

However, the use of such materials presents problems: the materials become wet very quickly (with the exception of old tires), losing their insulating capacity and increasing the weight of the container. When wet, most of them tend to rot very quickly. The solution is to put them inside a plastic bag (waterproof); however, in this case they tend to settle, leaving part of the walls without insulation.

With a view to overcoming these problems, the concept of "insulated pillows" was developed in various FAO/DANIDA fish technology workshops. This concept is very simple: the insulating material (e.g., coconut fibres) is placed inside one plastic tube of the type usually found to produce ordinary small polyethylene bags (10 cm in diameter); the insulating material is pressed before sealing the tube; the tube is sealed by heat at both ends (e.g., every 20 cm), and with some practice it is possible to produce a strip of "pillows". It is advisable to utilize a second tube to reduce the incidence of punctures due to fish spines and bones.



(a)

(b)

Figure 7.11 (a) Sketch of an artisanal insulated container (the "Mbegani fish container") developed and utilized in Tanzania; (b) The "Mbegani fish container" on a bicycle to distribute fresh fish. This container was initially developed at the FAO/DANIDA National Workshop on Fish Technology and Quality Control, held at Mbegani, Tanzania, May-June 1984

The strip of "insulated pillows" can then be placed between the internal and the external walls of the container. Once the container is finished with an insulated lid and handles, fish and ice can be put in a large resistant plastic bag, as shown in Figure 7.11 (a). The use of the plastic bag extends the lifespan of the container and improves fish quality.

This example indicates the type of practical problems found when developing an artisanal insulated fish container, and the possible solutions.

Why is ice not always used to chill fish when necessary

Despite the knowledge on the advantages of fish chilling, ice it is not as widely used as it should be, particularly at artisanal level in developing countries. Which are the main problems found in practice? Some of the problems that can be found are as follows:

(i) Ice should be produced mechanically

This obvious statement implies, *inter alia,* that it is not possible to produce ice artisanally for practical purposes (machines and energy are required). To produce ice under tropical conditions, from 55 to 85 kWh/l ton of ice (depending on the type of ice) are necessary whereas, in cold and temperate countries from 40 to 60 kWh are required for the same purpose. This may be a large power requirement for many locations in developing countries, particularly in islands and places relatively far from large cities or electricity networks. Ice plants require maintenance and hence trained people and spare parts (in many cases this requires access to hard currency).

A cold chain will also require chill rooms (onboard and on land), insulated containers, insulated trucks and other auxiliary equipment (e.g., water treatment units, electric generators). Besides increasing the cost, all this equipment will increase the technological difficulty associated with the fish cold chain.

(ii) Ice is produced and used within an economic context

In developed countries ice is very cheap and costs only a fraction of the price of fresh fish. In developing countries ice is very often expensive when compared with fresh fish prices.

A survey conducted in 1986 by the FAO/DANIDA Project on Training on Fish Technology and Quality Control on current fish and ice prices in fourteen African countries demonstrated that in all cases and for all the fish species, I kg of ice increased the fish price at least twice the rate recorded in developed countries. The cheaper the fish the worse the situation. For instance, in the case of small pelagics, the percentage of increase in the fish cost per kilogramme of ice added, was 40% for the "yaboy" of Senegal, 16-25% for the sardinella of Congo, and 66 % for the sardinella of Mauritania and the anchovy of Togo. The market price for fish, in this case, acts as a deterrent for the use of ice.

According to the relative cost of ice to fish, ice may or may not be used. For instance, in Accra, Ghana in 1992, it was found that using ice to chill small pelagics (Ghanian herring) in a proportion of 2 kg ice: 1 kg fish would increase the cost of fish by 32-40%. However, in the case of snapper, for the same ratio of ice to fish the cost increase would be in the range of 4.5-5.7%. The result is that ice chilling of snapper is relatively common in Accra, whereas ice is not utilized to chill small pelagics.

Very often fish compete with other sources of demand (soft drinks, beer), even if the ice machine was initially installed to supply ice for chilling fish. This and energy losses at the ice plants contribute to increase the market price of ice.

In addition to producing and utilizing ice on a sustainable basis, economic aspects must be considered (e.g., depreciation, reserves, investment). Moreover, in the case of ice manufacture there is a strong influence of the scale of production. Low ice prices in developed countries are also the result of large ice plants located at the fishing harbours that supply a large number of companies and fishing boats.

(iii) Practical constraints

Introduction of ice into fish handling systems that are not accustomed to using it can create practical problems. For instance, from Table 7.1 it is clear that the introduction of ice will increase the volume required for storage and distribution, and will reduce the effective fish hold in vessels. The use of ice will also increase the weight to be handled. This will have a number of implications such as an increased workload for the fishermen, fish processors and fishmongers, and an increase in costs and investment.

From Figures 7.3 and 7.4 it is clear that the total amount of ice needed per 1 kg of fish, in the complete cycle from the sea to the consumer will be much higher in tropical countries than in cold and temperate regions. As an indication, the average consumption of ice in the Cuban fishery industry was estimated at around 5 kg of ice per 1 kg of fish handled (including ice losses), although higher values (up to 8-10 kg of ice per 1 kg of fish) have been recorded in single industries in tropical countries; this necessitates large storage and transport capacities.

Freshwater or seawater utilized for producing ice should comply with standards (microbiological and chemical) for potable water and should be readily available in the volumes required. This is not always possible particularly in countries with energy problems (blackouts) and without (or with erratic) public tap- water distribution. If water has to be treated, this implies additional costs and additional equipment to operate and maintain.

Properly trained personnel are required to operate the ice plant and auxiliary equipment efficiently, and to handle ice and fish properly. Although many developing countries have made efforts to train people, in many cases there is a lack of technical personnel ranging from well trained fish technologists to refrigeration mechanics or electricians, or simply plant foremen.

Moreover, in many developing countries it is increasingly difficult to keep technical and professional schools operating in this field, thus jeopardizing the possibility of self-sustained training, and hence fishery industry developments.

(iv) Ice is not an additive

Knowledgeable people (e.g., fishmongers) are quickly aware of the fact that ice is not an additive. Therefore, when there is a delay in icing, ice is not usually utilized (even if available) because it will not improve fish quality. Consumers could also be intuitively aware of this fact, and they prefer to be presented with the fish as it is (e.g., at the terminal state of its quality) rather than in ice, because in this case ice will increase the price of fish but not enhance its quality. Due to the above and to the problems associated with the transition between artisanal and industrial or semi-industrial fisheries, already discussed, consumers in some countries (e.g., in Saint Lucia and Libya) tend to believe that iced fish is not fresh fish.

A need for chilled fish can develop if a market for iced fish (not just a market for "fresh fish") is developed, and to develop a market for iced fish where it does not already exist may be a very difficult and expensive endeavour as is the introduction of any other food product.

(v) Need for appropriate fish handling technologies

To chill and keep fish with ice is a very simple technique. A more complicated picture emerges when actual fish handling systems are analysed, including the economic aspect.

From a comparative study on the same fish handling operation, utilizing ice and insulated containers, carried out in both a developed and a developing country, it was seen that in developed countries, the more "appropriate" technology would aim at reducing wage costs (e.g., chutes to handle ice and fish, special tables to handle containers and boxes and conveyors to move them, machines that mix ice and fish automatically); in developing countries the main concern would be to reduce ice consumption, and to increase the fish : ice ratio in the containers (Lupin, 1986 b).

The same study found that a twentyfold difference in wage costs between developing countries and developed countries cannot offset a tenfold difference in the cost of ice. There is no "comparative advantage" in low wages in developing countries with regard to fresh fish handling. Advanced technology on fish handling from developed countries could make work easier for people in developing countries, but might not improve the economics of the operation as a whole.

There is obviously no single solution to the problems discussed above. However, it is clear that it is the problem to be solved in the coming decade in the field of fresh fish handling. With total catches having reached a plateau, losses due to the lack of ice utilization could be ill-afforded, and developing countries and artisanal fishermen in particular should not be deprived of potential market opportunities.

7.3 Improved catch handling in industrial fisheries

The aims of modern catch handling are the following:

- to maximize the quality of the landed fish raw material. It is of particular importance to provide a continuous flow in handling and to avoid any accumulation of unchilled fish, thereby bringing the important time-temperature phase under complete control.
- to improve working conditions onboard fishing vessels by eliminating those catch handling procedures which cause physical strain and fatigue to such a degree that no fishermen need to leave their occupation prematurely for health reasons.
- to give the fisherman the opportunity to concentrate almost exclusively on the quality aspects of fish handling.

To meet these aims, equipment and handling procedures that will eliminate heavy lifting, unsuitable working positions and rough handling of fish must be introduced. By doing so, the catch handling time is accelerated and the chilling process initiated much earlier than was previously the case (Olsen, 1992). The typical unit operations in catch handling are shown in Figure 7.12.

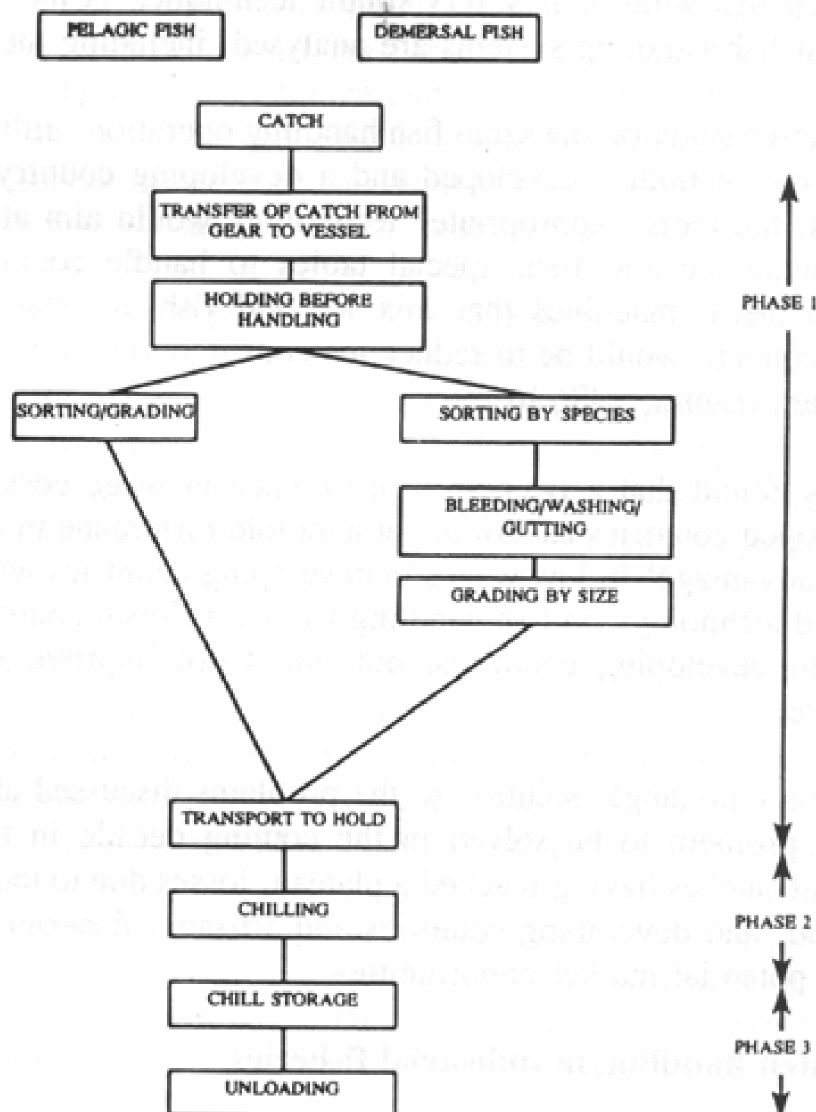


Figure 7.12 Typical unit operations in catch handling of pelagic and demersal fish

Important general aspects in modern catch handling are:

- phase one, which covers the time used for the necessary handling onboard, i.e., the time until the fish is placed in chilling medium, must be as short as possible. The fish temperature at time of capture can be high with consequent high spoilage rate.
- phase two - the chilling process - must be arranged so that a fast chilling rate is obtained for the whole catch. Maximum chilling rate will be obtained by a homogeneous mixing of fish and ice, where the individual fish is completely surrounded by ice and the heat transfer therefore is maximum, controlled by the conduction of heat through the meat to the surface. This ideal situation can be obtained during chilling of small pelagics in a chilled seawater (CSW) system; but by chilling demersal food fish in boxes with ice it is not always possible to obtain homogeneous fish/ice mixing. However, the appearance of fish completely surrounded by ice is often deteriorated due to discolorations and impression-marks. In practical life, icing is therefore often done by placing a single layer of fish on top of a layer of ice in the box even if it is bad practice from a temperature control and therefore shelf life point of view. Cooling is primarily achieved by melt-water dripping from the box stacked on top. This type of chilling will only function satisfactorily if fish boxes are shallow and have a perforated bottom.
- in phase three, which covers the chilled storage period, it is important that a homogeneous temperature at -1.5°-0°C is maintained in the fish until first hand sale. As this period may be extended for several days, this aspect has top priority.

Catch handling can be done in several ways ranging from manual methods to fully automated operations. How many operations will be used in practice and the order in which they are done depends on the fish species, the fishing gear used, vessel size, duration of the voyage and the market which has to be supplied.

Transferring catch from gear to vessel

Midwater trawlers and purse seiners fishing pelagic fish use tackling in lifts of up to 4 t, pumping or brailing for bringing the catch onboard. When lifting huge hauls (100 t or more) onboard by these methods, the danger of losing fish and gear always exists if the fish start to sink after having been brought to the surface. The speed of which the fish may sink depends on the species, catching depth and weather conditions during hauling.

Pumping the catch onboard using submersible pumps without bruising the fish can be difficult, as it is not easy to control the fish-to-water ratio during pumping.

In recent years, the so-called P/V pump (P/V - pressure/vacuum) has found increasing use. The P/V-pump principle is that an accumulation tank of 500-1500 1 size is alternately put under vacuum and pressure by a water-ring vacuum-pump (Figure 7.13). The fish, together with some water, are sucked through a hose and a valve into the tank of the system. When the tank is full, it is pressurized by changing the vacuum and pressure side connections from the tank to the pump and the fish/water mix flows through a valve and a hose into a strainer. The P/V-pump is claimed to handle the fish more gently than other fish pump types, but the capacity is generally lower, mostly because of the alternating way of operations. This problem can be solved by having two P/

V-tanks running in phase opposition using only one vacuum-pump.

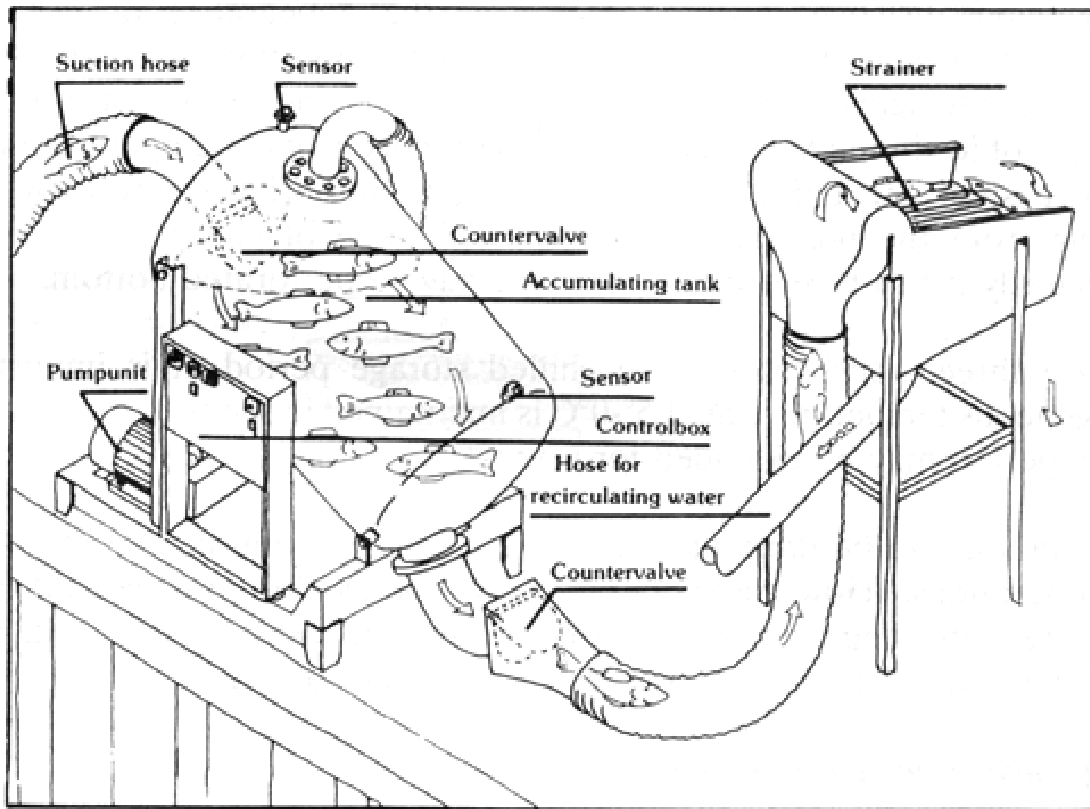


Figure 7.13 Working principle of a P/V pump

Small gillnetters (10-15 m) haul the nets with the net hauler, and very often store their catch in the net until landing. Here the net is drawn through a net shaker by two men in order to free the fish from the gear. It has been shown that the violent way in which the shaker works can be harmful to the men's hands, arms and shoulders. Ergonomic precautions have therefore been suggested to overcome this problem.

Trawlers and seiners (Danish and Scottish) tackle the catch into pounds. Commonly used pounds are those with a raised bottom which can be hoisted hydraulically. The purpose of these designs is to provide good working conditions for the crew (Figure 7.14). Also gillnetters may use a work-saving pound system, which is often connected with a conveyor to bring fish to the gutting-table.

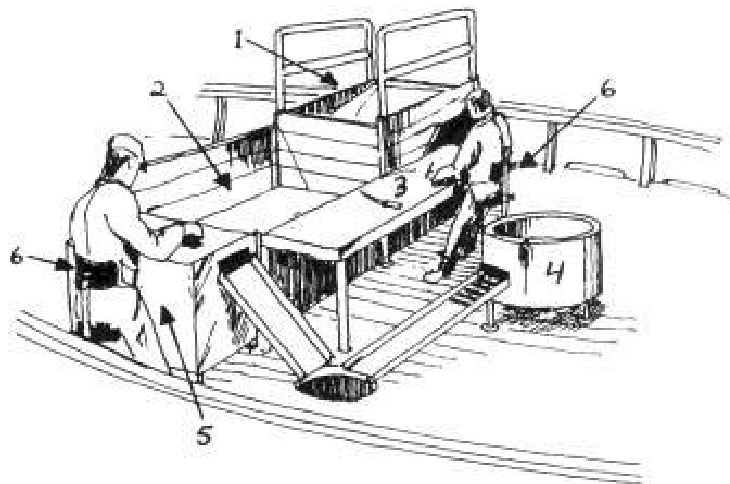


Figure 7.14 Deck lay-out for trawler using machine gutting of demersal fish
1. Tackle pound, 2. Hoisting pound, 3. Gutting table, 4. Bleeding/washing machine, 5. Gutting machine, 6. Chair.

Holding of catch before handling

When large catches are to be handled, or if for other reasons catch handling cannot start immediately, it is convenient and necessary to prechill the catch during holding in deck-pounds using ice or in tanks using Refrigerated Sea Water (RSW) or a mixture of ice and sea water (Chilled Sea Water, CSW).

Prechilling holding systems are mostly used on pelagic trawlers which grade their catches in size before storing in boxes or in portable CSW-containers. It is also essential to prechill when the pelagic fish are soft and feeding and therefore very prone to bellyburst. Prechilling tanks are unloaded by elevator or P/V-pumps. If no sorting is done onboard, the fish is conveyed directly for chilled storage in the hold. A system for holding demersal fish in tanks is shown in Figure 7.15.

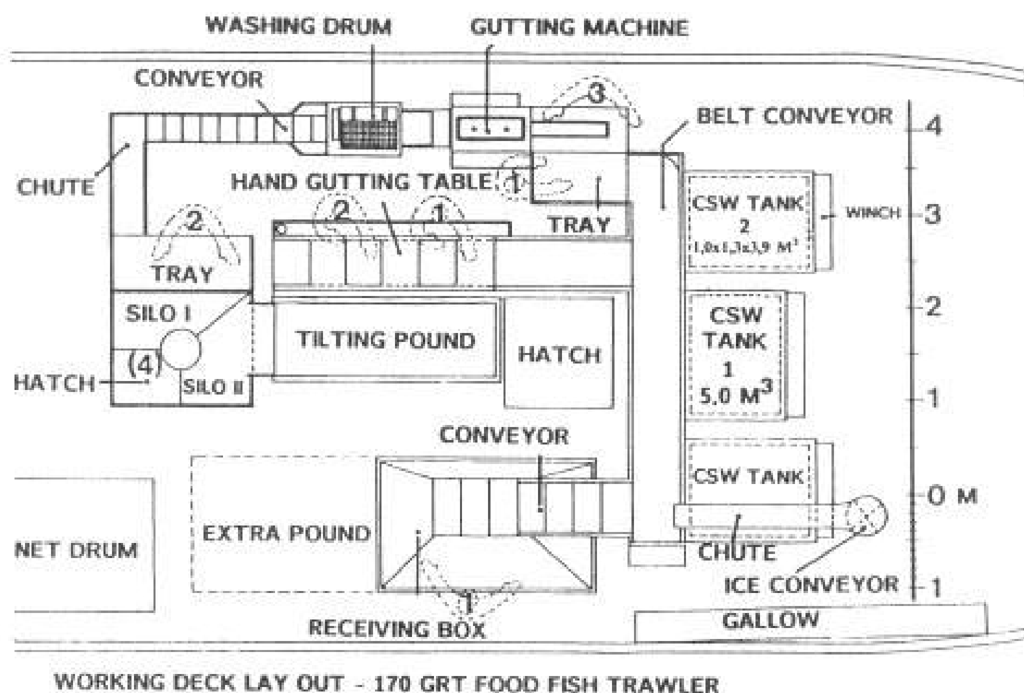


Figure 7.15 System comprising CSW raw material holding tanks before manual or machine gutting of fish

Sorting/grading

Pelagic fish are sometimes sorted or graded onboard according to size. The equipment used operates on the basis of thickness of fish using principles such as:

- vibrating, inclined diverging bars
- contrarotating, inclined, diverging rollers
- diverging conveyors where fish are being transported along a power driven V-belt.

Grading by thickness can meet the demand for the high capacity needed in pelagic fish handling, but it is generally accepted that the correlations between thickness and length or weight are not too good (Hewitt, 1980). The most important point, often forgotten, for making a grader function at its optimum is even feeding. This could be done with an elevator delivering to a (vibrating) water sprayed chute leading to the inlet guide chute of the grading machine.

Sometimes it is necessary to install a manual sorting conveyor before the grading machine for removal of larger fish and debris, e.g., in the fishery for argentine with by catch of grenadier.

Sorting and grading of demersal fish by species and by size is normally done by hand. However, some automatic systems of sorting according to width are in use. Static or dynamic weighing by marine weighing systems are also in use with good results. Research is under way using a computerized vision system for species and size grading.

Bleeding/gutting/washing

In order to obtain optimal quality in a white fillet, many white-fleshed demersal fish (but not all) need to be bled and gutted immediately after capture. The best procedures from an economic, biological and practical point of view are still under discussion (see section 3.2 on bleeding and section 6.4 on gutting).

The vast majority of fishermen are handling the fish in the easiest and also the fastest way, which means the fish are bled and gutted in one single operation. This may be done manually, but gutting machines have been introduced to obtain even more speed. The fish are transported to and from the fisherman by suitable conveyor systems. Using machines, round fish can be gutted with a speed of approximately 55 fish/minute for fish length up to 52 cm and 35 fish/minute for fish length up to 75 cm. Gutting by machine is 6-7 times faster than hand-gutting.

Existing gutting machines for round fish of the type using a circular saw blade for cutting and removing the guts destroy the valuable roe and liver. A new type of gutting machine which copies the manual gutting procedure is now available on the market. Gutting speed of this machine is 35-40 fish/minute and the roe and liver can be saved (Olsen, 1991). Flatfish can also be gutted by a recently developed machine. The speed of this machine is about 30 fish/minute.

After gutting, the fish are conveyed to the washing or bleeding operation. This may be done in pounds, often with raised bottom or in special bleeding tanks, frequently with a hydraulically-operated tilting system and rotating washing drums are also used (Figure 7.15); and special equipment such as the Norwegian and British fish washer may be used.

After catch handling (sorting, grading, gutting, etc.), the fish may be passed to an intermediate storage silo or batch holding system for the different sizes or grades before being dropped by chute to the hold, or the chutes may lead directly from the grading machines to the hold (Figure 7.16).

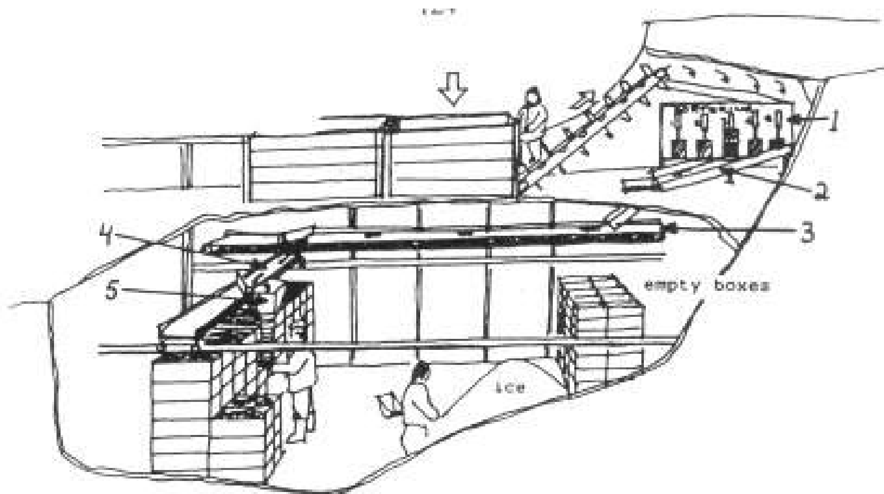


Figure 7.16 "Polar"-system. Mechanized sorting and boxing of herring 1. Herring sorting machine, 2,3,4. Conveyors, 5. Flexible dosing tube.

Chilling/chilled storage

Demersal fish have traditionally been stored on shelves or in boxes. Boxing has a big advantage over shelf storage as it reduces the static pressure on the fish and also facilitates unloading.

Shelf storage is done by alternating layers of ice and fish from one layer of ice and fish (single shelving 25 cm between shelves) up to ice/fish layers 100 cm deep. In practice, shelving often allows better temperature control than boxing and therefore also a longer storage life. Because excessive handling during unloading and excessive pressure on the fish have- a negative effect on quality, e.g., appearance, boxing is preferable to shelving, given proper icing.

In pelagic fisheries, boxed fish will be untouched until processed, but in demersal fisheries the catch is often only sorted by species onboard and not graded by size and weighed. These operations are carried out after landing before auction whereby some of the handling and quality advantages of boxing are lost.

In the near future when integrated quality assurance systems have been introduced, these unit operations will be carried out onboard and an informative label on each box will give details of factors of importance for first-hand sale (including freshness).

In general, two types of plastic fish boxes are used: stack-only and nest/stack boxes (Figures 17 a and 17 b).

To overcome some of the space problems in using stack-only boxes, the nest/stack type has been developed. These occupy only approximately a third of the space needed when stored empty compared to when the boxes are loaded with fish and ice.

Fig. 7.17 a Stack-only boxes

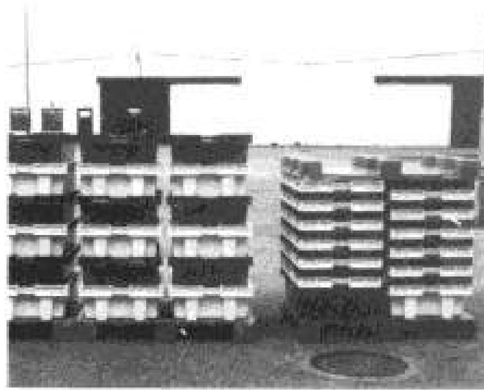


Figure 7.17 b Nest/stack boxes

This type of box is widely used in France, the Netherlands and Germany and also in some Danish ports. When a system tailor-made for a certain type of plastic box is designed, the quality advantages of using boxes can be fully utilized onboard. The key points to consider are:

1. The handling rate necessary to prevent quality loss because of delayed icing. Prechilling can be of advantage to compensate lack in handling rate.
2. Handling methods which make it possible to guarantee that the icing procedure is sufficient to chill the fish to 0°C and maintain this temperature until landing.
3. The hold construction must be constructed such that safe and easy stacking of the boxes can take place.
4. Hold insulation of a relatively high quality should be considered. A small mechanical refrigeration plant can be of advantage. Air temperature in the hold should be + 1°-3°C

RSW-storage (Refrigerated Sea Water is a well established practice which has been refined both theoretically and practically since its introduction in the 1960s in Canada where it was developed for salmon and herring storage (Roach et al, 1967). At the beginning, most RSW vessels were salmon- packers and because of some failures in design which were attributed either to insufficient refrigeration or circulation systems, a standard for control of RSW-systems was established. Since vessels are different, the RSW-installation has to be studied carefully in every fishery to determine its real capability. Therefore, methods for rating each individual system and vessel and providing general specifications and guidelines for the proper installation have been suggested by the Canadian technicians (Gibbard and Roach, 1976).

In order to obtain maximum shelf life from RSW-systems, temperature homogeneity in the region of -1°C is very important. The factors affecting temperature homogeneity were recently studied in Denmark (Kraus, 1992). The most important conclusions were that the inflow of the chilled seawater in the bottom of the tank must take place over the whole tank bottom area, and that filling capacity for securing water circulation and temperature homogeneity is dependent on fish species. The necessary chilling rate was suggested to be: fish temperature must be below 3°C within four hours and below 0°C after 16 hours, and the temperature should be kept between -1.5°C and 0°C until unloading.

The CSW system has also been developed in Canada as a much cheaper means an investment point of view - to obtain rapid uniform chilling of fish. The most popular method used is the so-called "Champagne" method where rapid heat transfer between fish and ice is obtained by agitation with compressed air introduced at the bottom of the tanks, instead of using circulation

pumps as in RSW and some earlier CSW designs (Figure 7.18) (Kelmann, 1977; Lee, 1985). An indication of the chilling rate for herring could be: reduction of fish temperature from 15°C to 0°C within two hours. The concept of a CSW system is to load well insulated tanks at the harbour with the amount of ice necessary to chill the catch to between 0' and - 1°C and maintain this temperature until unloading.

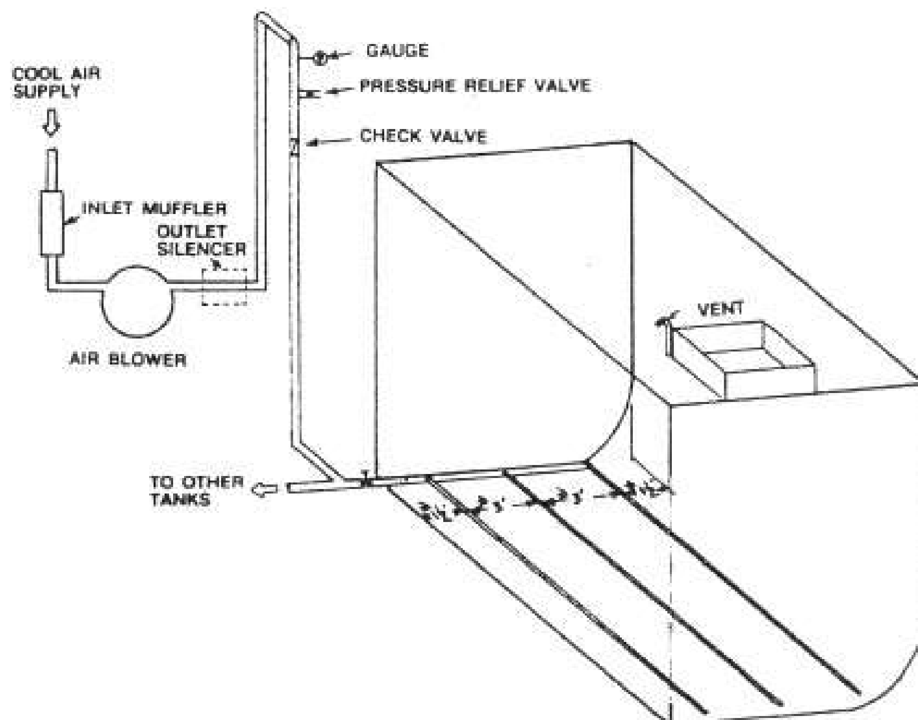


Figure 7.18 Chilled seawater system: piping layout

The Canadian west-coast fishermen are achieving this in practice by using a minimum of seawater when they start loading the tank and by forcing air through the ice-sea-water-fish-mixture only during loading, and stop forcing air immediately when the tankis full. Thereafter they will force the air only for 5-10 minutes with 3-4 hours' interval. The air agitation therefore only serves as a method to overcome local temperature differences in the tank. The objective is to obtain a uniform mixture of fish and ice in order to secure temperature homogeneity.

A proven rule-of-thumb for estimating the amount of ice necessary is simply to observe the amount of ice left in the tank at unloading, and compare it with temperature readings, which should be in the -1°C range measured in the landed fish. The starting situation should be conservative, which at sea-temperature around 12-14°C, for a trip lasting 7 days and with 10 cm polyurethane insulation, is 25% ice by weight of the tank capacity. The amount of ice is adjusted according to the observations on the following trips.

An analytical approach to estimate necessary ice quantities in a CSW tank system has been developed. The quantity of ice required takes into consideration tank size, catch volume, time at sea, water temperature, hold insulation and hold flooding strategy (Kolbe *et al.,* 1985).

CSW "Champagne" systems can also be used in small coastal vessels, e.g., in a fishery for small pelagic fish with vessels of 10-14 m length with a fish carrying capacity from 3 to 10 t fish (Roach, 1980).

Another way of loading a CSW tank, which is in practical use in Denmark, is to add the necessary amount of ice to the fish during loading by mixing a controlled stream of fish with a controlled stream of ice. The greatest amount of ice is added to the fish during loading. When the tank is full the voids are filled with seawater from a hose and the tank is left undisturbed, except for watercirculation by pumping or compressed air blowing for 5-10 minutes of 4-hour intervals. The ice is bulk-stored in the forward hold and the ice is shovelled into a conveyor flush with the floor. The conveyor then leads the ice to the mixing point at the deck.

The use of portable CSW containers for pelagic fish handling was tested in the early 1970s (Eddie and Hopper, 1974). The approximately 2 m^3 heat insulated containers were loaded with the necessary amount of ice from the harbour and agitated with compressed air in a similar way as for CSW-tanks. The main advantages with this method are that the fish will be undisturbed until processed and easily unloaded. The disadvantages are: marketing problems and reduced pay-load on existing vessels (Eddie, 1980). Portable 1.1m^3 CSW containers are used to a limited extent in combination with the earlier mentioned conveyor system originally laid-out for boxing without the above-mentioned reduced pay-load compared to boxing (Anon., 1986). Also, small coastal vessels can use insulated portable CSW containers (Figure 7.19).

Figure 7.19 Some of the 10 pieces of 200 l CSW containers placed on deck on a 15 GRT cod gillnet wooden boat

Unloading

Shelfed fish are unloaded, using baskets or boxes which are filled as the shelves are removed. The fish are tackled from the hold and emptied on a conveyor leading to the manual grading and weighing process.

Boxed fish iced in 20 or 40 kg boxes at sea will normally be unloaded in pallet loads of, for instance, twelve 40 kg boxes per pallet. Swedish boats use hydraulic deck-mounted cranes and a special pallet fork during unloading. An unloading rate of approximately 30 t/h is possible by this method.

Danish coastal vessels, landing their pelagic catches daily, use quay mounted P/V-pumps for

unloading their catches, which often are iced in pens in layers up to approximatly 1 m height. It is necessary only to add small quantities of water to make the pump function properly. The fish is delivered to a strainer from where a conveyer leads the fish to a size grader. The strained water is recirculated to the hold. Grading machines with up to 30 t/h are often installed.

In Scandinavia the 30-50 in RSW/CSW vessels still use brailing to a limited extent when unloading their catches at a rate of 30 to 50 t/h. The main disadvantage of this method is that very big hatches are needed to obtain reasonable unloading rates.

P/V-pumps have recently been introduced for unloading herring and mackerel. Thus vessels with small tanks, e.g., 30 in , and small hatches can also be unloaded at a rate similar to or higher than the above-mentioned brailing rate. P/V-pumping rates will typically be around 40-50 t/h. The fish can be transported directly in a tube system into the factory where representative samples are taken for quality assessment.

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8. ASSESSMENT OF FISH QUALITY

8.1. Sensory methods
8.2. Biochemical and chemical methods
8.3. Physical methods
8.4. Microbiological methods

Most often "quality" refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals. It is important to remember that "quality'' implies different things to different people and is a term which must be defined in association with an individual product type. For example, it is often thought that the best quality is found in fish which are consumed within the first few hours post mortem. However, very fresh fish which are in rigor mortis are difficult to fillet and skin and are often unsuitable for smoking. Thus, for the processor, slightly older fish which have passed through the rigor process are more desirable.

The methods for evaluation of fresh fish quality may be conveniently divided into two categories: sensory and instrumental. Since the consumer is the ultimate judge of quality, most chemical or instrumental methods must be correlated with sensory evaluation before being used in the laboratory. However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, etc., may be reduced.

8.1 Sensory methods

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing.

Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes.

Instruments capable of measuring parameters included in the sensory profile are the Instron, Bohlin Rheometer for measuring texture and other rheologic properties. Microscopic methods combined with image analysis are used to assess structural changes and "the artificial nose" to evaluate odour profile (Nanto et al., 1993).

Sensory process

In sensory analysis appearance, odour, flavour and texture are evaluated using the human senses. Scientifically, the process can be divided into three steps. Detection of a stimulus by the human sense organs; evaluation and interpretation by a mental process; and then the response of the assessor to the stimuli. Variations among individuals in the response of the same level of stimuli can vary and can contribute to a non-conclusive answer of the test. People can, for instance, differ widely in their response to colour (colour blindness) and also in their sensitivity to chemical stimuli. Some people cannot taste rancid flavour and some have a very low response to cold-storage flavour. It is very important to be aware of these differences when selecting and training judges for sensory analysis. Interpretation of the stimulus and response must be trained very carefully in order to receive objective responses which describe features of the fish being evaluated. It is very easy to give an objective answer to the question: is the fish in rigor (completely stiff), but more training is needed if the assessor has to decide whether the fish is *post* or pre-rigor. Subjective assessment, where the response is based on the assessor's preference for a product, can be applied in the fields like market research and product development where the reaction of the consumer is needed. Assessment in quality control must be objective.

Sensory methods

The analytical objective test used in quality control can be divided into two groups: discriminative tests and descriptive tests. Discriminative testing is used to determine if a difference exists between samples (triangle test, ranking test). Descriptive tests are used to determine the nature and intensity of the differences (profiling and quality tests). The subjective test is an affective test based on a measure of preference or acceptance.

Discriminative test
Is there a difference?

- **Triangle test**
- **Ranking**

Descriptive test
What is the difference or the absolute value and how big is it?

- **Quality index method**
- **Structured scaling**
- **Profiling**

Affective test
Is the difference of any significance?

- **Market test**

Figure 8.1 Methods of sensory analysis

In the following, examples of discriminative and descriptive testing will be given. For further information concerning market testing, see Meilgaard *et al.* (1991).

Quality assessment of fresh fish

Quality Index Method

During the last 50 years many schemes have been developed for sensory analysis of **raw fish.** The first modern and detailed method was developed by Torry Research Station (Shewan *et al.*, 1953). The fundamental idea was that each quality parameter is independent of other parameters. Later, the assessment was modified by collecting a group of characteristic features to be expressed in a score. This gives a single numerical value to a broad range of characteristics. In Europe today, the most commonly used method for quality assessment in the inspection service and in the fishing industry is the EU scheme, introduced in the council decision No. 103/76 January 1976 (Table 5.2). There are three quality levels in the EU scheme, E (Extra), A, B where E is the highest quality and below B is the level where fish is discarded for human consumption. The EU scheme is commonly accepted in the EU countries for sensory assessment. There is, however, still some discrepancy as the scheme

does not take account of differences between species into account as it only uses general parameters. A suggestion for renewal of the EU scheme can be seen in Multilingual Guide to EU Freshness Grades for Fishery Products (Howgate *et al.,* 1992),where special schemes for whitefish, dogfish, herring and mackerel are developed (Appendix E).

A new method, the Quality Index Method (QIM) originally developed by the Tasmanian Food Research unit (Bremner *et al.,* 1985)*,* is now used by the Lyngby Laboratory (Jonsdottir, 1992) for fresh and frozen cod, herring and saithe. In the Nordic countries and Europe it has also been developed for redfish, sardines and flounder.

Table 8.1 Quality assessment scheme used to identify the quality index demerit score (Larsen *et al.* 1992)

Quality parameter	Character	Score (ice/seawater)
General appearance	Skin	0 Bright, shining 1 Bright 2 Dull
	Bloodspot on gill cover	0 None 1 Small, 10-30% 2 Big, 30-50% 3 Very big, 50-100%
	Stiffness	0 Stiff, in *rigor mortis* 1 Elastic 2 Firm 3 Soft
	Belly	0 Firm 1 Soft 2 Belly burst
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Musty/sour 3 Stale meat/rancid
Eyes	Clarity	0 Clear 1 Cloudy

	Shape	0 Normal 1 Plain 2 Sunken
Gills	Colour	0 Characteristic, red 1 Faded, discoloured
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Sweaty/slightly rancid 3 Sour stink/stale, rancid
Sum of scores		(min. 0 and max. 20)

QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 4 demerit points (Jonsdottir, 1992). QIM is using a practical rating system, in which the fish is inspected and the fitting demerit point is recorded. The scores for all the characteristics are then summed to give an overall sensory score, the so-called quality index. QIM gives scores of zero for very fresh fish while increasingly larger totals result as fish deteriorate. The description of evaluation of each parameter is written in a guideline. For example, 0 demerit point for the appearance of the skin on herring means very bright skin only experienced in freshly caught herring. The appearance of the skin in a later state of decay turns less bright and dull and gives 2 demerit points. Most of the parameters chosen are equal to many other schemes. After the literal description, the scores are ranked for each description for all the parameters, giving scores 0-1, 0-2, 0-3 or 0-4. Parameters with less importance are given lower scores. The individual scores never exceed 4, so no parameter can excessively unbalance the score. A scheme for herring is shown in table 8.1; it is emphazised that it is neccessary to develop a new scheme for every species (the scheme for cod is seen in Appendix D).

There is a linear correlation between the sensory quality expressed as a demerit score and storage life on ice, which makes it possible to predict remaining storage life on ice. The theoretical demerit curve has a fixed point at (0,0) and its maximum has to be fixed as the point where the fish has been rejected by sensory evaluation of, e.g., the cooked product (see under structured scaling) or otherwise determined as the maximum keeping time. Using cooked evaluation the two parallel sensory tests demand an experienced sensory panel even though this is only required while developing the scheme, and later on it will not be necessary to assess cooked fish in order to predict the remaining shelf life. QIM does not follow the traditionally accepted S-curve pattern for deterioration of chilled fish during storage (Figure 5.1). The aim is a straight line which makes it possible to distiguish between fish at the start of the

plateau phase and fish near the end of the plateau phase (Figure 8.2).

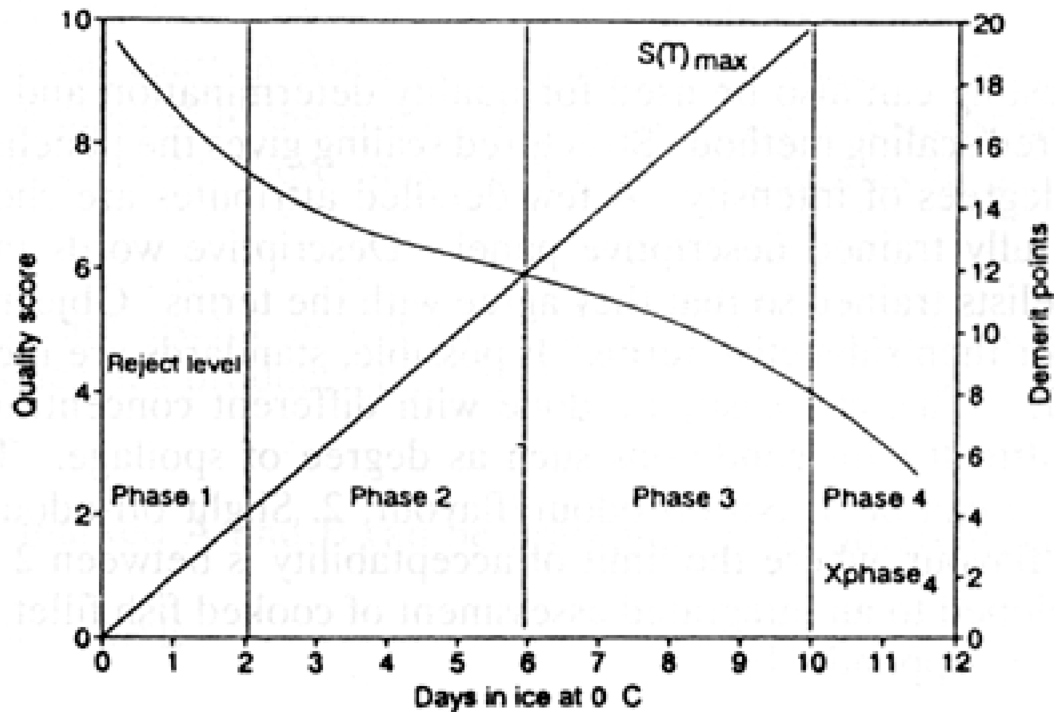


Figure 8.2 Combination of sensory curves for raw S(T) and cooked fish

When a batch of fish in Figure 8.2 reaches a sum of demerit points of 10, the remaining keeping time in ice will be 5 days. To predict remaining shelf life, the theoretical curve can be converted as shown in Figure 8.3.

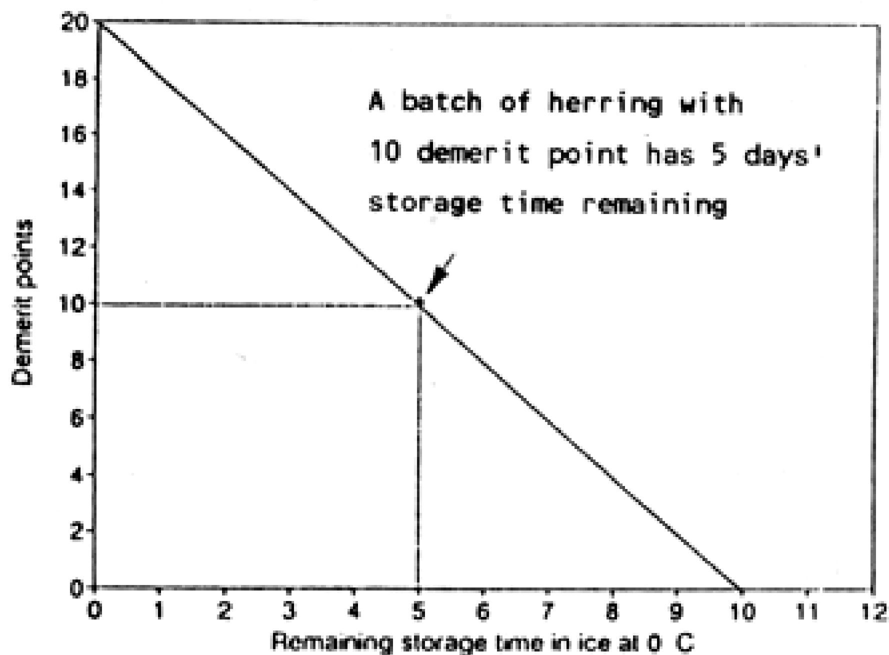


Figure 8.3 A curve to predict the storage time remaining for herring stored in ice or sea water at 0°C

A fish merchant may want to know how long his purchase will remain saleable if the fish are stored on ice immediately. A buyer at a fish market might be interested in the equivalent number of days on ice where the fish have been stored since they were caught, and thus how much marketable time on ice is left. These condition indicators can be extracted for a fish sample with a known rate of change in demerit points using the quality index method.

Structured Scaling

Descriptive testing can also be used for quality determination and shelf life studies applying a structured scaling method. Structured scaling gives the panelist an actual scale showing several degrees of intensity. A few detailed attributes are chosen often based on work from a fully trained descriptive panel. Descriptive words must be carefully selected, and panelists trained so that they agree with the terms. Objective terms should be preferred rather than subjective terms. If possible, standards are included at various points of the scale. This can easily be done with different concentrations of salt but might be more difficult with conditions such as degree of spoilage. The most simple method (Table 8.2) can be 1. No off-odour/flavour, 2. Slight off-odour/flavour and 3. Severe off-odour/flavour, where the limit of acceptability is between 2 and 3. This has been further developed to an integrated assessment of cooked fish fillet of lean and fatty fish (see example in Appendix E).

A 10-point scale is used as described under 5.1 Sensory changes, and an overall impression of odour, flavour and texture is evaluated in an integrated way. For statistics, t-test and analysis of variance can be used (see example in Appendix F).

Table 8.2 Evaluation of cooked fish

		Grade		Score
Acceptable	No off-odour/ flavour	I	Odour/flavour characteristic of species, very fresh, seaweedy Loss of odour/flavour Neutral	10 9 8 7 6
	Slight off-odour/ flavour	II	Slight off-odours/flavours such as mousy, garlic, bready, sour, fruity, rancid	5 4
Limit of acceptability				
Reject	Severe off-odour/ flavour	III	Strong off-odours/flavours such as stale cabbage, NH_3, H_2S or sulphides	3 2 1

Quality assessment of fish products

Assessment of **fishery products** can both be performed as a discriminative test and as a descriptive test.

Triangle test

The most used discriminative test in sensory analysis of fish is the triangle test (ISO standard 4120 1983), which indicates whether or not a detectable difference exists between two samples. The assessors receive three coded samples, are told that two of the samples are identical and one is different, and are asked to identify the odd sample.

Analysis of results from the triangle test is done by comparing the number of correct identifications with the number you would expect to obtain by chance

alone. In order to test this the statistical chart in Appendix A must be consulted. The number of correct identifications is compared to the number expected by use of a statistical table, e.g., if the number of responses is 12, there must be 9 correct responses to achieve a significant answer (1% level).

Triangle tests are useful in determining, e.g., if ingredient substitution gives a detectable difference in a product. Triangle tests are often used when selecting assessors to a taste panel.

The samples marked A and B can be presented in six different ways:

ABB BBA AAB
BAB ABA BAA

Equal numbers of the six possible combinations are prepared and served to the panel members. They must be served randomly, preferably as duplicates. The number of panel members should be no less than 12 (an example of a triangle test from the ISO standard can be seen in Appendix B).

Table 8.3 Example of score sheet: triangle test

TRIANGLE TEST	
Name:	Date:
Type of sample:	
Two of these three samples are identical, the third is different. Examine the samples from left to right and circle the number of the test sample which is different. It is essential you make a choice (guess if no difference is apparent).	
Test sample No.:	
Describe the difference:	

Ranking

In a ranking exercise, a number of samples are presented to the taste panel. Their task is to arrange them in order according to the degree to which they exhibit some specified characteristics, e.g, downward concentration of salt. Usually ranking can be done more quickly and with less training than evaluation by other methods. Thus ranking is often used for preliminary screening. The method gives no individual differences among samples and it is not suited for sessions where many criteria have to be judged simultaneously.

Profiling

Descriptive testing can be very simple and used for assessment of a single attribute of texture, flavour and appearance. Methods of descriptive analysis which can be used to generate a complete description of the fish product have also been developed. An excellent way of describing a product can be done by using flavour profiling (Meilgaard et al., 1991). Quantitative Descriptive Analysis provides a detailed description of all flavour characteristics in a qualitative and quantitative way. The method can also be used for texture. The panel members are handed a broad selection of reference samples and use the samples for creating a terminology that describes the product.

In Lyngby a descriptive sensory analysis for fish oil using QDA has been developed. A trained panel of 16 judges is used. Descriptive terms such as paint, nutty, grassy, metallic are used for describing the oil on an intensity scale. A moderately oxidized fish oil is given fixed scores and used as a reference.

Table 8.4 Profile of fish oil

Taste	Std					
Fresh fish	2					
Amine	1					
Oily	3					
Sweet	2					
Metallic	3					
Grassy	3					
Painty	2					
Fruity	2					

Remarks						
Taste as a whole (0 unacceptable - 9 neutral)	6					

Advanced multivariate analysis is used for statistics and makes it possible to correlate single attributes to oxidative deterioration in the fish oil. The results can be reported in a "spider's web" (se Figure 8.5). The panel uses an intensity scale normally ranging from 0 to 9.

Profiling can be used for all kinds of fishery products, even for fresh fish when special attention is placed on a single attribute.

The results of QDA can be analyzed statistically using analysis of variance or multivariate analysis (O'Mahony, 1986).

Statistics

In any experiment including sensory analysis the experimental design (e.g., number of panel members, number of samples, time aspects, hypotheses to test) and statistical principles should be planned beforehand. Failure to do so may often lead to insufficient data and non-conclusive experiments. A guide to the most used statistical methods can be seen in Meilgaard *et al.* (1991). A panel used for descriptive testing shall preferably consist of no less than 8-10 persons, and it should be remembered that the test becomes statistically much stronger if it is done in duplicate. This can often be difficult using sensory analysis on small fish. Thus the experiment must include a sufficient number ofsamples to remove the sources of variability, and the testing must be properly randomized. For further information see O'Mahony (1986) and Smith (1989).

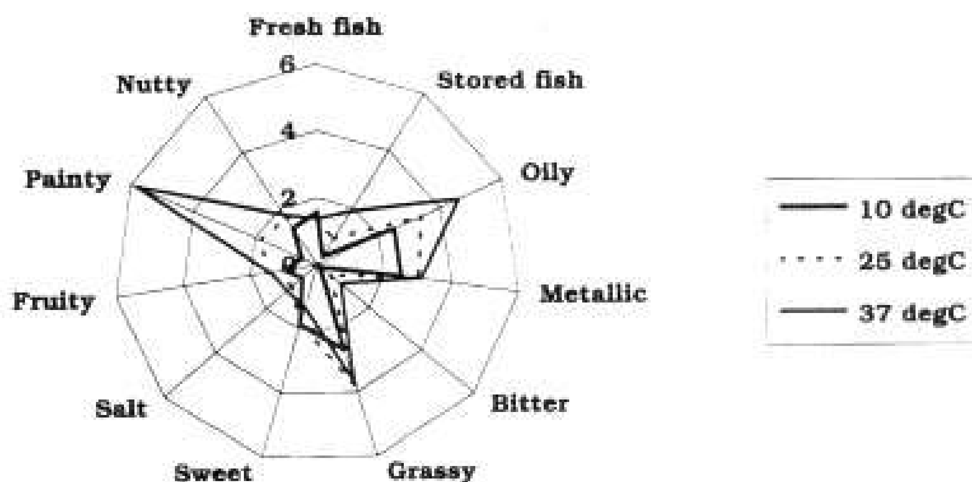


Figure 8.4 Flavour profiles of a fish oil after 2 weeks of storage at various temperatures (Rorbaek et al., 1993)

Training of assessors

Training of assessors for sensory evaluation is necessary in almost all sensory methods. The degree of training depends on the difficulty and complexity of the assessment. For example, for profiling a thorough training with presentation of a large range of samples is necessary in order to obtain proper definitions of the descriptors an equal use of the scoring system. The triangle test normally requires a minor degree of training.

Sensory quality control is often done by a few persons either at the fish market when buying fish or at quality inspection. The experience of these persons allows them to grade the fish. Starting as a fish inspector it is not necessary to know all the different methods of sensory assessment described in textbooks (Meilgaard et al., 1991), but some of the basic principles must be known. The assessor must be trained in basic tastes, the most common fish taste and must learn the difference between off- flavour and taints. This knowledge can be provided in a 2- day basic training course.

In bigger companies and for experimental work a further training of a sensory panel is necessary in order to have an objective panel. A laboratory panel must have 8-10 members, and the training and testing of panel members must be repeated regularly.

Facilities

The facilities required for sensory evaluation is described in textbooks on sensory evaluation.

The minimum requirement for evaluation is a preparation room and a room where the samples are served. The rooms should be well ventilated and provided with a good light (Howgate, 1994). There must be enough space on the tables for inspection of raw samples of fish.

Cooking and serving

The samples of fishery products should not be less than 50-100 g per person. Fillets can be served in loins and should be cooked to an internal temperature of 65°C. The samples should be kept warm when served, i.e., in insulated containers or on a hot plate. The fish can be heat treated by steaming in a

water bath, packed as boiled-in-the-bag in a plastic pouche or in alufoil. An oven (microwave or steam-oven) can also be used for heat treatment. The fish can be packed in plastic or put on a small porcelain plate covered with alufoil. For cod loins (2,5x1,5x6cm) on a porcelain plate covered with alufoil the heating time in a steam-oven (convectomate) at 100°C must be 10 minutes. The samples should be coded before serving.

8.2 Biochemical and chemical methods

The appeal of biochemical and chemical methods for the evaluation of seafood quality is related to the ability to set quantitative standards. The establishment of tolerance levels of chemical spoilage indicators would eliminate the need to base decisions regarding product quality on personal opinions. Of course, in most cases sensory methods are useful for identifying products of very good or poor quality. Thus, biochemical/ chemical methods may best be used in resolving issues regarding products of marginal quality. In addition, biochemical/ chemical indicators have been used to replace more time consuming microbiological methods. Such objective methods should however correlate with sensory quality evaluations and the chemical compound to be measured should increase or decrease with the level of microbial spoilage or autolysis. It is also important that the compounds to be measured must not be affected by processing (e.g., breakdown of amines or nucleotides in the canning process as a result of high temperatures).

The following is an overview of some of the most useful procedures for the objective measurement of seafood quality. Woyewoda *et al.* (1986) have produced a comprehensive manual of procedures (including proximate composition of seafood).

Amines - Total Volatile Basic Amines

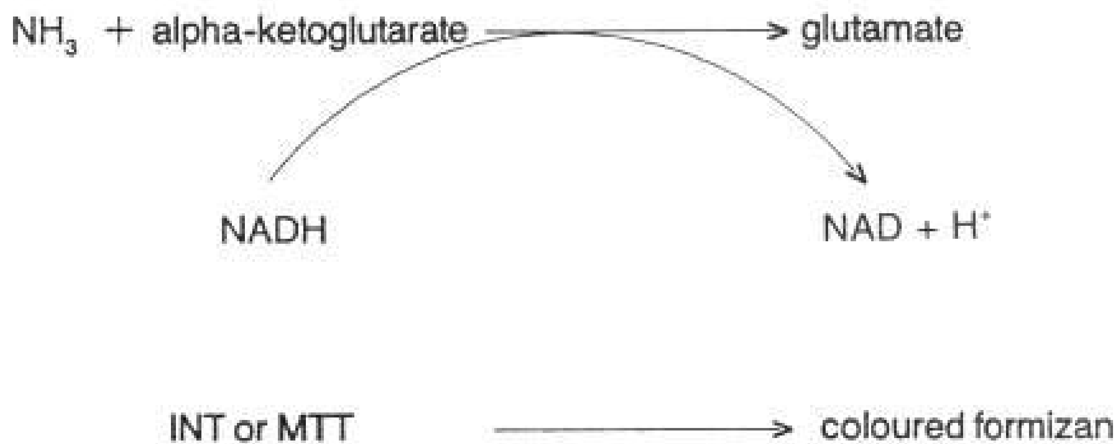
Total volatile basic amines (TVB) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of cod as well as several other species (Rehbein and Oehlenschlager, 1982). They are particularly useful for the measurement of quality in cephalopods such as squid (LeBlanc and Gill, 1984), industrial fish for meal and silage (Haaland and Njaa,

1988), and crustaceans (Vyncke, 1970). However, it should be kept in mind that TVB values do not reflect the mode of spoilage (bacterial or autolytic), and results depend to a great extent on the method of analysis. Botta *et al.* (1984) found poor agreement among six published TVB procedures. Most depend upon either steam distillation of volatile amines or microdiffusion of an extract (Conway, 1962); the latter method is the most popular in Japan. For a comparative examination of the most common procedures for TVB analysis, see Botta *et al.* (1984).

Ammonia

Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino- acids. It is also produced in the autolytic breakdown of adenosine monophosphate (AMP, Figure 5.4) in chilled seafood products. Although ammonia has been identified as a volatile component in a variety of spoiling fish, few studies have actually reported the quantification of this compound since it was impossible to determine its relative contribution to the overall increase in total volatile bases.

Recently, two convenient methods specifically for identifying ammonia have been made available. The first involves the use of the enzyme glutamate dehydrogenase, NADH and alpha-ketoglutarate. The molar reduction of NH_3 in a fish extract yields one mole of glutamic acid and NAD which can be monitored conveniently by absorbance measurements at 340 nm. Test kits for ammonia based on glutamate dehydrogenase are now available from Sigma (St. Louis, Missouri, USA) and Boehringer Mannheim (Mannheim, Germany). A third type of ammonia test kit is available in the form of a test strip (Merck, Darmstadt, Germany) which changes colour when placed in contact with aqueous extracts containing ammonia (ammonium ion). LeBlanc and Gill (1984) used a modification of the glutamate dehydrogenase procedure to determine the ammonia levels semi-quantitatively without the use of a spectrophotometer, but with a formazan dye, which changed colour according to the following reaction:



where INT is iodontrotetrazolium and MTT is 3 - [4,5-dimethylthiazol-2-yl] 2,5 diphenyl tetrazolium bromide

Ammonia has been found to be an excellent indicator of squid quality (LeBlanc and Gill, 1984) and comprised a major proportion of the TVB value for chilled short-finned squid (Figure 8.7). However, ammonia would appear to be a much better predictor of the latter changes in quality insofar as finfish are concerned. LeBlanc (1987) found that for iced cod, the ammonia levels did not increase substantially until the sixteenth day of storage. It would appear that at least for herring, the ammonia levels increase far more quickly than trimethylamine (TMA) levels which have traditionally been used to reflect the growth of spoilage bacteria on lean demersal fish species. Thus ammonia has potential as an objective quality indicator for fish which degrades autolytically rather than primarily through bacterial spoilage.

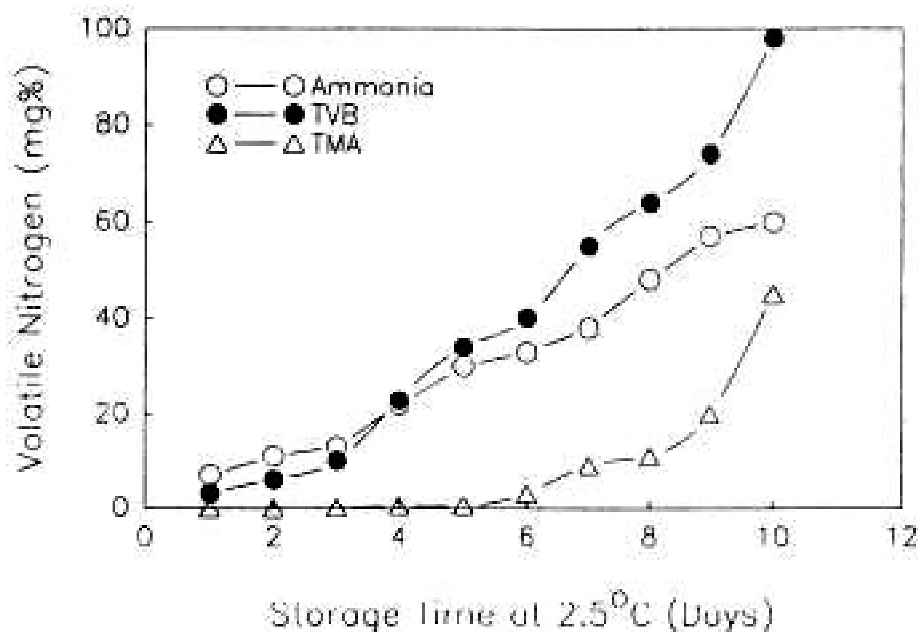


Figure 8.7 Effect of storage time on production of ammonia. TVB and TMA in

short finned squid *(Illex illecebrosus),* adapted from Gill (1990)

Trimethylainine (TMA)

Trimethylamine is a pungent volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. Although TMA is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good. This phenomenon is now thought to be due to the presence of small numbers of "specific spoilage" bacteria which do not always represent a large proportion of the total bacterial flora, but which are capable of producing large amounts of spoilage -related compounds such as TMA. One of these specific spoilage organisms, *Photobactetium phosphoreum,* generates approximately 10 - 100 fold the amount of TMA than that produced from the more commonly-known specific spoiler, *Shewanella putrefaciens* (Dalgaard, 1995) (in press).

As mentioned above, TMA is not a particularly good indicator of edibility of herring quality but is useful as a rapid means of objectively measuring the eating quality of many marine demersal fish. The correlations between TMA level or more preferably, TMA index (where TMA index = log (1 + TMA value)) and eating quality have been excellent in some cases (Hoogland, 1958; Wong and Gill, 1987). Figure 8.8 illustrates the relationship between odour score and TMA level for iced cod. The linear coefficient of determination was statistically significant at the P £ 0.05 level.

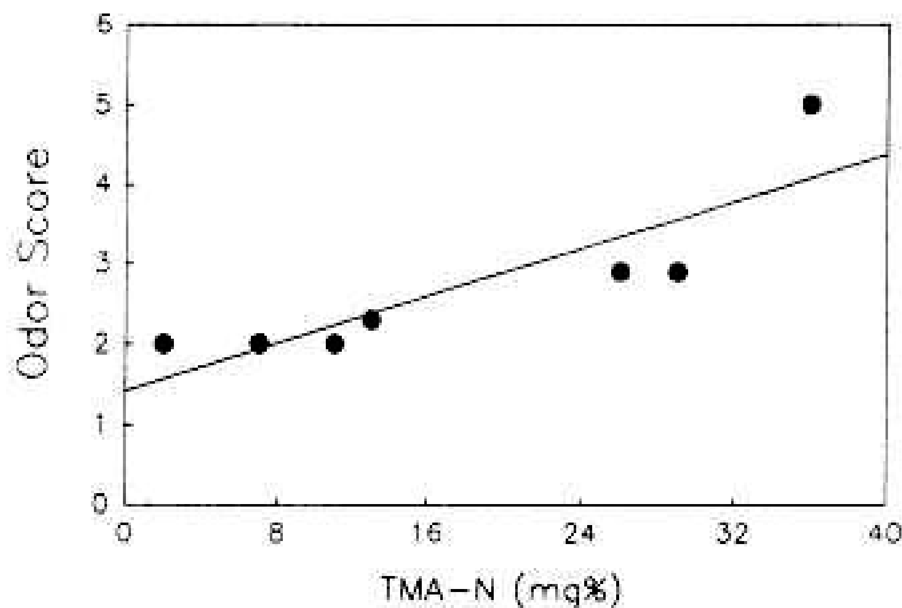


Figure 8.8 Relationship between odour score and TMA levels for iced cod. The

straight line was fitted by linear regression analysis (P£ 0.05) and all data points were averages of data obtained for three individual cod, adapted from Wong and Gill (1987)

The chief advantages of TMA analysis over the enumeration of bacterial numbers are that TMA determinations can be performed far more quickly and often reflect more accurately the degree of spoilage (as judged organoleptically) than do bacterial counts. For example, even high quality fillets cut with a contaminated filleting knife may have high bacterial counts. However, in such a case the bacteria have not had the opportunity to cause spoilage, thus TMA levels are bound to be low. The chief disadvantages of TMA analyses are that they do not reflect the earlier stages of spoilage and are only reliable for certain fish species. A word of caution should be given concerning the preparation of fish samples for amine analysis. TMA and many other amines become volatile at elevated pH. Most analytical methods proposed to date begin with a deproteinization step involving homogenization in perchloric or trichloroacetic acids. Volatilization of amines from stored samples may result in serious analytical errors. Therefore, samples should be neutralized to pH 7 immediately before analysis and should be left in their acidified form in sealed containers if being stored for extended time periods prior to analysis. It is also important to note that appropriate protection for hands and eyes be worn when handling perchloric and/or trichloroacetic acids. In addition, perchloric acid is a fire hazard when brought into contact with organic matter. Spills should be washed with copious quantities of water. Some of the methods of analysis reported to date include colorimetric (Dyer, 1945; Tozawa, 1971), chromatographic (Lundstrom and Racicot, 1983; Gill and Thompson, 1984) and enzymatic analysis (Wong and Gill, 1987; Wong *et al.,* 1988), to name but a few. For a more comprehensive review of the analytical techniques for TMA see the recent review articles: (Gill 1990, 1992).

Dimethylarnine (DMA)

As outlined in section 5.2, certain types of fish contain an enzyme, TMAO dimethylase (TMAO-ase), which converts TMAO into equimolar quantities of DMA and formaldehyde (FA). Thus for fish in the cod (gadoid) family, DMA is produced along with FA in frozen storage with the accompanying FA-induced toughening of the proteins. The amount of protein denaturation is roughly proportional to the amount of FA/DMA produced, but it is most common to monitor the quality of frozen-stored gadoid fish by measuring DMA rather than FA. Much of the FA becomes bound to the tissue and is thus not extractable and cannot be measured quantitatively. The most common method for DMA analysis is a colorimetric determination of the DMA in deproteinized fish extracts. The Dyer and Mounsey (1945) procedure is still in use today although

perhaps more useful is the colorimetric assay proposed by Castell *et al.* (1974) for the simultaneous determination of DMA and TMA, since both are often present in poor quality frozen fish. Unfortunately, many of the colorimetric methods proposed to date lack the specificity where mixtures of different amines are present in samples. The chromatographic methods including gas-liquid chromatography (Lundstrom and Racicot, 1983) and high performance liquid chromatography (Gill and Thompson, 1984) are somewhat more specific, and are not as prone to interferences as the spectrophotometric methods. Also, most of the methods proposed to date for the analysis of amines are destructive and not well suited for analyzing large numbers of samples. Gas chromatographic analysis of headspace volatiles has been proposed as a non-destructive alternative for amine determinations; however, none of the methods proposed to date are without serious practical limitations.

Dimethylamine is produced autolytically during frozen storage. For gadoid fish such as hake, it has been found to be a reliable indicator of FA-induced toughening (Gill et *al.,* 1979). Because it is associated with membranes in the muscle, its production is enhanced with rough handling and with temperature fluctuations in the cold storage facility. Dimethylamine has little or no effect on the flavour or texture of the fish *per se,* but is an indirect indicator of protein denaturation which is often traceable to improper handling before and/or during frozen storage.

BiogenicAmines

Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, presumably so that the organisms may raise the pH of the growth medium through the production of amines.

Histamine, putrescine, cadaverine and tyramine are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. Histamine has received most of the attention since it has been associated with incidents of scombroid poisoning in conjunction with the ingestion of tuna, mackerel, mahi-mahi (dolphinfish from Hawaii). However, the absence of histamine in scombroid fish (tuna, mackerel, etc.) does not ensure the wholesomeness of the product since spoilage at chill storage temperatures does not always result in the production of histamine. Mietz and Karmas (1977) proposed a chemical quality index based on biogenic amines which reflected the quality loss in canned tuna where:

$$\text{Quality Index} = \frac{\text{ppm histamine} + \text{ppm putrescine} + \text{ppm cadaverine}}{1 + \text{pm spermidine} + \text{ppm spermine}}$$

They found that as the quality index ratio increased, the sensory scores on the canned' product decreased. Later, Farn and Sims (1987) followed the production of histamine, cadaverine and putrescine in skipjack and yellowfin tuna at 20°C and found that cadaverine and histamine increased exponentially after an initial lag period of about 36 hours. However, putrescine increased slowly after an initial lag period of 48 hours. Levels of cadaverine and histamine increased to maximum levels of 5-6µg/g tuna but the authors reported that the absence of such amines in raw or cooked product did not necessarily mean that the products were not spoiled.

It is interesting to note that most of the biogenic amines are stable to thermal processing, so their presence in finished canned products is a good indication that the raw material was spoiled prior to heating.

Some of the methods for biogenic amine analysis include high pressure liquid chromatography (Mietz and Karmas, 1977), gas chromatography (Staruszkiewicz andBond, 1981), spectrofluorometric (Vidal-Carou *et al.,* 1990) and a newly-developed rapid enzymatic method for histamine using a microplate reader (Etienne and Bregeon, 1992).

Nucleotide Catabolites

A discussion of the analysis of nucleotide catabolites has been presented in section 5.2 -Autolytic Changes, although all of the catabolic changes are not due to autolysis alone. Most of the enzymes involved in the breakdown of adenosine triphosphate (ATP) to inosine monophosphate (IMP) are believed in most cases to be autolytic whereas the conversion of IMP to inosine (Ino) and then hypoxanthine (Rx) are believed mainly to be due to spoilage bacteria although Hx has been shown to accumulate slowly in sterile fish tissue. Since the levels of each of the catabolic intermediates rise and fall within the tissue as spoilage progresses, quality assessment must never be based upon levels of a single catabolite since the analyst has no way of knowing whether a single compound is increasing or decreasing. For example, if the IMP content of a fish sample were determined to be 5 µmoles/g tissue, the sample might well have been taken from a very fresh fish or a fish on the verge of spoilage, depending on whether or not AMP were present.

Thus, the analysis of the complete nucleotide catabolite profile is nearly always recommended. A complete analysis of nucleotide catabolites may be completed on a fish extract in 12-25 minutes using a high pressure liquid chromatographic (HPLC) system equipped with a single pump and spectrophotometric detector (wavelength 254 nm). Perhaps the simplest HPLC technique published to date is that proposed by Ryder (1985).

Several other approaches have been proposed for the analysis of individual or combination of nucleotide catabolites but none are more reliable than the HPLC approach. A word of caution is perhaps in order with regard to the quantitative analysis of nucleotide catabolites. Most methods proposed to date involve deproteinization of the fish samples by extraction with perchloric or trichloracetic acids. It is important that the acid extracts are neutralized with alkali (most often potassium hydroxide) as soon as possible after extraction to prevent nucleotide degradation in the extracts. Neutralized extracts appear to be quite stable even if held frozen for several weeks. One advantage of using perchloric acid is that the perchlorate ion is insoluble in the presence of potassium. Thus, neutralizing with KOH is a convenient method of sample "clean-up" before HPLC analysis and this procedure helps to extend the life of the HPLC column. Also, it should be noted that nucleotide determination on canned fish does not necessarily reflect the levels in the raw material. *Gill et al.* (1987) found recoveries of 50%, 75%, 64% and 92% for AMP, IMP, Ino and Hx standards which were spiked into canned tuna prior to thermal processing.

Several unusual but innovative approaches utilizing enzymatic assays have been proposed over the years and are presented in Table 8.3. All of the approaches to date rely on destructive sampling (tissue homogenization). It should be noted that regardless of the approach, enzymes denature with time and thus test kits, enzyme-coated strips, electrodes or sensors have a limited shelf life whereas the HPLC techniques do not.

Table 8.3 Fish Freshness Testing Using Enzyme Technology

Analyte (s)	**Principle**	**Advantages**	**Disadvantages**	**Reference**
Hx	enzymes (xanthine oxidase, X0) immobilized on a test strip	rapid simple to use outside the lab	semi -quantitative only capable of measuring Hx (later stages of spoilage)	Jahns *et al.* (1976)

Hx, Ino	test strip, with immobilized enzymes	rapid simple to use outside the lab	semi -quantitative poor reproducibility limited to Hx and Ino (later stages spoilage)	Ehira *et al.* (1986)
IMP, Ino, Hx	enzyme-coated oxygen electrode	rapid accurate	more complicated and time consuming than test strip technology	Karube *et al.* (1984)
K-index	coupled enzyme assay "KV-101 Freshness Meter"	rapid results comparable to HPLC	must purchase enzymes and reagents cost ?	commercially available from Orienta Electric, Niiza Saitama 352, Japan
K-index	enzyme-coated oxygen electrode "Microfresh"	rapid results comparable to HPLC	cost ?	commercially available from Pegasus Instruments, Agincourt, ON, Canada

The factors which have been shown to affect the nucleotide breakdown pattern include species, temperature of storage and physical disruption of the tissue. In addition, since nucleotide breakdown reflects the combined action of autolytic enzymes and bacterial action, the types of spoilage bacteria would no doubt affect the nucleotide patterns. The selection of which nucleotide or combination of nucleotide catabolites to be measured should be made carefully. For example, in certain cases one or two of the catabolites change rapidly with time of chilled storage, whereas the remaining components may change very little. The technical literature should be consulted for guidance on this matter. An excellent overview on the biological and technological factors affecting the nucleotide catabolites as quality indicators was presented by Frazer Hiltz *et al.* (1972).

Ethanol

Ethanol has been used for many years as an objective indicator for seafood quality although it is not nearly as common as the analysis of TMA. Since

ethanol can be derived from carbohydrates via anaerobic fermentation (glycolysis) and/or deamination and decarboxylation of amino-acids such as alanine, it is a common metabolite of a variety of bacteria. It has been used to objectively measure the quality of a variety of fish including canned tuna (Iida *et al.,* 1981 a, 1981b; Lerke and Huck, 1977), canned salmon (Crosgrove, 1978; Hollingworth and Throm, 1982), raw tuna (Human and Khayat, 1981), redfish, pollock, flounder and cod (Kelleher and Zall, 1983).

To date, the simplest and perhaps most reliable means of measuring ethanol in fish tissue is the use of the commercial enzyme test kits available from Boehringer Mannheim (German) or Diagnostic Chemicals (Charlottetown, P.E. I., Canada). One advantage of using ethanol as a spoilage indicator is that it is heat-stable (although volatile) and may be used to assess the quality of canned fish products.

Measurements of oxidative rancidity

The highly unsaturated fatty acids found in fish lipids (section 4.2) are very susceptible to oxidation (section5.4).The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential to oxidize iodide to iodine or to oxidize iron(II) to iron(III). The concentration of the hydroperoxides may be determined by titrimetricor by spectrophotometric methods, giving the peroxide value (PV) as milliequivalents (mEq) peroxide per 1 kg of fat extracted from the fish. A method for PV- determination by iodometry has been described by Lea (1952), and for determination by spectrophotometry of iron (III)thiocyanate by Stine *et al.* (1954).The methods for PV-determination are empirically based, and comparisons between PVs are only possible for results obtained using identical methods. For instance, the thiocyanate-method may give values 1.5 - 2 times higher than the iodine titration method (Barthel and Grosch, 1974).

For several reasons, interpretation of the PV as an index of quality is not straightforward. First, the hydroperoxides are odour- and flavour-less, thus the PV is not related to the actual sensory quality of the product analyzed. However, the peroxide value may indicate a potential for a later formation of sensorial-objectionable compounds. Second, lipid hydroperoxides break down with time, and a low PV at a certain point during the storage of a product can indicate both an early phase of autoxidation and a late stage of a severely oxidized product, where most hydroperoxides have been broken down (Kanner and Rosenthal, 1992),e.g., in dried, salted fish (Smith *et al.,* 1990).

In later stages of oxidation *secondary oxidation products will* usually be present and thus be indicative of a history of autoxidation. These products (section *5.4)*

comprise aldehydes, ketones, short chain fatty acid and others, many of which have very unpleasant odours and flavours, and which in combination yield the fishy and rancid character associated with oxidized fish lipid. Some of the aldehydic secondary oxidation products react with thiobarbituric acid, forming a reddish coloured product that can be determined spectrophotometrically. Using this principle, a measure of thiobarbituric acid-reactive substances (TBA-RS) can be obtained. Several method variations exist; one method for fish lipids is described by Ke and Woyewoda *(1979),* and for fish by Vyncke *(1975).* The results are expressed in terms of the standard (di-)aldehyde used, malonaldehyde, and reported as micromoles malonaldehyde present in 1 g of fat. *(A note of caution:* Sometimes the TBA-results may be expressed as mg malonaldehyde in 1 g of fat, or as amount of malonaldehyde (μmol or μg) in relation to amount of tissue analyzed.) Several reports (e.g., by Hoyland and Taylor *(1991)* and by Raharjo *et al. (1993))* speak of some correlation between TBA-RS and sensory assessments, but other authors fail to find a correlation (e. g., Boyd *et al., 1993).* Thus, caution is necessary in interpretation of TBA-RS values into measures of sensory quality.

Provided that the PV has not been lowered through extended storage or high temperature exposure, the PV (by iodometric titration) should not be above 10-20 meq/kg fish fat (Connell, *1975).*

Examples of guidelines for TBA-RS-values: foods with TBA-RS above 1-2 μmol MDA-equiv per g fat (Connell, *1975)* or above 10,μmol MDA-equiv per 1 kg fish (Ke *et al.,* 1976) will probably have rancid flavours.

Modern instrumental methods allow analysis of better defined oxidation products (specific hydroperoxides, actual content of malonaldehyde), but for general quality estimation, methods that determine a broader range of oxidation products (such as PV and TBA-RS) are to be preferred, although these methods have their limitations as discussed above. Headspace analysis of the volatile oxidation products gives results correlating well with sensory evaluation (e.g., in catfish (Freeman and Hearnsberger, *1993)),* but the method requires access to gas chromatographic equipment.

8.3 Physical methods

Electrical Properties

It has long been known that the electrical properties of skin and tissue change after death, and this has been expected to provide a means of measuring *post mortem* changes or degree of spoilage. However, many difficulties have been encountered in developing an instrument: for example, species variation;

variation within a batch of fish; different instrument readings when fish are damaged, frozen, filleted, bled or not bled; and a poor correlation between instrument reading and sensory analysis. Most of these problems, it is claimed, are overcome by the GR Torrymeter (Jason and Richards, 1975). However, the instrument is not able to measure quality or freshness of a single fish, although it may find application in grading batches of fish, as shown in Figure 8.9.

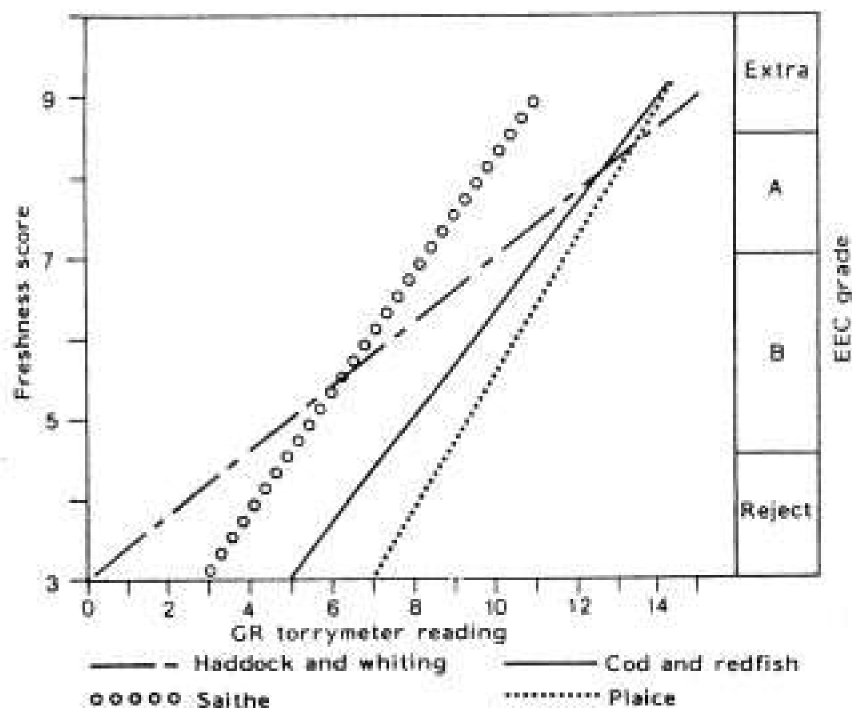


Figure 8.9 Relationship between GR Torrymeter readings of various species of fish and freshness, adapted from Cheyne (1975)

Until recently, no instruments have been capable of on-line determination of quality although this type of mechanized quality evaluation would be highly desirable on the processing floor. The RT Freshness Grader development began in 1982 and, by 1990, a production model capable of sorting 70 fish per minute over 4 channels was made available. The developer was Rafagnataekni Electronics (Reykjavik, Iceland) based the sensing unit on the GR Torrymeter.

pH and Eh

Knowledge about the pH of fish meat may give valuable information about its condition. Measurements are carried out with a pH-meter by placing the electrodes (glass-calomel) either directly into the flesh or into a suspension of fish flesh in distilled water. Measurements of Eh are not carried out routinely, but it is likely that a freshness test can be based on this principle.

Measuring Texture

Texture is an extremely important property of fish muscle, whether raw or cooked. Fish muscle may become tough as a result of frozen storage or soft and mushy as a result of autolytic degradation. Texture may be monitored organoleptically but there has for many years been a need for the development of a reliable objective rheological test which would accurately reflect the subjective evaluation of a well-trained panel of judges. Gill *et al.* (1979) developed a method for evaluating the formaldehyde-induced toughening of frozen fish muscle. The method utilized an Instron Model TM equipped with a Kramer shear cell with 4 cutting blades. This method correlated well with data obtained from a trained texture panel. A method for measuring hardness/ softness of fish flesh, designated as compressive deformability, has been reported by Johnson *et al.* (1980). An accuratelycut fish sample is compressed by a plunger, and the stress-strain curve recorded. A modulus of deformability is calculated from the recorded graph. The results from such measurements may, however, be difficult to interpret.

Another method, measuring the shear force of fish flesh, has been investigated by Dunajski (1980). From this work, it has been concluded that a thin-bladed shear force cell of the Kramer type can be applied.

These are but a few of the examples cited in the literature and until recently all involved expensive equipment and destructive sampling. Therefore, Botta (1991) developed a rapid non-destructive method for the measurement of cod fillet texture. It is a small, portable penetrometer which measures both firmness and resilience. Each test takes only 2-3 seconds to complete and results appear to correlate well with subjective texture grades.

8.4 Microbiological methods

The aim of microbiological examinations of fish products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality of the fish including temperature abuse and hygiene during handling and processing. Microbiological data will in general not give any information about eating quality and freshness. However, as outlined in sections 5 and 6, the number of specific spoilage bacteria will be related to the remaining shelf life and this can be predicted from such numbers (see Figure 5.8).

Traditional bacteriological examinations are laborious, time-consuming, costly and require skill in execution and interpretation of the results. It is recommended that such analyses be limited in number and extent. Various

rapid microbiological methods have been developed during the last decade and some of these automated procedures may be of use when large numbers of samples are to be analyzed.

Total counts

This parameter is synonymous with Total Aerobic Count (TAC) and Standard Plate Count (SPC). The total count represents, if carried out by traditional methods, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. This figure is seldom a good indicator of the sensoric quality or expected shelf life of the product (Huss *et al.,* 1974). In ice-stored Nile perch, the total count was 109 cfu/g for days before the fish was rejected (Gram *et al., 1989)* and in lightly preserved fish products high counts prevail for long time before rejection. If a count is made after systematic sampling and a thorough knowledge of the handling of the fish before sampling, temperature conditions, packaging etc., it may give a comparative measure of the overall degree of bacterial contamination and the hygiene applied. However, it should also be noted that there is no correlation between the total count and presence of any bacteria of public health significance. A summary of different methods used for fish and fish products is given in Table 8.4.

Common plate count agars (PCA) are still the substrates most widely used for determination of total counts. However, when examining several types of seafood a more nutrient rich agar (Iron Agar, Lyngby, Oxoid) gives significantly higher counts than PCA (Gram, 1990). Furthermore, the iron agar yields also the number of hydrogen sulphide producing bacteria, which in some fish products are the specific spoilage bacteria. Incubation temperature at and above 30°C are inappropriate when examining seafood products held at chill temperatures. Pour plating and a 3-4 day incubation at 25°C is relevant when examining products where psychrotrophs are the most important organisms, whereas products where the psychrophilic *Photobacterium phosphoreum* occurs should be examined by surface plating and incubation at maximum 15° C.

Several attempts have been made to ease the procedures for determination of the content of bacteria (Fung *et al., 1987).* Both *Redigel* (RCR Scientific) and *Petrifilm*™ SM (3M Company) are dried agars with a gelling agent to which the sample is added directly. The main advantage of Redigel and Petrifilm compared to conventional plate counts in addition to the costs, is the ease of handling. However, all agar-based methods share a common drawback in the lengthy incubation required.

Microscopic examination of foods is a rapid way of estimating bacterial levels. By phase contrast microscopy the level of bacteria in a sample can be determined within one log-unit. One cell per field of vision equals approximately 5 -10^5cfu/ml at 1000 X magnification. The staining of cells with acridine orange and detection by fluorescence microscopy has earned widespread acceptance as the direct epifluorescence filter technique (DEFT). Whilst microscopic methods are very rapid, the low sensitivity must be considered their major drawback.

Bacterial numbers have been estimated in foods by measuring the amount of bacterial adenosine triphosphate (ATP) (Sharpe *et al., 1970)* or by measuring the amount of endotoxin (Gram-negative bacteria) by the *Limulus* amoebocytes lysate (LAL) test (Gram, *1992).* The former is very rapid but difficulties exist in separating bacterial and somatic ATP.

Table 8.4 Methods for determination of the content of bacteria in seafood

Method	**Temperature, °C**	**Incubation**	**Sensitivity, cfu/g**
Plate count or Iron agar	15-25	3-5 days	10
"Redigel"/"Petrifilm™ SM"	15-25	3-5 days	10
Microcolony-DEFT	15-30	3-4 hours	10^4-10^5
DEFT	-	30 min.	10^4-10^5
ATP	-	1 hour	10^4-10^5
Limulus lysate test	-	2-3 hours	10^3-10^4
Microcalorimetry/Dye reduction Conductance/Capacitance	15-25	4-40 hours	10

Several methods (microcalorimetry, dye reduction, conductance and capacitance) used for rapid estimation of bacterial numbers are based on the withdrawal of a sample, incubation at high temperature (20-25°C) and detection of a given signal. In microcalorimetry the heat generated by the sample is compared to a sterile control, whereas in conductance and capacitance

measurements of the change in electrical properties of the sample, as compared to a sterile control, is registered. The time taken before a significant change occurs in the measured parameter (heat, conductance, etc.) is called the Detection Time (DT). The DT is inversely related to the initial number of bacteria, i.e., early reaction indicates a high bacterial count in the sample. However, although the signal obtained is reversely proportional to the bacterial count done by agar methods, it is only a small fraction of the microflora that give rise to the signal and care must be taken in selection of incubation temperature and substrate.

Spoilage bacteria

The total number of bacteria on fish rarely indicates sensoric quality or expected storage characteristics (Huss *et al.,* 1974). However, it is recognized that certain bacteria are the main cause of spoilage (see section 5.3). Different peptone-rich substrates containing ferric citrate have been used for detection of H_2S-producing bacteria such as *Shewanella putrefaciens,* which can be seen as black colonies due to precipitation of FeS (Levin, 1968; Gram *et al.,* 1987). Ambient spoilage is often caused by members of *Vibrionaceae* that also will form black colonies on an iron agar to which an organic sulphur source is added (e.g., Iron Agar, Lyngby). No selective or indicative medium exists for the *Pseudomonas spp.* that spoil some tropical and freshwater fish or for *Photobacterium phosphoreum* that spoil packed fresh fish. At the Technological Laboratory, Lyngby, a conductance- based method for specific detection of P. *phosphoreum is* currently being developed (Dalgaard, personal communication). The presence or absence of pathogenic bacteria is often evaluated by methods based on immuno- or molecular biology techniques. Such techniques may also be developed for specific spoilage bacteria, and the Technological Laboratory has been currently investigating the use of antibodies specific for S. *putrefaciens* (Fonnesbech *et al.,* 1993).Also, a gene-probe which is specific for S. *putrefaciens* has been developed but has not been tried on fish products (DiChristina and DeLong, *1993).*

Spoilage reactions

Several spoilage reactions can be used for evaluation of the bacteriological status of fish products. As described above, agars on which H_2S producing organisms are counted have been developed. During spoilage of white lean fish, one of the major spoilage reactions is the bacteriological reduction of trimethylamine oxide to trimethylamine (Liston, 1980; Hobbs and Hodgkiss, 1982).When TMAO is reduced to TMA several physical changes occur: the redox-potential decreases, the pH increases and the electrical conductance increases. The measurement of such changes in a TMAO containing substrate

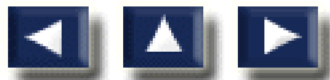
inoculated with the sample can be used to evaluate the level of organisms with spoilage potential; thus the more rapid the change occurs the higher the level of spoilage organisms.

Several authors have inoculated a known amount of sample in a TMAO-containing substrate and recorded the time taken until a significant change in conductivity occurs (Gibson *et al.,* 1984;Gram, *1985;* Jorgensen *et al.,* 1988). This time, the detection time, has been found to be inversely proportional to the number of hydrogen sulphide producing bacteria in fresh aerobically-stored fish, and rapid estimation of their numbers can be given within 8-36 hours.

The changes in redox-potential in a TMAO-containing substrate can be recorded either by electrodes or by observing the colour of a redox-indicator (Huss *et al.,* 1987).As with the conductimetric measurements, the time taken until a significant change is recorded is inversely proportional to the initial amount of bacteria.

Pathogenic bacteria

Several pathogenic bacteria may either be present in the environment or contaminate the fish during handling. A detailed description of these organisms, their importance, and detection methods is given by Huss (1994).



9. ASSURANCE OF FRESH FISH QUALITY

The artisanal fisherman, fishing for a few hours and returning to sell his catch on the beach while the fish is still alive or very fresh, does not need a complicated quality assurance system. His customers know very well the quality of the fish, and most often the fish are caught, sold and consumed within the same day. However, no food production, processing or distribution company can be self-sustained in the medium- or long-term, unless the issues of quality are properly recognized and addressed and an appropriate quality system is put into operation in the processing establishment. The need for effective quality assurance systems is further underlined by the fact that global demand for fish and fishery products is continuously growing while production level is approaching its maximum with limited possibilities for future increase. The need for improved utilization of present harvest including a reduction of fish wasted due to spoilage is therefore a strong incentive to introduce an effective quality assurance system. Further benefits are increasing efficiency, increasing employee satisfaction and lower costs to the processing industry.

Traditionally, fish processors have regarded quality assurance as the responsibility of the regulatory governmental agency, and the means used by these agencies have been the formulation of food laws and regulations, inspection of facilities and processes and final product testing. The processors' own efforts have in many cases been based entirely on final product testing. Such a system is costly, ineffective, provides no guarantee of quality but merely a false sense of security.

At this point, a distinction needs to be drawn between Quality Assurance and Quality Control. Unfortunately, these two terms have been used indiscriminately and the difference between them has become blurred. According to International Standards (ISO 8402), **Quality Assurance (QA)** is "all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality". In other words, QA is a strategic management function which establishes policies, adapts programmes to meet established goals, and provides confidence that these

measures are being effectively applied.

Quality Assurance is the modern term for describing the control, evaluation and audit of a food processing system. The primary function is to provide confidence for both management and the ultimate customer that the company is supplying products with the desired quality which has been specified in trade agreements between the producer and the customer. Only by having a planned QA- programme can a firm continue to succeed in supplying the customer with the desired products.

A large part of a quality assurance programme is built around **Quality Control (QC).** QC is "the operational techniques and activities that are used to fulfil requirements for quality" (ISO 8402), i.e., a tactical function which carries out the programmes established by the QA. Thus quality control is quite often equated with "inspection" or measurements within a quality assurance programme. Thus QC means to regulate to some standard, most often associated with the processing line, i.e., specific processes and operations. QC is the tool for the production worker, to help him operate the line in accordance with the predetermined parameters for any given quality level.

In contrast to the principles in traditional quality programmes relying heavily on control of end- products, a preventative strategy based on a thorough study of prevailing conditions is much more likely to provide a better guarantee of quality, and even at a reduced cost. Such a strategy was first introduced by microbiologists more than 20 years ago to increase safety of food products and is named the Hazard Analysis Critical Control Point (HACCP) System. The principles of the HACCP system can very easily be used also in the control of other aspects of quality.

The principles of the HACCP system are now being introduced in food production in many parts of the world. One reason for this development is that a number of national food legislations today are placing full responsibility for food quality on the producer (e.g., EEC Council Directive no. 91/493/EEC) and the use of the HACCP system is required (EEC 1993, 1994).

The Hazard Analysis Critical Control Point (HACCP) system

The main elements of the HACCP system are:

A. Identify potential hazards. Assess the risk (likelihood) of occurrence.
B. Determine the Critical Control Points (CCPs). Determine steps that can be controlled to eliminate or minimize the hazards. A CCP that can completely control a hazard, is designated CCP-1, while a CCP that

minimizes, but not completely controls a hazard is designated a CCP-2.

C. Establish the criteria (tolerances, target level) that must be met to ensure that a CCP is under control.
D. Establish a monitoring system.
E. Establish the corrective action when CCP is not under control.
F. Establish procedures for verification.
G. Establish documentation and record-keeping.

For detailed information on introduction and application of the HACCP system, Huss (1994) should be consulted.

The great advantage of the HACCP system is that it constitutes a scientific and systematic, structural, rational, multi-disciplined, adaptable and cost-effective approach of preventive quality assurance. Properly applied, there is no other system or method which can provide the same degree of safety and assurance of quality, and the daily running cost of a HACCP system is small compared with a large sampling programme.

By using the HACCP concept in food processing it is possible to assure and - as all actions and measurements are recorded - to document assurance of a quality standard as specified in the product specification.

Application of the HACCP system for fresh or frozen fish production

A starting point in design and implementation of any quality programme is to achieve a complete and correct definition and description of the product. Further, it must be ensured that each and every quality attribute is included and is written such that any ambiguity is avoided. Thus the critical limits for defects such as presence of bones, pieces of skin and membranes on skinless fillets, maximum permitted short weights, etc., must be clearly stated. When this task is completed, and the processes within the operation have been considered, it is possible to identify the hazards to be controlled. A list of possible hazards and Critical Control Points in production and processing of fresh and frozen boneless fillets is given in Table 9.1.

In most presentations it is recommended that hazards are limited to safety hazards and decomposition (spoilage). However, in the present presentation commercial quality (defects) have also been included as hazards.

When all hazards, defects and Critical Control Points (CCP) have been identified, an appropriate monitoring and checking system must be established at each CCP. This includes:

a. a detailed description of control measure, frequency of control and nomination of who is responsible
b. establishment of critical limits for each control measure
c. records to be kept for all actions and observations
d. establishment of a corrective action plan.

Table 9.1 Hazards and Critical Control Points (CCP) in production and processing of fresh and frozen boneless fish fillets

Processing flow	**Hazard**	**Preventive Measure**	**Degree of control**
LIVE FISH	Contamination (chemicals, enteric pathogens) biotoxins	Avoid fishing in contaminated areas and areas where biotoxins are prevalent	CCP-2
CATCH			
CATCH HANDLING	Growth of bacteria Gaping in fillets Discolorations	Short handling time Avoid rough handling	CCP-1 CCP-2
CHILLING	Growth of bacteria	Low temperature	CCP-1
LANDING			
ARRIVAL OF RAW MATERIAL AT FACTORY	Substandard quality entering processing	Ensure reliable source (HACCP-plan onboard or list of approved suppliers) Sensory evaluation	CCP-2
CHILLING	Growth of bacteria (deterioration)	Ensure low temperature	CCP-1
PROCESSING: De-icing Washing Filleting Skinning,Trimming Candling	Pieces of skin, bones and membranes left on fillet Visible parasites left on fillet	Proper setting of machinery Instructions of personnel Ensure light intensity on candling table Frequent change of personnel	CCP-2 CCP-2

Weighing Packaging	Short weights/over weights Deterioration during (fresh/frozen) storage	Ensure accuracy of scales Ensure adequate packaging material and method (e.g., vacuum)	CCP-1 CCP-2
All processing steps	Growth of bacteria Contamination (enteric bacteria)	Short processing time Factory hygiene/ sanitation water quality	CCP-1 CCP-2 CCP-1
CHILLING/FREEZING - STORAGE	Deterioration	Ensure correct (low) temperature	CCP-1

A precise and detailed description of all CCPs is not possible as the individual and local situation may vary. However, some general points are considered as follows:

LIVE FISH - before being caught. The hazards are presence of biotoxins and contamination with chemicals and/or enteric pathogens:

a. control measures are monitoring of the environment (fishing areas) for pollution and presence of biotoxins. The government will be responsible for this activity in most countries and regular surveys should be carried out
b. critical limits should be set by national governments
c. results of surveys should be published at regular intervals
d. corrective action is restricted fishing in grossly polluted areas

CATCH HANDLING - hazards are growth of bacteria (causing histamine formation and/or decomposition), discoloration and gaping in fillets:

a. control measures are restricted time for catch handling (time from catch to chilling) and visual check that crew are following prescribed procedures to avoid rough handling. The control should be continuous and the skipper or first mate on deck is responsible
b. time for catch handling is limited to max 3 h
c. a detailed log on each haul, proper marking of boxes or containers for identification of lot, time (day and hour) for catch, catch handling time, deviations - if any - from prescribed procedure
d. corrective actions are check of product (sorting) and rejection of low quality product

CHILLING - the hazard is growth of bacteria:

a. control measures are continuous recording of temperature (automatic) or visual control of icing of the fish. The skipper or chief is responsible
b. the critical limit for fish temperature is 1°C
c. a log on temperature and icing observations must be kept
d. corrective action is checking of fish from period out of control, sorting and rejection of low quality fish. Identification of reason(s) for temperature out of control

ARRIVAL OF RAW MATERIAL AT FACTORY - the hazard is risk of substandard quality entering processing:

a. control measures are check of identity of raw material, sensory assessment (visual) and control of fish temperature of all arriving raw material. Processing manager or person specially designated may be responsible
b. no low quality fish will be accepted (company specification)
c. a log on all daily actions and observations Must be kept
d. rejection of low quality fish. Identify reason for low quality. Change of supplier

CHILLING - the hazard is growth of bacteria (deterioration):

a. control measures are continuous recording (automatic) of temperatures in chill room and check on icing of fish. Accuracy of thermometer must be checked regularly against mercury-thermometers. Responsible person is the processing manager or designated person
b. chill room temperature must be £ 5°C
c. a continuous log on temperatures and observations must be kept
d. if temperatures are out of control, all products must be reinspected, sorted and low quality material rejected

PROCESSING Filleting, skinning/ trimming - the hazards are pieces of skin, bones and membranes left on fillet:

a. control measures are daily check of machinery for correct setting. Instructions of personnel. A sample of x kilo of fillet is taken x times daily for careful visual examination. Frequency of sampling is company policy, on-line electronic control is possible (Pau and Olafsson, 1991). Line manager is responsible for the on-line control, while QC-manager is responsible for collecting and examination of samples (verification)
b. critical limits are specified in product specification by the buyer
c. records on all actions and observations
d. sort and reprocess fillets with defects. Identify reason for processing out

of control

Candling - the hazard is visible parasites left on fillet:

a. control measures are continuous candling of all fillets, packaging personnel is instructed to observe for parasites. The sample taken for control of bones, membranes and skin is also checked for parasites and same person is responsible. The production manager is responsible for the on-line control while the QC manager is responsible for collecting and examination of samples (verification)
b. critical limits may be set by buyer or by company policy. See also Codex Alimentarius and EEC regulations
c. records on all actions and observation
d. fillets with visible parasites are reprocessed or rejected. Adjustment of candling light. Frequent change of personnel

Weighing - the hazards are short weight or over-weight:

a. control measures are frequent (1-2-3 times daily) check of weighing procedures, control weighing of samples and daily check of accuracy of scales. Line operator is responsible
b. critical limits are specified by company policy or buyer
c. daily records of all actions and observation
d. reweighing of products processed when out of control. Identification of reason for deviation

Packaging - the hazard is spoilage in frozen storage if packaging (packaging material, vacuum) is inadequate:

a. the processing manager must ensure daily that packaging is in agreement with product specification

All processing steps - the hazards are 1) growth of bacteria and 2) (gross) contamination by enteric pathogens:

a. control measure for 1) is establishment of short processing time - which must be checked on a daily basis by the line manager. For control of contamination, the personal hygiene must be supervised continuously by production manager, and prescribed procedures must be followed (medical certificate, report on illness, dress, etc.). Microbiological control of water quality must be carried out on a regular basis (daily - weekly - monthly - depending on the source of water) and is the responsibility of the QC-manager. If chlorination of water is applied, the level of free

chlorine must be determined on a daily basis
b. critical limits for water quality are standards for drinking water. Limits for chlorine is 0.5 mg/l. No person with gastro-intestinal disorder must work in direct contact with unwrapped fish
c. records on tests for water quality. Actions and observations on personal hygiene must be recorded
d. corrective action is microbiological testing of products. Rejection of all contaminated products

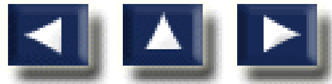
CHILLING /FREEZING - the hazard is deterioration:

a. continuous temperature control (automatic recording) or frequent check of icing. Accuracy of thermometers must be checked regularly against an accurate mercurythermometer. Foreman in charge of stores is responsible
b. critical limits are + 1°C for chilled fish and -18°C for frozen fish
c. log on all temperature readings must be kept
d. corrective action is reinspection of fish exposed to elevated temperature - and rejection of low quality products

In order to be effective, the HACCP system needs to be applied from origin of food (catch) to consumption. In the case of fresh fish, the situation is most often that the fish change owner at the time of landing. Here, the new owner (the processor) must ensure that the fish are supplied from a reliable source (fisherman) who also applies the HACCP principles. If this is possible, the processor has the situation under control and needs only occasionally to verify the quality on arrival to the factory by checking quality (sensory evaluation) and temperature of fish on arrival. In this case it is not a critical situation and this step can be designated a Control Point (CP) only.

The situation is very different if the processor needs to buy fish from a number of unknown fishermen (auction system). This will require constant checking of fish quality on arrival to the factory in order to ensure compliance with all the requirements of the product. In this case, it is therefore a critical Control Point, and since there is still a risk of substandard quality entering the processing line, it is a CCP-2.

Most on-line control (continuous control of temperatures, quality of work, sensory quality of product) should be the responsibility of the processing manager.



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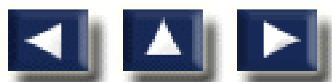


APPENDIX A

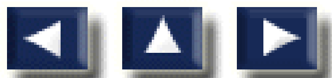
Triangle Test for Difference: Critical Number (Minimum) of Correct Answers

Entries are the minimum number of correct responses required for significance at the stated significance level (i.e., column) for the corresponding number of respondents "n" (i.e., row). Reject the assumption of "no difference" if the number of correct responses is greater than or equal to the tabled value.

Significance level (%)					Significance level (%)				
n	**10**	**5**	**1**	**0.1**	**n**	**10**	**5**	**1**	**0.1**
3	3	3	-	-	**26**	13	14	15	17
4	4	4	-	-	**27**	13	14	16	18
5	4	4	5	-	**28**	14	15	16	18
					29	14	15	17	19
					30	14	15	17	19
6	5	5	6	-	**31**	15	16	18	20
7	5	5	6	7	**32**	15	16	18	20
8	5	6	7	8	**33**	15	17	18	21
9	6	6	7	8	**34**	16	17	19	21
10	6	7	8	9	**35**	16	17	19	22
11	7	7	8	10	**36**	17	18	20	22
12	7	8	9	10	**42**	19	20	22	25
13	8	8	9	11	**48**	21	22	25	27
14	8	9	10	11	**54**	23	25	27	30
15	8	9	10	12	**60**	26	27	30	33
16	9	9	11	12	**66**	28	29	32	35
17	9	10	11	13	**72**	30	32	34	38
18	10	10	12	13	**78**	32	34	37	40

19	10	11	12	14	**84**	35	36	39	43
20	10	11	13	14	**90**	37	38	42	45
					96	39	41	44	48
21	11	12	13	15					
22	11	12	14	15					
23	12	12	14	16					
24	12	13	15	16					
25	12	13	15	17					

Note: For values of n not in the table compute



$$z = (k - (1/3)n) / \sqrt{(2/9)n}$$

where k is the number of correct answers.

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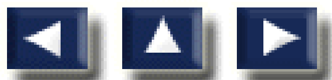


APPENDIX B

Practical example of the application of the simple triangular test ("forced choice" option)*

A manufacturer who changes the formulation of his product wishes to know whether the new product will be similar to the old from the sensory point of view. He has at his disposal 12 assessors.

Two batches are prepared, one representing the old formulation (A) and one the new formulation (B).

Presuming that each assessor makes only one assessment, it will be necessary to prepare 18 test samples of A and 18 test samples of B in two series of six sets, as follows:

two sets ABB
two sets AAB
two sets ABA
two sets BAA
two sets BBA
two sets BAB

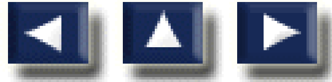
These sets are distributed at random among the assessors.

The test supervisor chooses a significance level of 5%, i.e., he accepts a risk of 5% of concluding that the test will reveal a difference when there is none.

The number of correct answers (8) is referred to the table.

With 12 replies, it is concluded that the two products are different at the 5% significance level.

* From ISO 4120-1983(E)



APPENDIX C

Guide to EEC Freshness Grades

In order to be placed in grade E, A, B or Unfit (C) the fish should possess the following characteristics. The descriptive terms are meant to be guides and not all the characteristics described will necessarily occur together in every fish. Gill odours are particularly discriminatory.

White fish: cod, saithe, haddock, whiting plaice redfish ling hake

		E	A	B	Unit(C)
Skin		bright; shining; iridescent (not redfish) or opalescent; no bleaching	waxy; slight loss of bloom; very slight bleaching	dull;	dull; gritty; marked bleaching and shrinkage
Outer slime		transparent; water white	milky	yellowish-grey; some clotting	yellow-brown; very clotted and thick
Eyes		convex; black pupil; translucent cornea	plane; slightly opaque pupil; slightly opalescent	slightly concave; grey pupil; opaque cornea	completely sunken; grey pupil opaque discoloured cornea
Gills		dark red or bright red; mucus translucent	red or pink; mucus slightly opaque	brown/grey and bleached; mucus opaque and thick	brown or bleached; mucus yellowish grey and clotted

Peritoneum (in gutted fish)		glossy; brilliant; difficult to tear from flesh	slightly dull; difficult to tear from flesh	gritty; faily easy to tear from flesh	gritty; easily torn from flesh
Gill and internal odours	all except plaice	fresh; seaweedy; shellfishy	no odour; neutral odour; trace musty, mousy, milky, capryllic, garlic or peppery	definite musty, mousy, milky, capryllic, garlic or peppery; bready; malty; beery; lactic; slightly sour	acetic: butyric; fruity; turnipy; amines; sulphide; faecal
Gill and internal odours	plaice	fresh oil; metallic; fresh-cut grass; earthy; peppery	oily; seaweedy; aromatic; trace musty, mousy, or critic	oily; definite musty, mousy, or critic; bready; malty; beery; slightly rancid; painty	muddy; grassy; fruity; acetic; butyric, rancid; amines; sulphide; faecal

Dogfish

	E	A	B	Unit(c)
Eyes	convex; very bright and iridescent; narrow pupils	convex to flat; green; clear but some loss of brightness and irridescence; oval pupils	flat to sunken; some yellowing; slithgly cloudy	sunken; yellow; cloudy
Appearance	in or partly in rigor; small amount of clear slime on skin	loss of rigor; no slime on skin and particularly no slime in mouth or at gill openings	sticky slime in	copious slime in mouth and gill openings; obvious flattering of snout
Odour	fresh marine	ordourless or slightly musty; not ammoniacal	ammoniacal; sour	very ammoniacal; very sour

Herring

	E	A	B	Unit(c)
Skin	full bloom; bright; shining; iridescent; clean	slight dullness and loss of bloom	difinite dullness and loss of bloom	dull; no bloom
Outer slime	transparent or water white	milky; slight browning	brownish	brown
Gills covers	silvery	silvery; slight browning; slight bright red blood stain	some browning and blood staining	very brown and blood stained
Eyes	convex	plane	slightly concave	concave; sunken
Firmness	very stiff and firm	fairly stiff and firm	stiffness nearly absent, fairly soft	soft or very soft
Gill odours	fresh seaweedy	less fresh seaweedy, slight oily	slight stale seaweedy; definite oily; trace or slight H_2S (sulphide), "salt cured" or rancid oil	definite H_2S (sulphide); rancid oil; amines; faecal; sour

Mackerel

	E	A	B	Unit(c)
Skin	strong blue and turquoise colours; iridescence over all body; well defined lateral line; reticulations on upper surface; clear distinction between upper and lower surfaces	loss of bright colours with fading of reticulations; pale golden tinge on lower surface	golden tinge over all body; skin wrinkles on flexing; washed-out appearance of colours; patchy iridescence	yellow slime; little distinction between upper and lower surfaces
Texture of body	stiff	firm	some softening	limp and floppy

Eyes	bulging with protruding lens; shiny jet black/ blue pupil with metallic brown iris; transparant eye cap	convex; slight clouding of lens and wrinkling of iris; clouding of eye cap	plane; cloudy lens with black specks in iris; pale golden eye cap	sunken eye covered with yellow slime
Gills appearance	uniformly dark red/purple with free blood and water clear slime	loss of colour with red/brown slime; pale margins to gills	further loss of colour with patchy bleaching; increase in red/ brown slime	bleached; thick yellow slime
Gill odour	fresh seaweed; sharp; halogens; pepper; fresh-cut grass; metallic;blood; fresh, sweet oil	dull; muddy; musty mousy; cardboard; fish oil	yeast; sour rotten fruit; "wet dogs"; old grass cuttings; strong oily	compost heap; rotten turnips; sour cheese; ammonia; sulphides; rancid oil

1. INTRODUCTION

World catches of fish have increased in the 1970s and 1980s but seem to have stabilized since 1988 to just under 100 million t. As the human population is ever increasing, it means that less fish will be available *per caput* every year. Nevertheless, a large part of this valuable commodity is wasted: it has been estimated by FAO that post-harvest losses (discards at sea and losses due to deterioration) remain at a staggering 25 % of the total catch. Better utilization of the aquatic resources should therefore aim primarily at reducing these enormous losses by improving the quality and preservation of fish and fish products and by upgrading discarded low value fish to food products. Very often, ignorance and lack of skill in fish handling or in the administration of fisheries are among the causes for lack of progress in this direction.

FAO has long recognized the need for training in fish technology, and since 1971 a series of training courses, financed by the Danish International Development Agency (DANIDA), has been conducted in the developing countries. In 1988 a training manual entitled "Fresh fish - quality and quality changes" was published. This book has been extensively used and is now out of print. This present book is a revised and updated version of the first publication. It still only deals with fresh fish, as it is felt that a solid background knowledge of the raw material is essential for further development in preservation of and adding value to the product. In the context of this book, fresh fish is either fish kept alive until it is consumed, or dead fish preserved only by cold water or ice.

The book describes fundamentals in fish biology, chemical composition of fish and *post mortem* changes, with a view to explaining the rationale for optimal catch handling procedures and obtaining maximum shelf life. The effect of various factors (temperature, atmosphere, etc.) on fresh fish quality is discussed as are the various sensory, chemical and micro-biological methods for assessing fish quality. Wherever possible, data on tropical fish have been included.

Two new chapters, not included in the first publication, have been added. One is a description of the practical application of new and improved fish handling methods (Chapter 7) and the other is the application of the Hazard Analysis Critical Control Point (HACCP) system in a quality assurance programme for fresh and frozen fish (Chapter 9).

Fresh fish handling procedures encompass all the operations aimed at maintaining food safety and quality characteristics from the time fish is caught until it is consumed. In practice, it means reducing the spoilage rate as much as possible, preventing contamination with undesirable microorganisms, substances and foreign bodies and avoiding physical damage of edible parts.

The immediate effect of fish handling procedures (e.g., washing, gutting, chilling) on quality can easily be assessed by sensory methods. Fish quality, in terms of safety and keeping time, is highly influenced by non-visible factors such as autolysis and contamination and growth of microorganisms. These effects can only be assessed long after the damage has occurred, and the proper procedures must thus be based on knowledge about the effects of the many different factors involved. Large or small improvements are usually feasible when analysing current fish handling methods.

It is hoped that the reading of this book, combined with practical training, will be helpful in providing the stimulus which is often necessary to promote development in fisheries.

APPENDIX D

Evaluation form for raw cod Quality assessment of cod - Raw fish

Name:
Date:

Quality parameter	Characteristics	Grading	
Skin	Surface appearance	0 1 2 3	Bright shining Waxy, slight loss of bloom Dull, some bleaching Dull, gritty
	Slime	0 1 2 3	Transparent or water white Milky Yellow-grey Yellow-brown
	Stiffness	0 1	Firm Soft
Flesh	Stiffness	0 1	Rigor Post-rigor
Eyes	Clarity	0 1 2	Translucent cornea Opalescent cornea Opaque cornea
	Shape of Pupil	0 1 2	Convex Plane Sunken
	Colour	0 1 2	Bright red Pink Faded/discoloured

	Odour	0 1 2 3	Fresh/seaweed Fishy Stale Spoilt
	Mucus	0 1 2	Absent Moderate Excessive
Flesh colour	In open surfaces	0 1 2	Translucent Grey Yellow-brown
Blood	In throat cut	0 1 2	Red Dark red Brown
Character sum			

Instruction: Please assess the fish using the list of descriptive terms provided above. Rate the fish on each of the quality parameters and circle the corresponding score on the scale.

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2. AQUATIC RESOURCES AND THEIR UTILIZATION

More than two-thirds of the world's surface is covered by water and the total yearly production of organic material in the aquatic environment has been estimated at about 40 000 million t (Moeller Christensen, 1968). Tiny microscopic plants, the phytoplankton, are the primary producers of organic material using the energy supplied by the sun (see Figure 2.1).

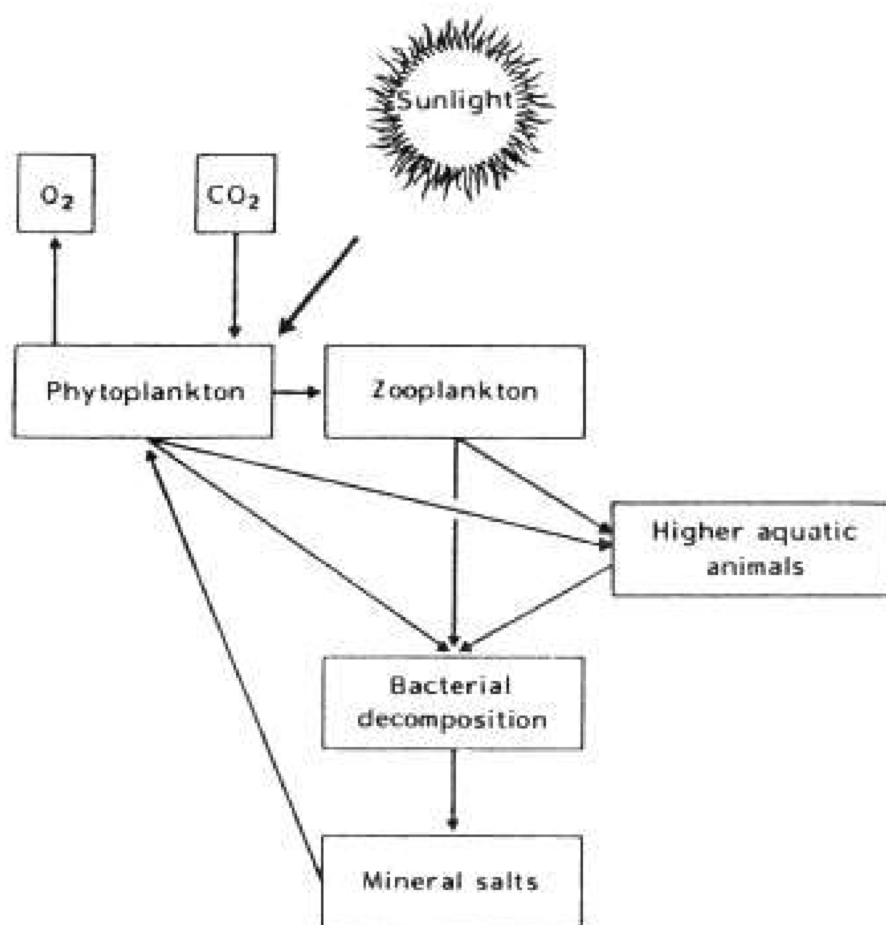


Figure 2.1 The annual aquatic production of organic material is estimated at 40 000 million t (Moeller Christensen, 1968)

This enormous primary production is the first link in the food chain and forms the basis for all life in the sea. How much harvestable fish results from this primary production has been the subject of much speculation. However, there are great difficulties in estimating the ecological efficiency, i.e., the ratio of total production at each successive trophic level. Gulland (1971) reports a range from 10 to 25 % but suggests 25 % as the absolute upper limit of ecological efficiency; for example, not all of the production at one trophic level is consumed by the next. Ecological efficiency also varies between levels, being higher at the lower levels of the food chain with smaller organisms using proportionally more of their food intake for growth rather than for maintenance. Diseases, mortality, pollution, etc. may also influence ecological efficiency. As an example, the conditions in the North Sea, an area with very rich fishing waters, are shown in Figure 2.2.

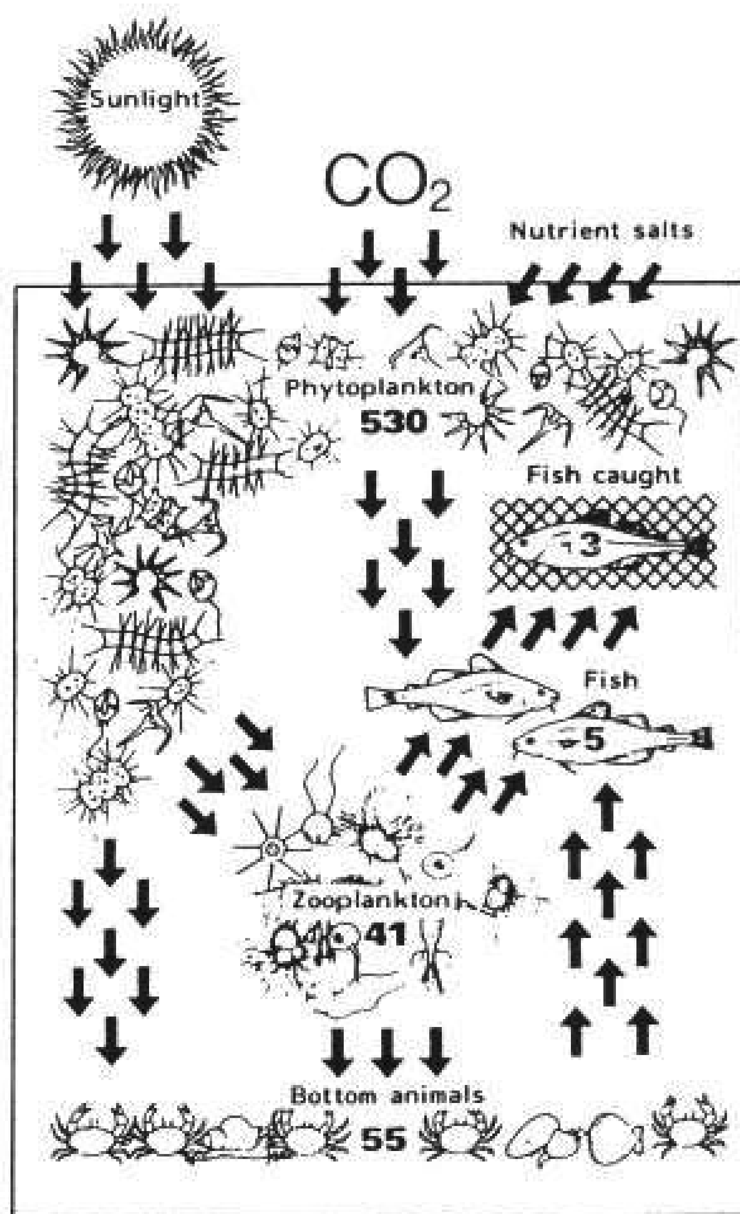


Figure 2.2 Annual production (in million t) in the North Sea, one of the richest

fishing grounds in the world (Moeller Christensen and Nystroem, 1977)

Since production is greater in the early stages of the food chain, the potential catch is also greater if harvesting is carried out at these stages.

Up to 1970, the world catch of marine fish continued to rise at an overall rate of 6 percent per year, according to FAO statistics. Great optimism was expressed by various authors who estimated the potential world catch to be somewhere between 200 million t/year to 2 thousand t/year (Gulland, 1971); most of this wide variation being due to uncertainties concerning the trophic level at which the harvest would be taken. The world fish catch since 1970 is shown in Figure 2.3.

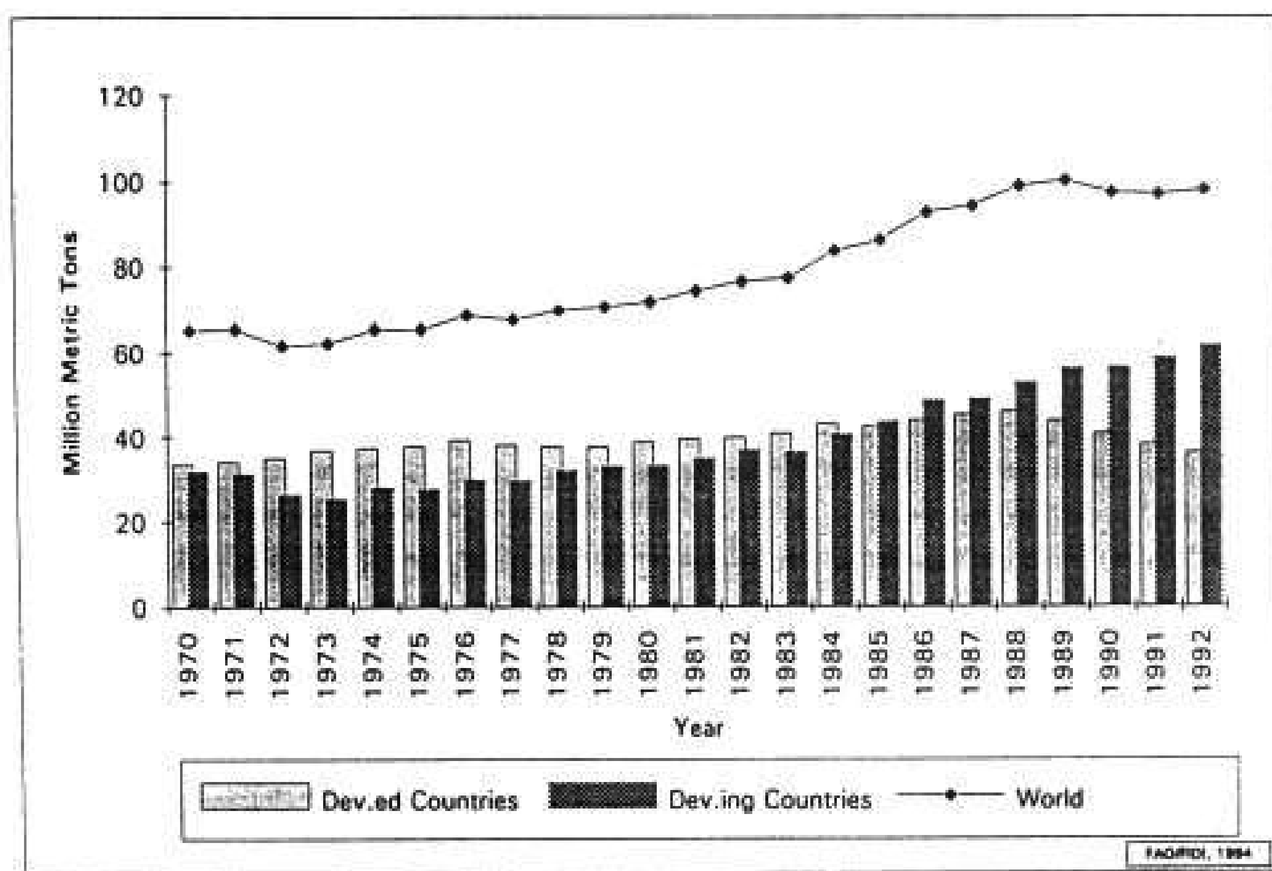


Figure 2.3 Total world fish catch from 1970 to 1992 (FAO, 1994 a)

It is clear from Figure 2.3 that the yearly increase in catches has slowed down since 1970, and the total catch reached a peak of 100 million t in 1989. Since then it has started to drop as a number of fish stocks have begun to collapse, in many cases due to overfishing. However, a slight upward trend is noticed for 1992 and for 1993 world catch is estimated to reach 101 million t. While total catch has started to decline since the peak in 1989, the catch from developing

countries as a group is still increasing and since 1985 has exceeded that from developed countries. Thus in 1992 little more than 60 % of the total world catch was taken by developing countries, and it is estimated that this figure will increase to 66% in 1993. This also means that an increasing part of the world fish catch is taken from warm tropical waters.

Are we then reaching the limits of production from "wild" aquatic resources now or do the optimistic predictions from the 1970s still hold? The answer to this question is not only in the affirmative, but for many resources the limit was reached decades earlier than the peak in global landings (FAO, 1993 a). A combination of factors has helped to mark the depletion of many conventional resources. One of these is that continued investments in fishing fleets throughout the world has meant that although catch rates and abundance of high value fish species have often declined, the overall level of fishing effort has increased so that roughly similar levels of landings are being taken at much greater cost to many fishing nations.

The real problems with decreasing fish stocks are familiar. First there is "the tragedy of the commons" - whatever lacks a known owner, whether buffalo or fish - which everyone will race to exploit and ultimately destroy.

The next problem which can be identified is the exceptionally poor management of the aquatic resources. What has been done has been too late and too little. The 1982 Law of the Sea, which extended the territorial seas from 12 to 200 miles, gave the coastal States an opportunity to take a protective interest in their fishing grounds. Instead, many of them rushed to plunder the resources by offering generous subsidies and tax relief for new vessels. Also, the much used quota-system is subject to severe criticism. Often, the net result is increased fishing and increased waste, as perfectly good fish are thrown overboard if quotas are already reached. Many fish stocks (such as pollack, haddock and halibut off New England) are now considered "commercially extinct"; that is, there are now too few fish to warrant catching.

The typical history of the use of a single fish stock has been illustrated as shown in Figure 2.4.

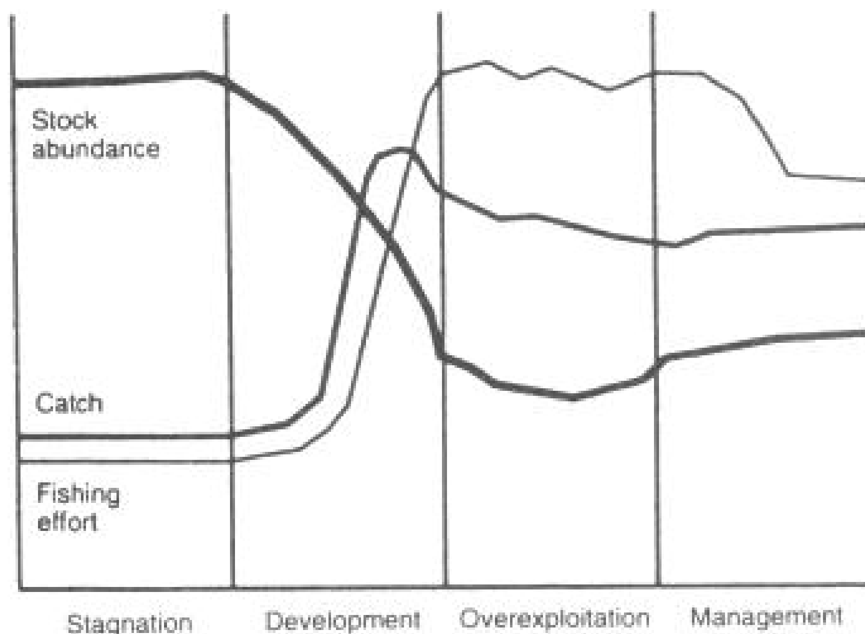


Figure 2.4 Schematic changes in stock abundance, catch and fishing effort in situations of development, overexploitation and management of fisheries. (SOURCE: Danish International Development Agency, DANIDA, 1989)

From an initial stage of under-utilization the fishing passes through a phase of rapid expansion until the limit of the resource is reached. This is then followed by a period o overfishing with high fishing effort, but reduced catches until finally - and hopefully - a phase of proper management is reached. Details on resource management are beyond the scope of this book, but should include the concept of sustainability, environmental aspects and responsible fishing. However, in an FAO publication (FAO, 1994) it is stated that change from a focus on short-term development of fishing fleets to proper management is a necessary, but insufficient condition for sustainable development. In the same report it is further stated that "Sustainable Development" as promoted at the United Nations Conference on Environment and Development (UNCED) in 1992 cannot be achieved under open-access regimes, whether these are within or outside national territorial waters.

In contrast, the world aquaculture production inclusive of aquatic plants has steadily increased over the last decade totalling 19.3 million t in 1992, almost half of this (49% is produced in marine aquaculture, 44% in inland aquaculture, and the rest in brackish environment. About 49% of world aquaculture production are fish. Production of aquati plants is increasing rapidly and reached 5.4 million t in 1992, while smaller increases if production of molluscs and crustaceans are seen (Figure 2.5). The total value of the aquaculture production is estimated to more than $US 32.5 billion in 1992.

To summarize, it can be said that further increases in supply of fish can be

expected from better utilization/ reduction of losses and further expansion of aquaculture.

Table 2.1 shows the breakdown of world fish production.

Table 2.1 Breakdown of world fish production (percentage of world total in live weight) (FAO, 1993 a)

Year	For human consumption					Other purposes Animal Feed
	Total	Fresh	Freezing	Curing	Canning	
1982	71.1	19.4	25.3	12.8	13.6	28.9
1992	72.8	27.0	24.1	9.3	12.4	27.2

Table 2.1 shows relatively modest differences in the breakdown of the fish production during the decade 1982-92. However, there was a significant increase in fresh fish consumption. Total fish for human consumption increased by 1.2% while fish used for curing and canning continued to decrease.

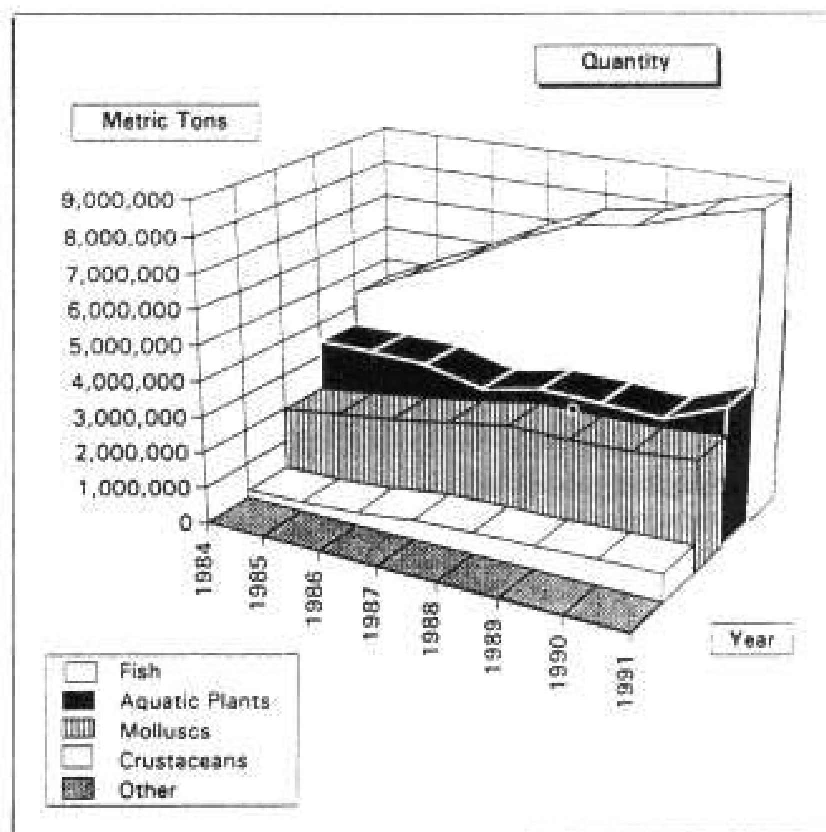
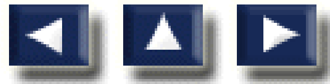


Figure 2.5 World Aquaculture Production by species category, 1984-91 (FAO, 1993 c)

In value terms, fishery exports reached an estimated $US 40.1 billion in 1993 (FISHDAB, 1994). Exports of fish and fishery products from developing countries continued to increase reaching a total value of $US 19.4 billion in 1993. In the same year exports from developed countries dropped by 5% to an estimated total value of $US 20.7 billion. Developing countries recorded an increasingly positive trade balance in fish trade, which reached $US 12.7 billion in 1993 (FISHDAB, 1994).

It should be noted that Table 2.1 does not give a true picture of the amount of fish available for human food. An enormous amount of fish is wasted due to discards on board or post-harvest losses during processing and distribution. It has been estimated that the global amount of discards is in the range of 17-39 million t/year with an average of 27 million t/year (Alverson et al., 1994). It has been further estimated that the total post-harvest losses in fish products are about 10 % (James, D., personal communication 1994). These high losses are mainly due to problems of fisheries management, and lack of proper technology and of economic incentives.



APPENDIX E

Evaluation of cooked fish

NAME:______________________________ DATE:____________________SPECIES:________________________

INSTRUCTION: Choose the best description to characterize the code
Write the corresponding value in the column to the right for the description under the right code.
Please note the choice between two sets of description for each of the three parameters:
Uppermost: Fat species (e.g., herring, sardine, anchovy, sardine, mackerel, trout, salmon ,etc.).
Lowermost: Lean species (e.g., cod, haddock, saithe, plaice, hake, etc.).

WHOLE FISH ☐

FILLET ☐

CODE INDICATION

Parameter		10	8	6	4	2	0	Code indication
SMELL	FAT	Fresh oil, marine creamy, weak odour	Oily, rancid, musty, burnt, slightly brown oil	Rancid, cheesy, slightly sour	Very rancid, sweaty, cheesy, sour, stale meat	Very rancid sour	spoilt, rotten	
	LEAN	Species-specific.	Fresh fish, seaweed	Condensed milk, slightly fishy, slightly sour	Sour, stale, cabbage, sulphide	Rotten, spoilt, strong ammonia		
TASTE	FAT	Fresh oil, sweet, meaty, creamy	Oily, sweet, meaty, creamy, burnt, neutral	Oily, sweet, stale meat, creamy, rancid, sour	Rancid, sweet, cheesy, slightly bitter, sour fruit	Very rancid, bitter		
	LEAN	Meaty flavour, sweet, watery, initially no sweetness	Sweet and characteristic but reduces in intensity	Insipid - neutral, slightly fishy	Musty, fishy, slight sour, some off-flavours	Slight rotten, sour, sulphide	Rotten, spoilt	
TEXTURE	FAT	Firm, elastic, springy, flaky, juicy	Firm, springy, juicy, slightly dry, a bit stringy/fibrous	Less firm, less juicy, a bit stringy/fibrous	Dry and fibrous	Dry and tough		
	LEAN	Firm, elastic springy, flaky, juicy	Firm, springy, juicy	Less firm, less juicy	Softer, gritty	Very soft	Slippery, sloppy, greasy	

10	8	6	4	2	0
			Rejection level	OVERALL QUALITY SCORE	

3. BIOLOGICAL ASPECTS

3.1. Classification
3.2. Anatomy and physiology
3.3. Growth and reproduction

3.1 Classification

Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Thurman and Webber, 1984).

Five vertebrate classes have species which could be called fish, but only two of these groups - the sharks and rays, and the bonyfish - are generally important and widely distributed in the aquatic environment. The evolutionary relationship between the various groups of fish is shown in Figure 3.1.

Fish are the most numerous of the vertebrates, with at least 20 000 known species, and more than half (58 %) are found in the marine environment. They are most common in the warm and temperate waters of the continental shelves (some 8 000 species). In the cold polar waters about 1 100 species are found. In the oceanic pelagic environment well away from the effect of land, there are only some 225 species. Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1 000 m depth) the number of species increases. There are some 1 000 species of so-called mid- water fish (Thurman and Webber, 1984).

Classifying all these organisms into a system is not an easy task, but the taxonomist groups organisms into natural units that reflect evolutionary relationships. The smallest unit is the species. Each species is identified by a scientific name which has two parts the genus and the specific epithet (binominal nomenclature). The genus name is always capitalized and both are italicized. As an example, the scientific (species) name of the common dolphin is *Delphinus delphis.* The genus is a category that contains one or more species, while the next step in the hierarchy is

the family which may contain one or more genus. Thus the total hierarchical system is: Kingdom: Phylum: Class: Order: Family: Genus: Species.

The use of common or local names often creates confusion since the same species may have different names in different regions or, conversely, the same name is ascribed to several different species, sometimes with different technological properties. As a point of reference the scientific name should, therefore, be given in any kind of publication or report the first time a particular species is referred to by its common name. For further information see the International Council for the Exploration of the Sea "List of names of Fish and Shellfish" (ICES, 1966); the "Multilingual Dictionary of Fish and Fish Products" prepared by the Organisation for Economic Cooperation and Development (OECD, 1990) and the "Multilingual Illustrated Dictionary of Aquatic Animals and Plants" (Commission of the European Communities, 1993).

The classification of fish into cartilaginous and bony (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently (section 5) and vary with regard to chemical composition (section 4).

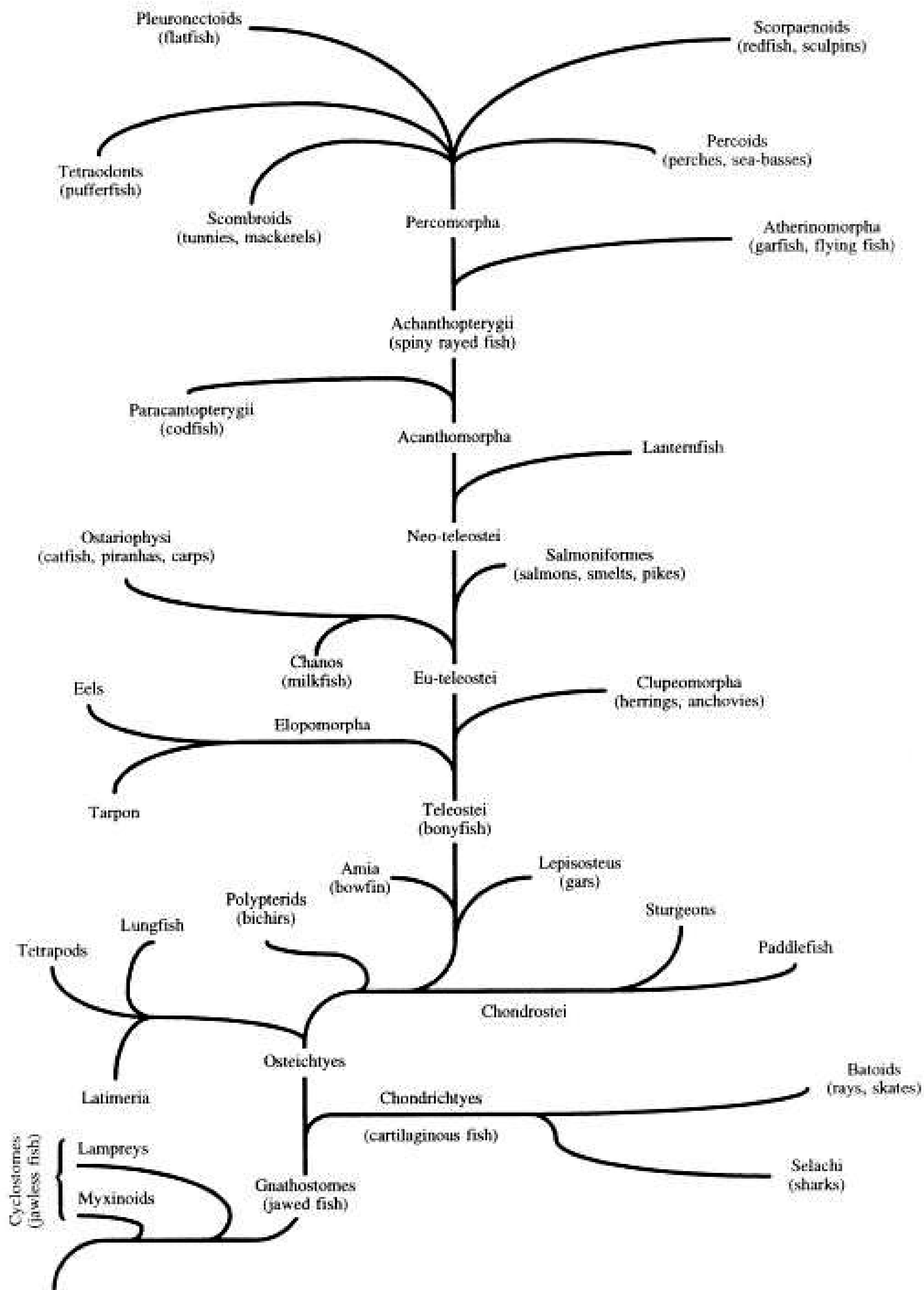


Figure 3.1 Simplified phylogenetic tree of the fishes. (Examples of food-fish, using common English names are shown in parantheses). (SOURCE: N. Bonde (1994),

Geological Inst., Copenhagen)

Furthermore, fish can be divided into fatty and lean species, but this type of classification is based on biological and technological characteristics as shown in Table 3.1.

Table 3.1 Classification of fish

Scientific grouping	Biological characteristics	Technological characteristics	Examples
Cyclostomes	jawless fish		lampreys, slime-eels
Chondrichthyes	cartilaginous fish	high urea content in muscle	sharks, skate, rays
Teleostei or bony fish	pelagic fish	fatty fish (store lipids in body tissue)	herring, mackerel, sardine tuna, sprat
	demersal fish	lean (white) fish (store lipids in liver only)	cod, haddock, hake grouper, seabass

3.2 Anatomy and physiology

The skeleton

Being vertebrates, fish have a vertebral column - the backbone - and a cranium covering the brain. The backbone runs from the head to the tail fin and is composed of segments (vertebrae). These vertebrae are extended dorsally to form neural spines, and in the trunk region they have lateral processes that bear ribs (Figure 3.2). The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes) (see also Figure 3.3). Usually, there is also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise prepared for food.

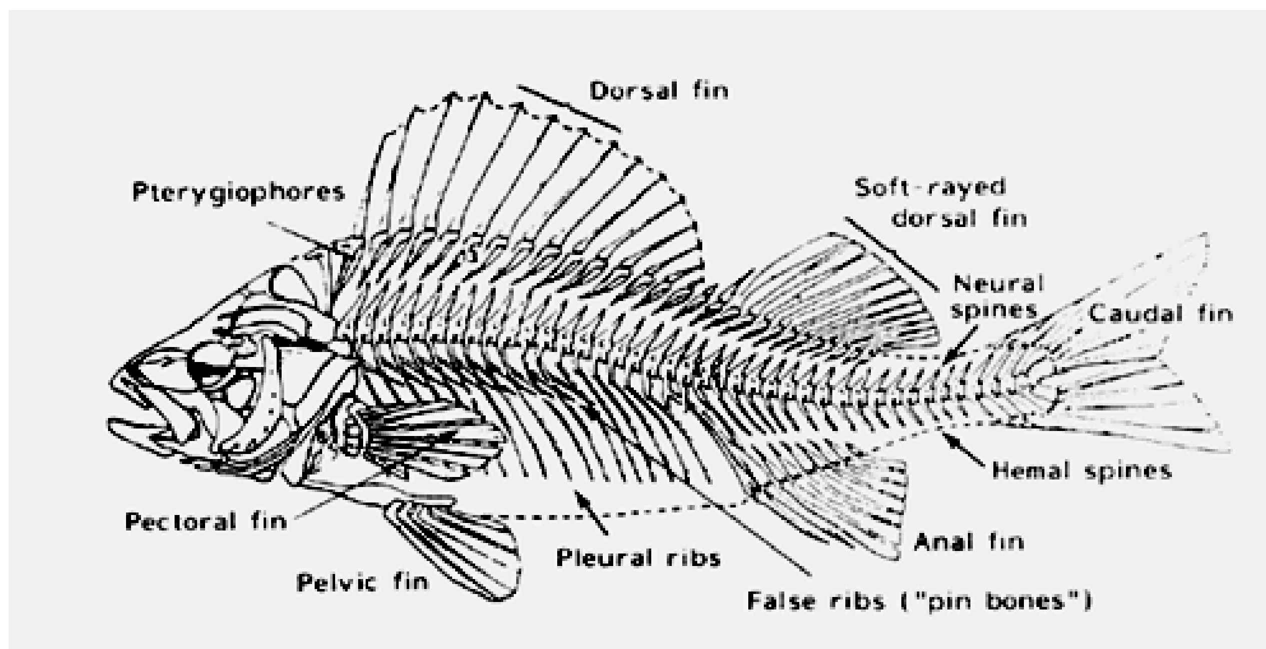


Figure 3.2 Skeleton of bonyfish (Eriksson and Johnson, 1979)

Muscle anatomy and function

The anatomy of fish muscle is different from the anatomy of terrestrial mammals, in that the fish lacks the tendinous system connecting muscle bundles **to the skeleton of the** animal. Instead, fish has muscle cells running in parallel and connected to sheaths of connective tissue (myocommata), which are anchored to the skeleton and the skin. The bundles of parallel muscle cells are called myotomes (Figure 3.3).

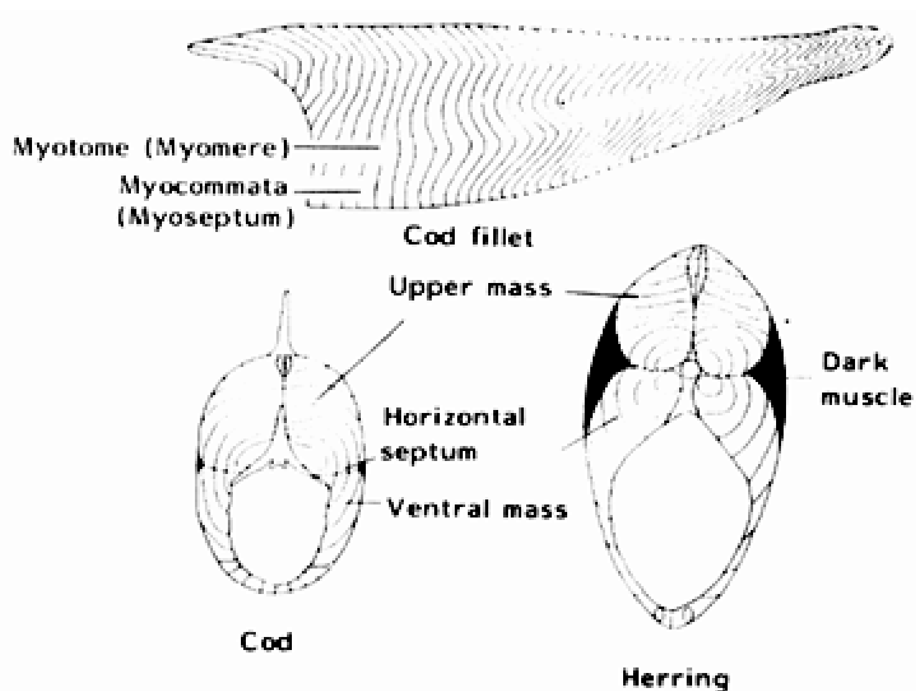


Figure 3.3 Skeletal musculature of fish (Knorr, 1974)

All muscle cells extend the full length between two myocommata, and run parallel with the longitudinal direction of the fish. The muscle mass on each side of the fish makes up the fillet, of which the upper part is termed the dorsal muscle and the lower part the ventral muscle.

The fillet is heterogenous in that the length of the muscle cells vary from the head end (anterior) to the tail end (posterior). The longest muscle cells in cod are found at about the twelfth myotome counting from the head, with an average length around 10 mm in a fish that is 60 cm long (Love, 1970). The diameter of the cells also vary, being widest in the ventral part of the fillet.

The myocommata run in an oblique, almost "plow-like" pattern perpendicular to the long axis of the fish, from the skin to the spine. This anatomy is ideally suited for the flexing muscle movements necessary for propelling the fish through the water.

As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, i.e., the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc., and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating system making the muscle appear striated upon microscopic examination (Figure 3.4).

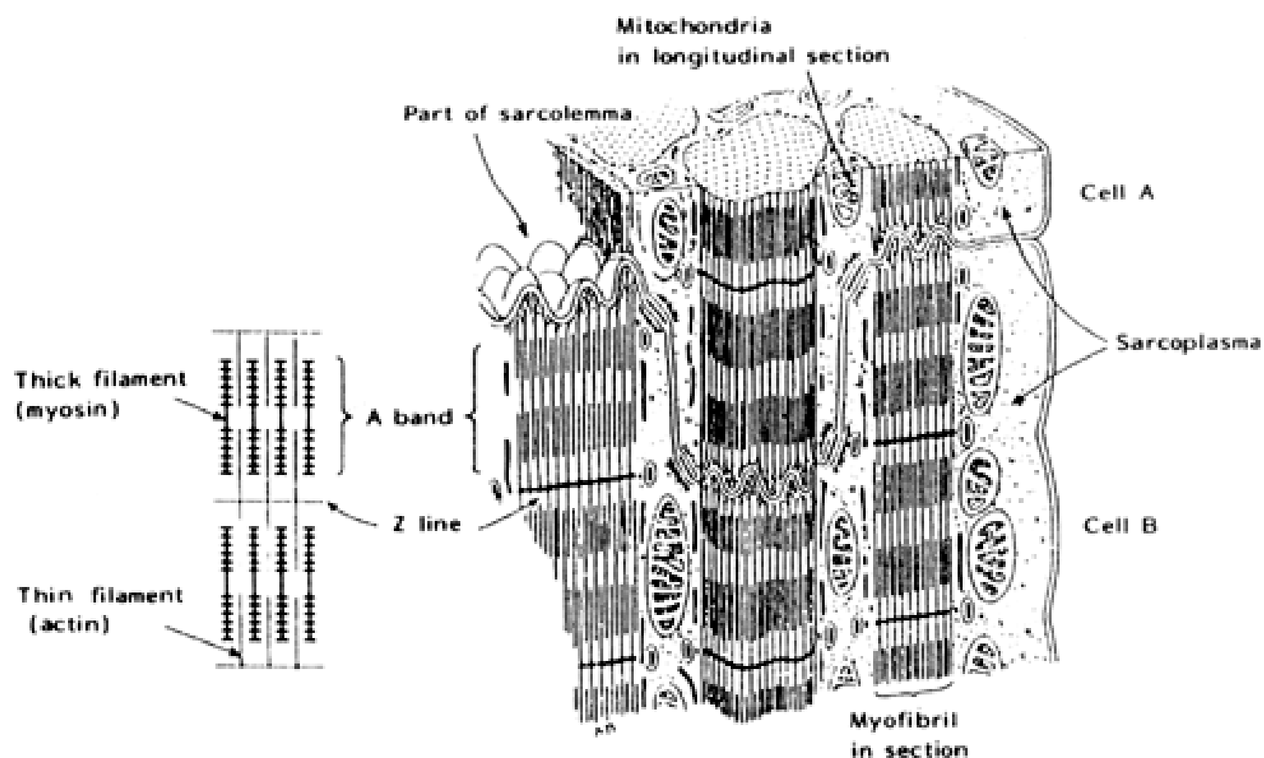


Figure 3.4 Section of a cell showing various structures including the myofibrils (Bell *et al., 1976)*

Most fish muscle tissue is white but, depending on the species, many fish will have a certain amount of dark tissue of a brown or reddish colour. The dark muscle is located just under the skin along the side of the body.

The proportion of dark to light muscle varies with the activity of the fish. In pelagic fish, i.e., species such as herring and mackerel which swim more or less continuously, up to 48 % of the body weight may consist of dark muscle (Love, *1970).* In demersal fish, i.e., species which feed on the bottom and only move periodically, the amount of dark muscle is very small.

There are many differences in the chemical composition of the two muscle types, some of the more noteworthy being higher levels of lipids and myoglobin in the dark muscle.

From a technological point of view, the high lipid content of dark muscle is important because of problems with rancidity.

The reddish meat colour found in salmon and sea trout does not originate from myoglobin but is due to the red carotenoid, astaxanthin. The function of this pigment has not been clearly established, but it has been proposed that the carotenoid may play a role as an antioxidant. Further, the accumulation in the muscle may function as a depot for pigment needed at the time of spawning when the male develops a strong red colour in the skin and the female transport carotenoids into the eggs. The latter seems to depend heavily on the amount of carotenoids for proper development after fertilization. It is clearly seen that the muscle colour of salmonids fades at the time of spawning.

The fish cannot synthesize astaxanthin and is thus dependent on ingestion of the pigment through the feed. Some salmonids live in waters where the natural prey does not contain much carotenoid, e.g., in the Baltic Sea, thus resulting in a muscle colour less red than salmonids from other waters. This may be taken as an indication that the proposed physiological function of astaxanthin in salmonids explained above may be less important.

In salmon aquaculture, astaxanthin is included in the feed, as the red colour of the flesh is one of the most important quality criteria for this species.

Muscle contraction starts when a nervous impulse sets off a release of Ca + + from the sarcoplasmic reticulum to the myofibrils. When the Ca + + concentration increases at the active enzyme site on the myosin filament, the enzyme ATP-ase is activated. This ATP-ase splits the ATP found between the actin and myosin filaments, causing a release of energy. Most of this energy is used as contractile

energy making the actin filaments slide in between the myosin filaments in a telescopic fashion, thereby contracting the muscle fibre. When the reaction is reversed (i.e., when the Ca + + is pumped back, the contractile ATP-ase activity stops and the filaments are allowed to slip passively past each other), the muscle is relaxed.

The energy source for ATP generation in the light muscle is glycogen, whereas the dark muscle may also use lipids. A major difference is, further, that the dark muscle contains much more mitochondria than light muscle, thus enabling the dark muscle to operate an extensive aerobic energy metabolism resulting in CO_2 and H_2O as the end products. The light muscle, mostly generating energy by the anaerobic metabolism, accumulates lactic acid which has to be transported to the liver for further metabolization. In addition, the dark muscle is reported to possess functions similar to those are found in the liver.

The different metabolic patterns found in the two muscle types makes the light muscle excellently fitted for strong, short muscle bursts, whereas the dark muscle is designed for continual, although not so strong muscle movements.

Post mortem the biochemical and physiological regulatory functions operating in vivo ceases, and the energy resources in the muscle are depleted. When the level of ATP reaches its minimum, myosin and actin are interconnected irreversibly, resulting in rigor mortis. This phenomenon is further described in section 5.

The cardiovascular system

The cardiovascular system is of considerable interest to the fish technologist since it is important in some species to bleed the fish (i.e., remove most of the blood) after capture.

The fish heart is constructed for single circulation (Figure 3.5). In bony fish it consists of two consecutive chambers pumping venous blood toward the gills via the ventral aorta.

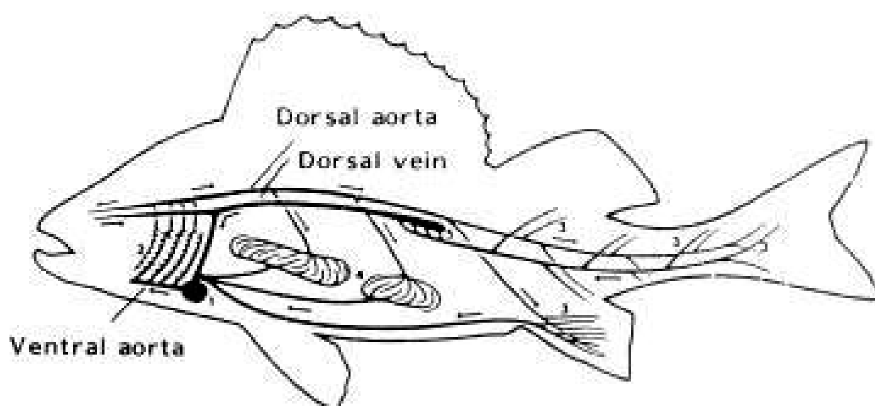


Figure 3.5 Blood circulation in fish (Eriksson and Johnson, 1979)

Notes:

1. The heart pumps blood toward the gills.
2. The blood is aerated in the gills.
3. Arterial blood is dispersed into the capillaries where the transfer of oxygen and nutrients to the surrounding tissue takes place.
4. The nutrients from ingested food are absorbed from the intestines, then transported to the liver and later dispersed in the blood throughout the body.
5. In the kidneys the blood is "purified" and waste products are excreted via the urine.

After being aerated in the gills, the arterial blood is collected in the dorsal aorta running just beneath the vertebral column and from here it is dispersed into the different tissues via the capillaries. The venous blood returns to the heart, flowing in veins of increasingly larger size (the biggest is the dorsal vein which is also located beneath the vertebral column). The veins all gather into one blood vessel before entering the heart. The total volume of the blood in fish ranges from 1.5 to 3.0 % of the body weight. Most of it is located in the internal organs while the muscular tissues, constituting two- thirds of the body weight, contain only 20 % of the blood volume. This distribution is not changed during exercise since the light muscle in particular is not very vascularized.

During blood circulation the blood pressure drops from around 30 mg Hg in the ventral aorta to 0 when entering the heart (Randall, 1970). After the blood has passed through the gills, the blood pressure derived from the pumping activity of the heart is already greatly decreased. Muscle contractions are important in pumping the blood back to the heart and counterflow is prevented by a system of paired valves inside the veins.

Clearly, the single circulation of fish is fundamentally different from the system in mammals (Figure 3.6), where the blood passes through the heart twice and is propelled out into the body under high pressure due to the contractions of the heart.

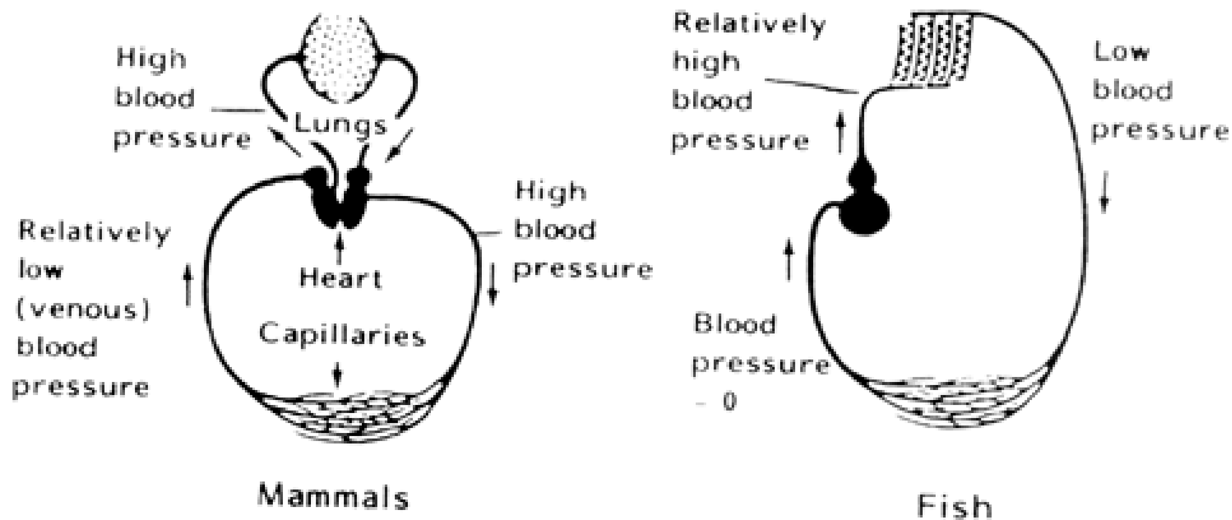


Figure 3.6 Blood circulation in fish and mammals (Eriksson and Johnson, 1979)

In fish, the heart does not play an important role in the transportation of blood from the capillaries back to the heart. This has been confirmed in an experiment where the impact of different bleeding procedures on the colour of cod fillets was examined. No difference could be found regardless of whether the fish had been bled by means of cutting the throat in front of or behind the heart before gutting, or had not been cut at all before slaughter.

In some fisheries, bleeding of the fish is very important as a uniform white fillet is desirable. In order to obtain this, a number of countries have recommended that fish are bled for a period (15-20 min) prior to being gutted. This means that throat cutting and gutting must be carried out in two separate operations and that special arrangements (bleeding tanks) must be provided on deck. This complicates the working process (two operations instead of one), time-consuming for the fishermen and increases the time-lag before the fish is chilled. Furthermore it requires extra space on an otherwise crowded working deck.

Several researchers have questioned the necessity of handling the fish in a two-step procedure involving a special bleeding period (Botta *et al.,* 1986; Huss and Asenjo, 1977 a; Valdimarsson *et al.* 1984). There seems to be general agreement about the following:

- bleeding is more affected by time onboard prior to bleeding/gutting than by the actual bleeding/gutting procedure.
- best bleeding is obtained if live fish are handled, but it is of major importance to cut the fish before it enters rigor mortis since it is the muscle contractions that force the blood out of the tissues.

Disagreement exists as to the cutting method. Huss and Asenjo (1977 a) found best

bleeding if a deep throat cut including the dorsal aorta was applied, but this was not confirmed in the work of Botta *et al.* (1986). The latter also recommended to include a bleeding period (two-step procedure) when live fish were handled (fishing with pound net, trap, seine, longline or jigging), while Valdimarsson *et al.* (1984) found that the quality of dead cod (4 h after being brought onboard) was slightly improved using the two-step procedure. However, it should be pointed out that the effect of bleeding should also be weighted against the advantages of having a fast and effective handling procedure resulting in rapid chilling of the catch.

Discoloration of the fillet may also be a result of rough handling during catch and catch handling while the fish is still alive. Physical mishandling in the net (long trawling time, very large catches) or on the deck (fishermen stepping on the fish or throwing boxes, containers and other items on top of the fish) may cause bruises, rupture of blood vessels and blood oozing into the muscle tissue (haematoma).

Heavy pressure on dead fish, when the blood is clotted (e.g., overloading of fish boxes) does not cause discoloration, but the fish may suffer a serious weight loss.

Other organs

Among the other organs, only the roe and liver play a major role as foodstuffs. Their size depends on the fish species and varies with life cycle, feed intake and season. In cod the weight of the roe varies from a few percent up to 27 % of the body weight and the weight of the liver ranges from 1 to 4.5 %. Likewise, the composition can change and the oil content of the liver vary from 15 to 75 %, with the highest values being found during autumn (Jangaard *et al.,* 1967).

3.3 Growth and reproduction

During growth it is the size of each muscle cell that increases rather than the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a size characteristic of the species and is this not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to rear female fish in aquaculture.

Every year mature fish use energy to build up the gonads (the roe and milk). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake (Figure 3.7).

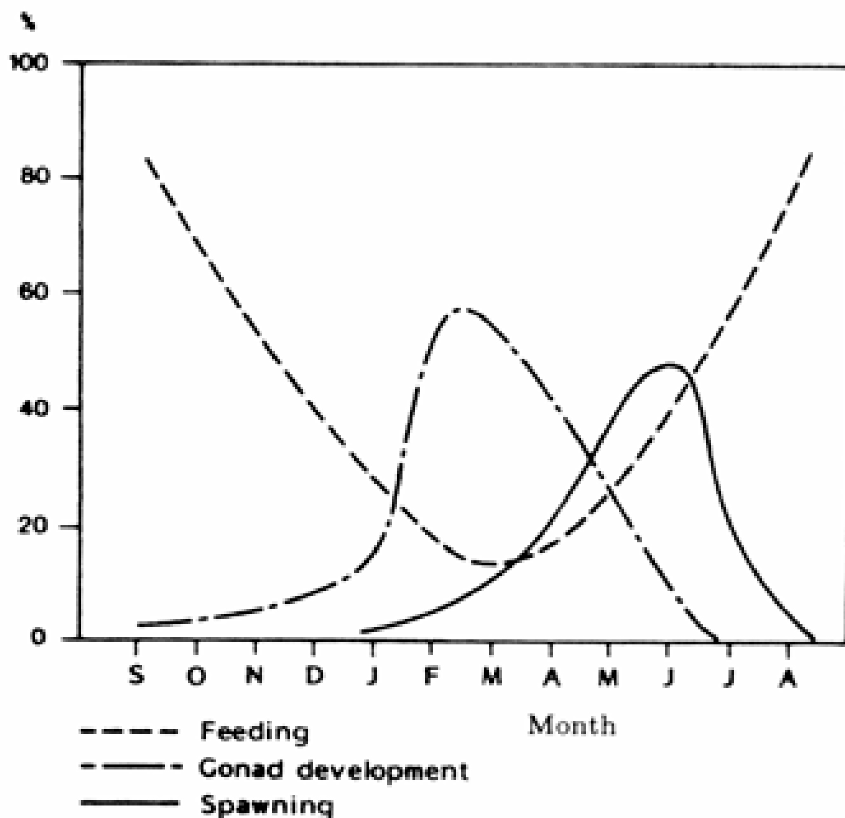


Figure 3.7 Relation between feeding cycle (percentage sample with food in stomach) and reproductive cycle (gonad development), percentage fish with ripening gonads (spawning, percentage ripe fish) of haddock *(Melanogrammus aeglefinus).* It should be noted that the development of the gonads takes place while the fish is starving (Hoar, 1957).

In North Sea cod it was found that prior to spawning the water content of the muscle increases (Figure 3.8) and the protein content decreases. In extreme cases the water content of very large cod can attain 87 % of the body weight prior to spawning (Love, 1970).

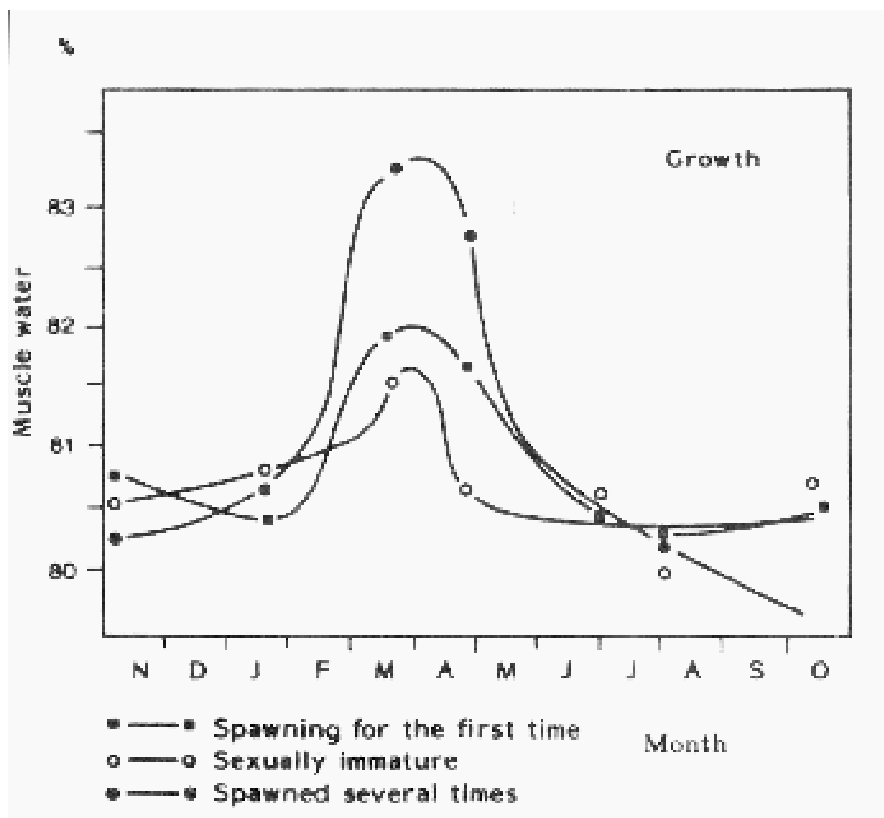
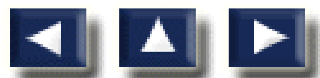


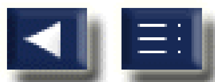
Figure 3.8 Water content of cod muscle *(Gadus morhua)* (Love, 1970)

The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity (Figure 3.7), while some have ripe ovaries for nearly the whole year.

The depletion of the reserves of the fish during gonadal development can be extremely severe, especially if reproduction is combined with migration to the breeding grounds. Some species, e.g., Pacific salmon *(Oncorhynchus* spp.), eel *(Anguilla anguilla)* and others, manage to migrate only once, after which they degenerate and die. This is partly because these species do not eat during migration so that, in the case of a salmon, it can lose up to 92 % of its lipid, 72 % of its protein and 63 % of its ash content during migration and reproduction (Love, 1970).

On the other hand, other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about eight years before spawning causes its death, and other species can live even longer (Cushing, 1975). In former times, 25-year-old herring *(Clupea harengus)* were not unusual in the Norwegian Sea, and plaice *(Pleuronectes platessa) up* to 35 years old have been found. One of the oldest fish reported was a sturgeon *(Acipenser sturio)* from Lake Winnebago in Wisconsin. According to the number of rings in the otolith, it was over 100 years old.





APPENDIX F

Quality test using structured scaling

Two samples (A and B) of cooked fish were evaluated by 10 assessors using the scheme fig. 8.3. The following overall quality score was obtained.

	Treatments		Difference
Assessor	A	B	A - B
1	7	7	0
2	8	7	1
3	6	5	1
4	7	5	2
5	7	5	2
6	8	6	2
7	8	6	2
8	8	6	2
9	7	5	2
10	8	7	1
Total	74	59	15
Mean	7.4	15.9	1.5

Standard deviation

$$s = \sqrt{(\Sigma\ difference^2 - \Sigma\ difference^2/n)}/(n - 1)$$

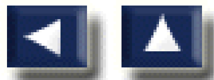
n is number of assessor

$$t = \text{Mean difference} / (s/\sqrt{n})$$

t = 1.5/(1.643/3.165) = 2.889

From Table with n - 1 degree of freedom (probability 0.05%) t is 2.622.

Sample A is significantly different as t(2.889)> t table(2.622)



3. BIOLOGICAL ASPECTS

3.1. Classification
3.2. Anatomy and physiology
3.3. Growth and reproduction

3.1 Classification

Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Thurman and Webber, 1984).

Five vertebrate classes have species which could be called fish, but only two of these groups - the sharks and rays, and the bonyfish - are generally important and widely distributed in the aquatic environment. The evolutionary relationship between the various groups of fish is shown in Figure 3.1.

Fish are the most numerous of the vertebrates, with at least 20 000 known species, and more than half (58 %) are found in the marine environment. They are most common in the warm and temperate waters of the continental shelves (some 8 000 species). In the cold polar waters about 1 100 species are found. In the oceanic pelagic environment well away from the effect of land, there are only some 225 species. Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1 000 m depth) the number of species increases. There are some 1 000 species of so-called mid- water fish (Thurman and Webber, 1984).

Classifying all these organisms into a system is not an easy task, but the taxonomist groups organisms into natural units that reflect evolutionary relationships. The smallest unit is the species. Each species is identified by a scientific name which has two parts the genus and the specific epithet (binominal nomenclature). The genus name is always capitalized and both are italicized. As an example, the scientific (species) name of the common dolphin is *Delphinus delphis.* The genus is a category that contains one or more species, while the next step in the hierarchy is

the family which may contain one or more genus. Thus the total hierarchical system is: Kingdom: Phylum: Class: Order: Family: Genus: Species.

The use of common or local names often creates confusion since the same species may have different names in different regions or, conversely, the same name is ascribed to several different species, sometimes with different technological properties. As a point of reference the scientific name should, therefore, be given in any kind of publication or report the first time a particular species is referred to by its common name. For further information see the International Council for the Exploration of the Sea "List of names of Fish and Shellfish" (ICES, 1966); the "Multilingual Dictionary of Fish and Fish Products" prepared by the Organisation for Economic Cooperation and Development (OECD, 1990) and the "Multilingual Illustrated Dictionary of Aquatic Animals and Plants" (Commission of the European Communities, 1993).

The classification of fish into cartilaginous and bony (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently (section 5) and vary with regard to chemical composition (section 4).

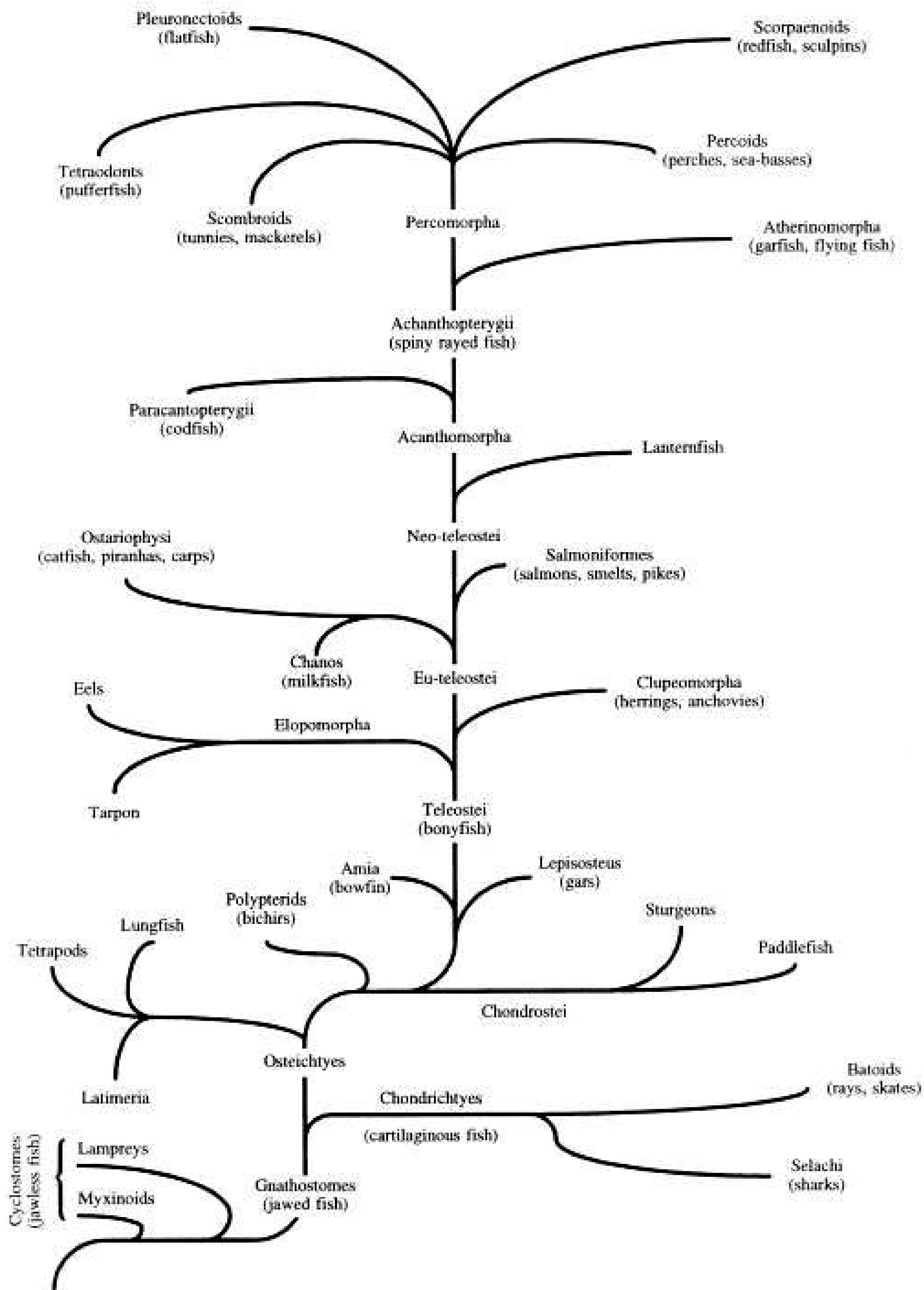


Figure 3.1 Simplified phylogenetic tree of the fishes. (Examples of food-fish, using common English names are shown in parantheses). (SOURCE: N. Bonde (1994),

Geological Inst., Copenhagen)

Furthermore, fish can be divided into fatty and lean species, but this type of classification is based on biological and technological characteristics as shown in Table 3.1.

Table 3.1 Classification of fish

Scientific grouping	**Biological characteristics**	**Technological characteristics**	**Examples**
Cyclostomes	jawless fish		lampreys, slime-eels
Chondrichthyes	cartilaginous fish	high urea content in muscle	sharks, skate, rays
Teleostei or bony fish	pelagic fish	fatty fish (store lipids in body tissue)	herring, mackerel, sardine tuna, sprat
	demersal fish	lean (white) fish (store lipids in liver only)	cod, haddock, hake grouper, seabass

3.2 Anatomy and physiology

The skeleton

Being vertebrates, fish have a vertebral column - the backbone - and a cranium covering the brain. The backbone runs from the head to the tail fin and is composed of segments (vertebrae). These vertebrae are extended dorsally to form neural spines, and in the trunk region they have lateral processes that bear ribs (Figure 3.2). The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes) (see also Figure 3.3). Usually, there is also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise prepared for food.

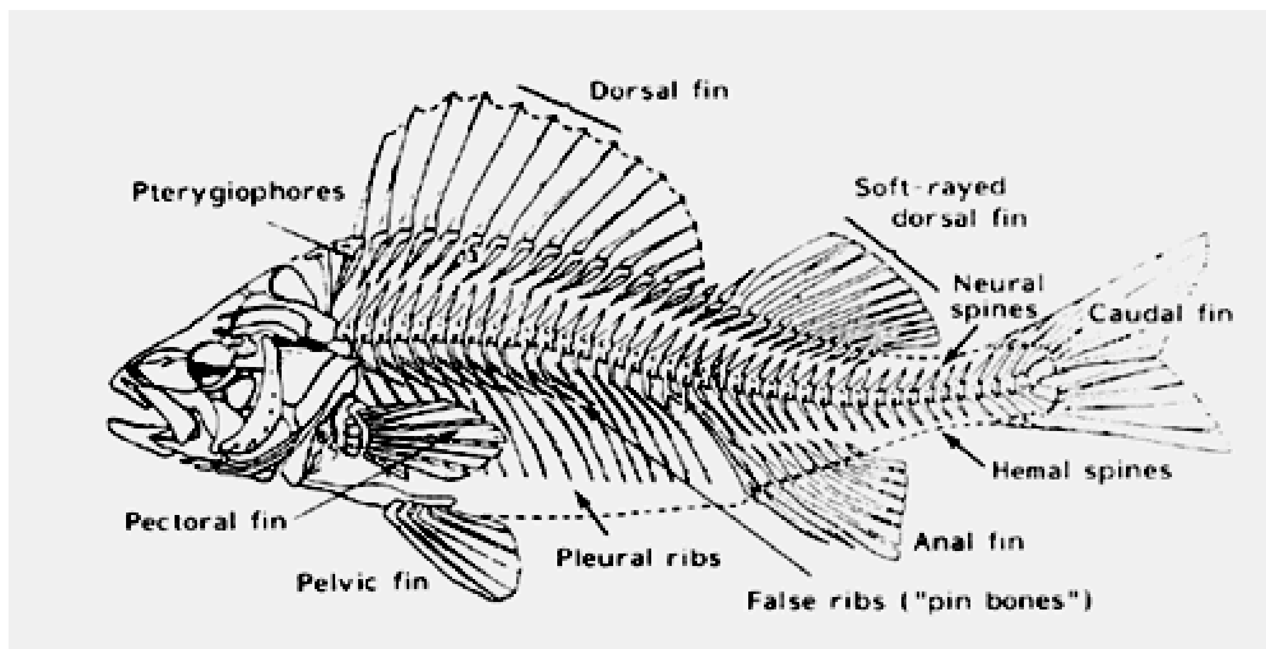
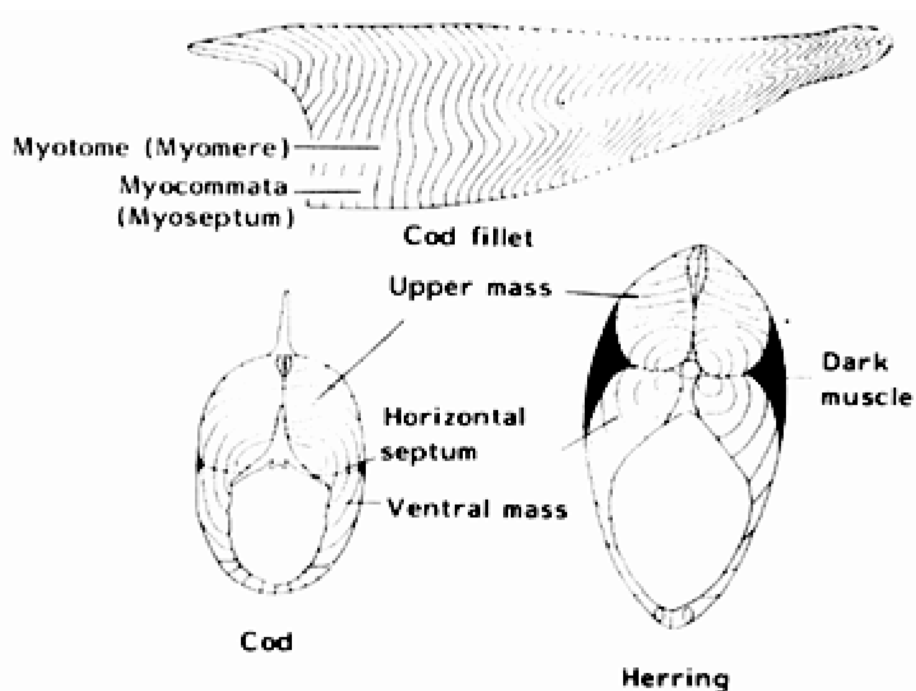


Figure 3.2 Skeleton of bonyfish (Eriksson and Johnson, 1979)

Muscle anatomy and function

The anatomy of fish muscle is different from the anatomy of terrestrial mammals, in that the fish lacks the tendinous system connecting muscle bundles **to the skeleton of the** animal. Instead, fish has muscle cells running in parallel and connected to sheaths of connective tissue (myocommata), which are anchored to the skeleton and the skin. The bundles of parallel muscle cells are called myotomes (Figure 3.3).



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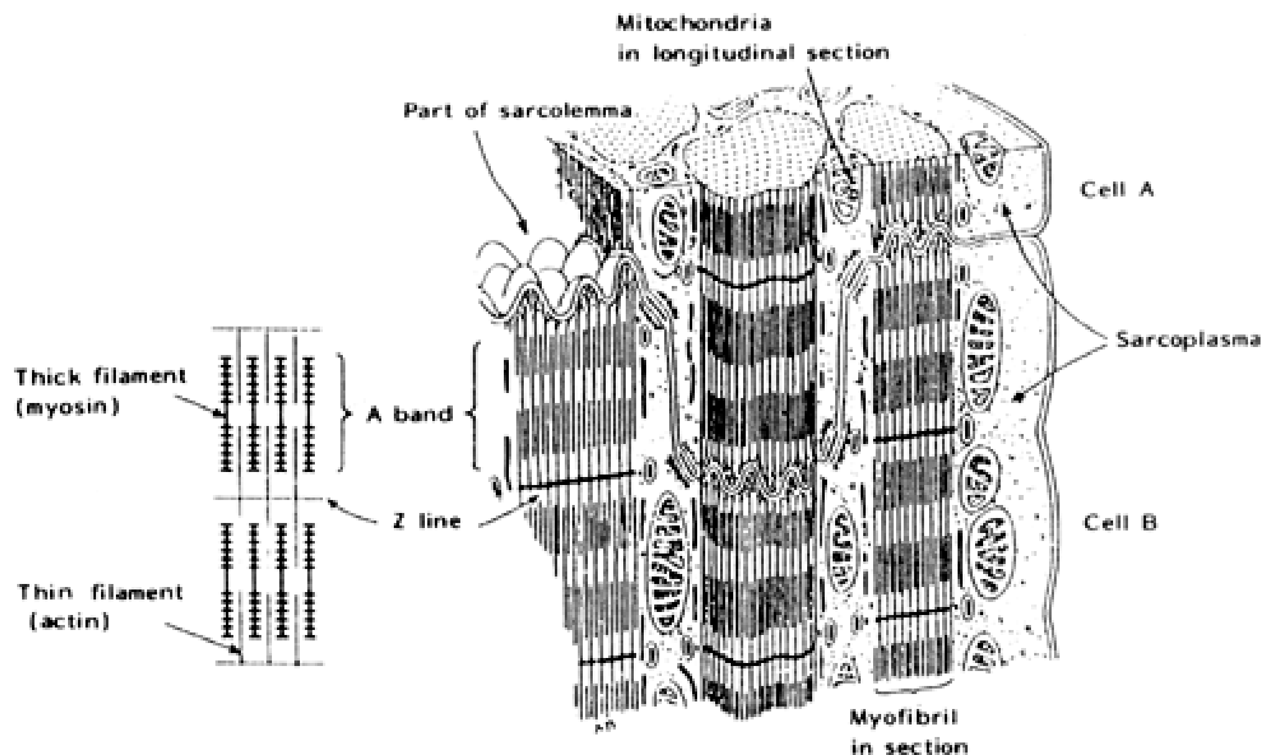
As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, i.e., the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc., and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating system making the muscle appear striated upon microscopic examination (Figure 3.4).



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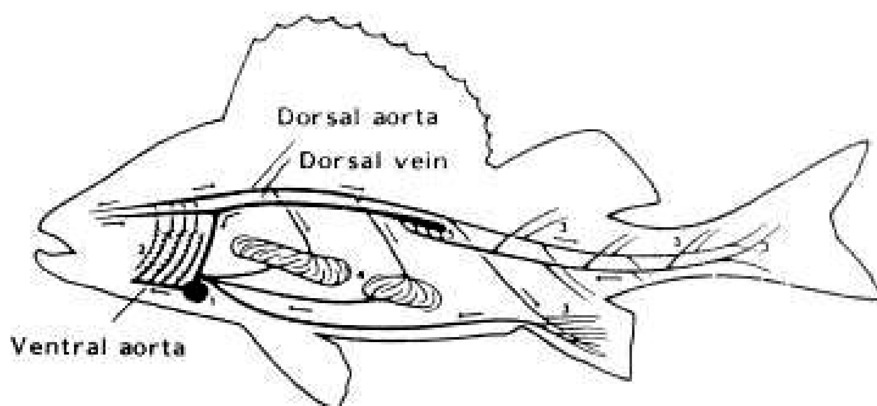

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5. In the kidneys the blood is "purified" and waste products are excreted via the urine.

After being aerated in the gills, the arterial blood is collected in the dorsal aorta running just beneath the vertebral column and from here it is dispersed into the different tissues via the capillaries. The venous blood returns to the heart, flowing in veins of increasingly larger size (the biggest is the dorsal vein which is also located beneath the vertebral column). The veins all gather into one blood vessel before entering the heart. The total volume of the blood in fish ranges from 1.5 to 3.0 % of the body weight. Most of it is located in the internal organs while the muscular tissues, constituting two- thirds of the body weight, contain only 20 % of the blood volume. This distribution is not changed during exercise since the light muscle in particular is not very vascularized.

During blood circulation the blood pressure drops from around 30 mg Hg in the ventral aorta to 0 when entering the heart (Randall, 1970). After the blood has passed through the gills, the blood pressure derived from the pumping activity of the heart is already greatly decreased. Muscle contractions are important in pumping the blood back to the heart and counterflow is prevented by a system of paired valves inside the veins.

Clearly, the single circulation of fish is fundamentally different from the system in mammals (Figure 3.6), where the blood passes through the heart twice and is propelled out into the body under high pressure due to the contractions of the heart.

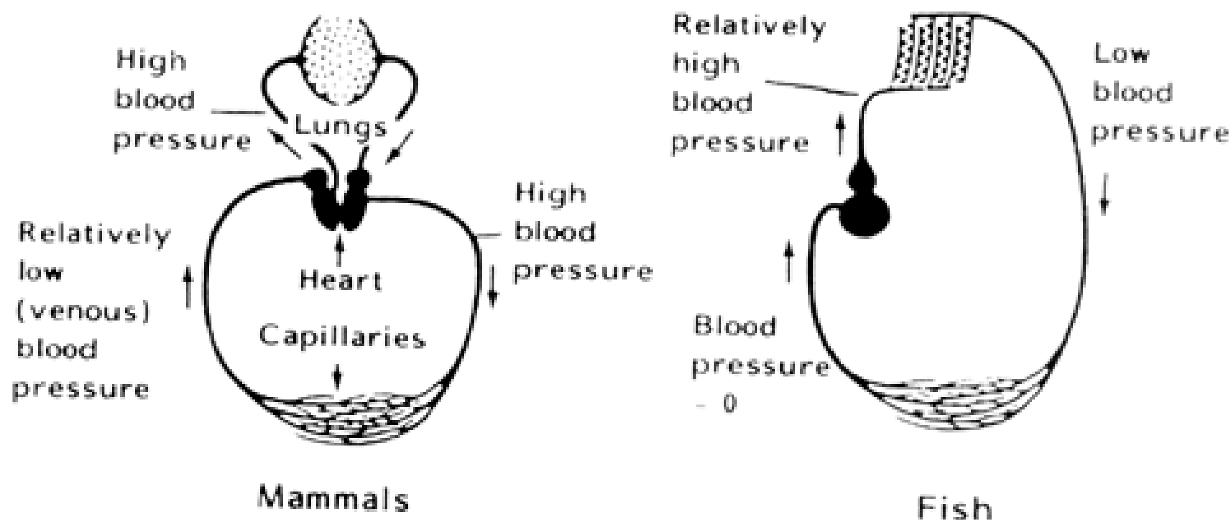


Figure 3.6 Blood circulation in fish and mammals (Eriksson and Johnson, 1979)

In fish, the heart does not play an important role in the transportation of blood from the capillaries back to the heart. This has been confirmed in an experiment where the impact of different bleeding procedures on the colour of cod fillets was examined. No difference could be found regardless of whether the fish had been bled by means of cutting the throat in front of or behind the heart before gutting, or had not been cut at all before slaughter.

In some fisheries, bleeding of the fish is very important as a uniform white fillet is desirable. In order to obtain this, a number of countries have recommended that fish are bled for a period (15-20 min) prior to being gutted. This means that throat cutting and gutting must be carried out in two separate operations and that special arrangements (bleeding tanks) must be provided on deck. This complicates the working process (two operations instead of one), time-consuming for the fishermen and increases the time-lag before the fish is chilled. Furthermore it requires extra space on an otherwise crowded working deck.

Several researchers have questioned the necessity of handling the fish in a two-step procedure involving a special bleeding period (Botta *et al.,* 1986; Huss and Asenjo, 1977 a; Valdimarsson *et al.* 1984). There seems to be general agreement about the following:

- bleeding is more affected by time onboard prior to bleeding/gutting than by the actual bleeding/gutting procedure.
- best bleeding is obtained if live fish are handled, but it is of major importance to cut the fish before it enters rigor mortis since it is the muscle contractions that force the blood out of the tissues.

Disagreement exists as to the cutting method. Huss and Asenjo (1977 a) found best

bleeding if a deep throat cut including the dorsal aorta was applied, but this was not confirmed in the work of Botta *et al.* (1986). The latter also recommended to include a bleeding period (two-step procedure) when live fish were handled (fishing with pound net, trap, seine, longline or jigging), while Valdimarsson *et al.* (1984) found that the quality of dead cod (4 h after being brought onboard) was slightly improved using the two-step procedure. However, it should be pointed out that the effect of bleeding should also be weighted against the advantages of having a fast and effective handling procedure resulting in rapid chilling of the catch.

Discoloration of the fillet may also be a result of rough handling during catch and catch handling while the fish is still alive. Physical mishandling in the net (long trawling time, very large catches) or on the deck (fishermen stepping on the fish or throwing boxes, containers and other items on top of the fish) may cause bruises, rupture of blood vessels and blood oozing into the muscle tissue (haematoma).

Heavy pressure on dead fish, when the blood is clotted (e.g., overloading of fish boxes) does not cause discoloration, but the fish may suffer a serious weight loss.

Other organs

Among the other organs, only the roe and liver play a major role as foodstuffs. Their size depends on the fish species and varies with life cycle, feed intake and season. In cod the weight of the roe varies from a few percent up to 27 % of the body weight and the weight of the liver ranges from 1 to 4.5 %. Likewise, the composition can change and the oil content of the liver vary from 15 to 75 %, with the highest values being found during autumn (Jangaard *et al.,* 1967).

3.3 Growth and reproduction

During growth it is the size of each muscle cell that increases rather than the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a size characteristic of the species and is this not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to rear female fish in aquaculture.

Every year mature fish use energy to build up the gonads (the roe and milk). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake (Figure 3.7).

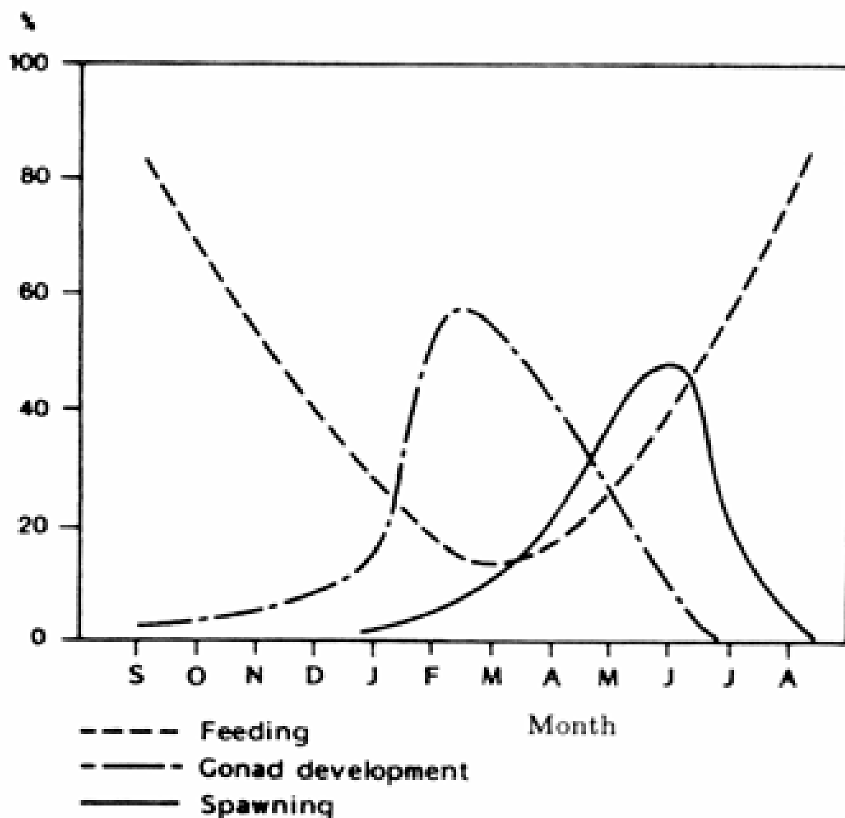


Figure 3.7 Relation between feeding cycle (percentage sample with food in stomach) and reproductive cycle (gonad development), percentage fish with ripening gonads (spawning, percentage ripe fish) of haddock *(Melanogrammus aeglefinus).* It should be noted that the development of the gonads takes place while the fish is starving (Hoar, 1957).

In North Sea cod it was found that prior to spawning the water content of the muscle increases (Figure 3.8) and the protein content decreases. In extreme cases the water content of very large cod can attain 87 % of the body weight prior to spawning (Love, 1970).

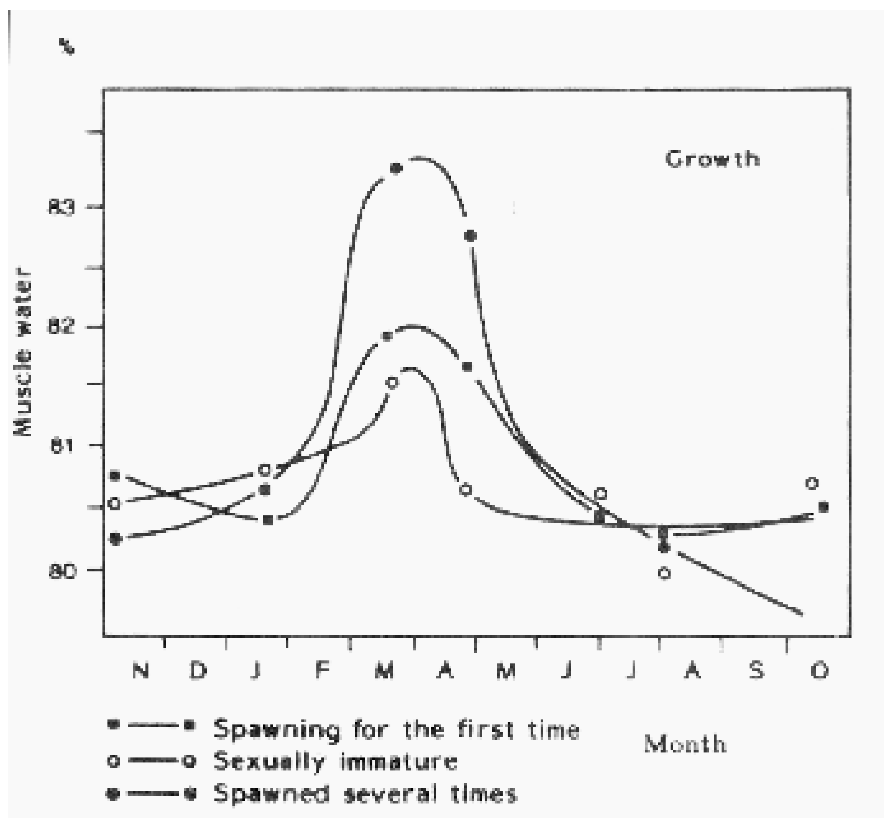
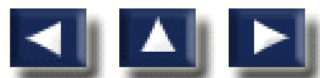


Figure 3.8 Water content of cod muscle *(Gadus morhua)* (Love, 1970)

The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity (Figure 3.7), while some have ripe ovaries for nearly the whole year.

The depletion of the reserves of the fish during gonadal development can be extremely severe, especially if reproduction is combined with migration to the breeding grounds. Some species, e.g., Pacific salmon *(Oncorhynchus* spp.), eel *(Anguilla anguilla)* and others, manage to migrate only once, after which they degenerate and die. This is partly because these species do not eat during migration so that, in the case of a salmon, it can lose up to 92 % of its lipid, 72 % of its protein and 63 % of its ash content during migration and reproduction (Love, 1970).

On the other hand, other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about eight years before spawning causes its death, and other species can live even longer (Cushing, 1975). In former times, 25-year-old herring *(Clupea harengus)* were not unusual in the Norwegian Sea, and plaice *(Pleuronectes platessa) up* to 35 years old have been found. One of the oldest fish reported was a sturgeon *(Acipenser sturio)* from Lake Winnebago in Wisconsin. According to the number of rings in the otolith, it was over 100 years old.



4. CHEMICAL COMPOSITION

4.1. Principal constituents
4.2. Lipids
4.3. Proteins
4.4. N-containing extractives
4.5. Vitamins and minerals

4.1 Principal constituents

The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment and season.

The principal constituents of fish and mammals may be divided into the same categories, and examples of the variation between the constituents in fish are shown in Table 4.1. The composition of beef muscle has been included for comparison.

Table 4.1 Principal constituents (percentage) of fish and beef muscle

Constituent	Fish (fillet)			Beef (isolated muscle)
	Min.	Normal variation	Max.	
Protein	6	16-21	28	20

Lipid	0.1	0.2-25	67	3
carboydrate		<0.5		1
Ash	0.4	1.2-1.5	105	1
Water	28	66-81	96	75

SOURCES: Stansby, 1962; Love, 1970

As can be seen from Table 4.1, a substantial normal variation is observed for the constituents of fish muscle. The minimum and maximum values listed are rather extreme and encountered more rarely.

The variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Fish will have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Usually spawning, whether occurring after long migrations or not, calls for higher levels of energy. Fish having energy depots in the form of lipids will rely on this. Species performing long migrations before they reach specific spawning grounds or rivers may utilize protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish. Most species, in addition, do usually not ingest much food during spawning migration and are therefore not able to supply energy through feeding.

During periods of heavy feeding, at first the protein content of the muscle tissue will increase to an extent depending upon how much it has been depleted, e.g., in relation to spawning migration. Then the lipid content will show a marked and rapid increase. After spawning the fish resumes feeding behaviour and often migrates to find suitable sources of food. Plankton-eating species such as herring will then naturally experience another seasonal variation than that caused by spawning, since plankton production depends on the season and various physical parameters in the oceans.

The lipid fraction is the component showing the greatest variation. Often, the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of spawning. Figure 4.1 shows the characteristic variations in the North Sea herring (4.1a) and mackerel (4.1b).

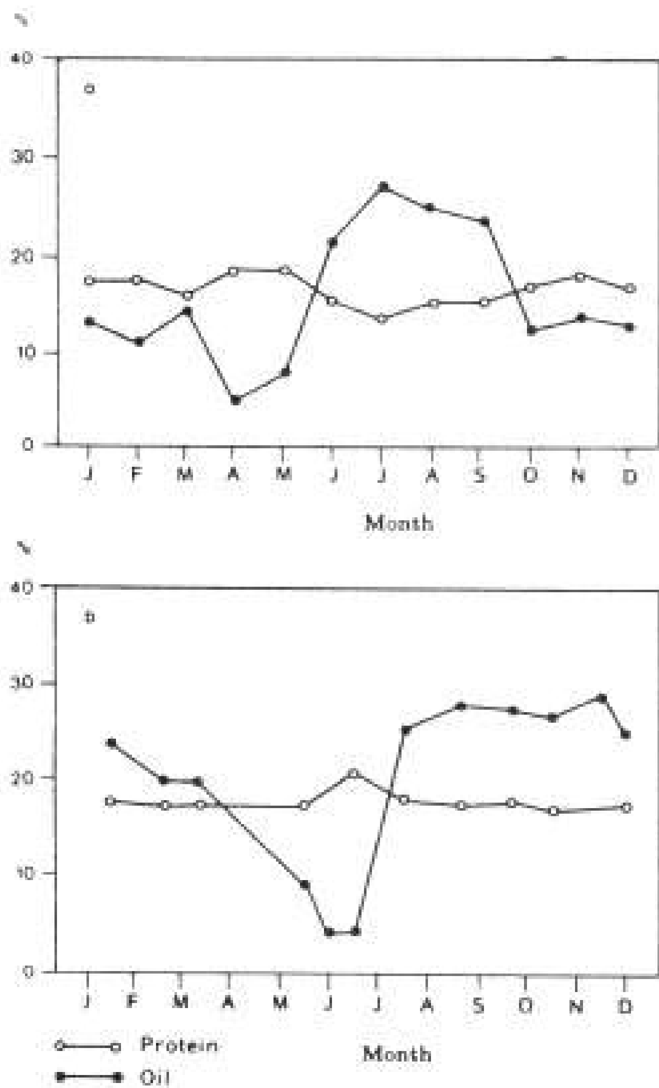


Figure 4.1 Seasonal variation in the chemical composition of (a) herring fillets *(Clupea harengus)* and (b) mackerel fillets *(Scomber scombrus).* Each point indicates the mean value of eight fillets

Although the protein fraction is rather constant in most species, variations have been observed such as protein reduction occuring in salmon during long spawning migrations (Ando *et al.,* 1985 b; Ando and Hatano, 1986) and in Baltic cod during the spawning season, which for this species extends from January to June/July (Borresen, 1992). The latter variation is illustrated in Figure 4.2.

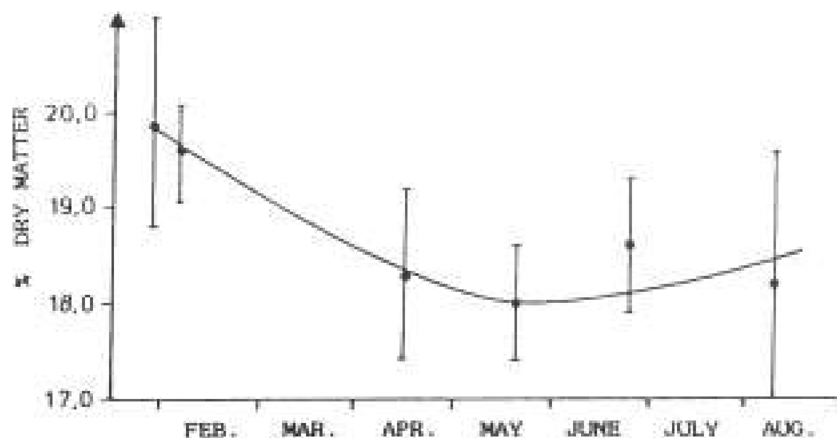


Figure 4.2 Variation in percentage dry matter in muscle of Baltic cod Vertical bars represent standard deviation of the mean value. (Borresen, 1992)

Some tropical fish also show a marked seasonal l variation in chemical composition. West African shad *(Ethmalosa dorsalis)* shows a range in fat content of 2-7 % (wet weight) over the year with a maximum in July (Watts, 1957). Corvina *(Micropogon furnieri)* and pescada-foguete*(Marodon ancylodon)* captured off the Brazilian coast had a fat content range of 0.2-8.7 % and 0.1-5.4 % respectively (Ito and Watanabe, 1968). It has also been observed that the oil content of these species varies with size, larger fish containing about 1 % more oil than smaller ones. Watanabe (1971) examined freshwater fish from Zambia and found a variation from 0.1 to 5.0 % in oil content of four species including both pelagics and demersals.

A possible method for discriminating lean from fatty fish species is to term fish that store lipids only in the liver as lean, and fish storing lipids in fat cells distributed in other body tissues as fatty fish. Typical lean species are the bottom-dwelling ground fish like cod, saithe and hake. Fatty species include the pelagics like herring, mackerel and sprat. Some species store lipids in limited parts of their body tissues only, or in lower quantities than typical fatty species, and are consequently termed semi- fatty species (e.g., barracuda, mullet and shark).

The lipid content of fillets from lean fish is low and stable whereas the lipid content in fillets from fatty species varies considerably. However, the variation in the percentage of fat is reflected in the percentage of water, since fat and water normally constitute around 80 % of the fillet. As a rule of thumb, this can be used to estimate the fat content from an analysis of the amount of water in the fillet. In fact, this principle is being utilized with success in a fat-analysing instrument called the Torry Fish Fat Meter, where it is the water content that is actually being measured (Kent et *al.,* 1992).

Whether a fish is lean or fatty the actual fat content has consequences for the technological characteristics postmortem. The changes taking place in fresh lean fish may be predicted from knowledge of biochemical reactions in the protein fraction, whereas in fatty species changes in the lipid fractions have to be included. The implication may be that the storage time is reduced due to lipid oxidation, or special precautions have to be taken to avoid this.

The variations in water, lipid and protein contents in various fish species are shown in Table 4.2.

Table 4.2 Chemical composition of the fillets of various fish species

Species	**Scientific name**	**Water %**	**Lipid %**	**Protein %**	**Energy value(kJ/ 100 g)**
Blue whiting a)	Micromesistius poutassou	79-80	1.9-3.0	13.8-15.9	314-388
Cod a)	Gadus morhua	78-83	0.1-0.9	15.0-19.0	295-332
Eel a)	Anguilla anguilla	60-71	8.0-31.0	14.4	
Herring a)	Clupea harengus	60-80	0.4-22.0	16.0-19.0	
Plaice a)	Pleuronectes platessa	81	1.1-3.6	15.7-17.8	332-452
Salmon a)	Salmo salar	67-77	0.3-14.0	21.5	
Trout a)	Salmo trutta	70-79	1.2-10.8	18.8-19.1	
Tuna a)	Thunnus spp.	71	4.1	25.2	581
Norway lobster a)	Nephrops norvegicus	77	0.6-2.0	19.5	369
Pejerrey b)	Basilichthys bornariensis	80	0.7-3.6	17.3-17.9	
Carp b)	Cyprinus carpio	81.6	2.1	16.0	
Sabalo c)	Prochilodus platensis	67.0	4.3	23.4	
Pacu c)	Colossoma macropomum	67.1	18.0	14.1	
Tambaqui c)	Colossoma brachypomum	69.3	15.6	15.8	
Chincuiña c)	Pseudoplatystoma tigrinum	70.8	8.9	15.8	
Corvina c)	Plagioscion squamosissimus	67.9	5.9	21.7	
Bagré c)	Ageneiosus spp.	79.0	3.7	14.8	

SOURCES: a) Murray and Burt, 1969, b)Poulter and Nicolaides, 1995 a. c) Poulter and Nicolaides, 1985 b

The carbohydrate content in fish muscle is very low, usually below 0.5 %. This

is typical for striated muscle, where carbohydrate occurs in glycogen and as part of the chemical constituents of nucleotides. The latter is the Source of ribose liberated as a consequence of the autolytic changes *post mortem*.

As demonstrated above, the chemical composition of the different fish species will show variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. These factors are observed in wild, free-living fishes in the open sea and inland waters. Fish raised in aquaculture may also show variation in chemical composition, but in this case several factors are controlled, thus the chemical composition may be predicted. To a certain extent the fish farmer is able to design the composition of the fish by selecting the farming conditions. It has been reported that factors such as feed composition, environment, fish size, and genetic traits all have an impact on the composition and quality of the aquacultured fish (Reinitz et al., 1979).

The single factors having the most pronounced Impact on the chemical composition is considered to be the feed composition. The fish farmer is interested in making the fish grow as fast as possible on a minimum amount of feed, as the feed is the major cost component in aquaculture. The growth potential is highest when the fish is fed a diet with a high lipid content for energy purposes and a high amount of protein containing a well balanced composition of amino acids.

However, the basic metabolic pattern of the fish sets some limits as to how much lipid can be metabolized relative to protein. Because protein is a much more expensive feed ingredient than lipid, numerous experiments have been performed in order to substitute as much protein as possible with lipids. Among the literature that may be consulted is the following: Watanabe *et al., 1979;* Watanabe, *1982;* Wilson and Halver, *1986;* and Watanabe *et al., 1987.*

Usually most fish species will use some of the protein for energy purposes regardless of the lipid content. When the lipid content exceeds the maximum that can be metabolized for energy purposes, the remainder will be deposited in the tissues, resulting in a fish with very high fat content. Apart from having a negative impact on the overall quality, it may also decrease the yield, as most surplus fat will be stored in depots in the belly cavity, thus being discarded as waste after evisceration and filleting.

A normal way of reducing the fat content of aquacultured fish before harvesting is to starve the fish for a period. It has been demonstrated for both fatty and lean fish species that this affects the lipid content (see, e.g., Reinitz, 1983; Johansson and Kiessling, 1991*;* Lie and Huse, 1992).

It should be mentioned that in addition to allowing for the possibility of, within certain limits, predetermining the fish composition in aquaculture operations, keeping fish in captivity under controlled conditions also offers the possibility of conducting experiments in which variation in chemical composition observed in wild fish may be provoked. The experiments may be designed such that the mechanisms behind the variations observed in wild fish may be elucidated.

4.2 Lipids

The lipids present in teleost fish species may be divided into two major groups: the phospholipids and the triglycerides. The phospholipids make up the integral structure of the unit membranes in the cells; thus, they are often called structural lipids. The triglycerides are lipids used for storage of energy in fat depots, usually within special fat cells surrounded by a phospholipid membrane and a rather weak collagen network. The triglycerides are often termed depot fat. A few fish have wax esters as part of their depot fats.

The white muscle of a typical lean fish such as cod contains less than 1 % lipids. Of this, the phospholipids make up about 90 % *(Ackman, 1980).* The phospholipid fraction in a lean fish muscle consists of about 69 % phosphatidyl-choline, 19 % *phosphatidyl-ethanolamine* and 5 % phosphatidyl-serine. In addition, there are several other phospholipids occurring in minor quantities.

The phospholipids are all contained in membrane structures, including the outer cell membrane, the endoplasmic reticulum and other intracellular tubule systems, as well as membranes of the organelles like mitochondria. In addition to phospholipids, the membranes also contain cholesterol, contributing to the membrane rigidity. In lean fish muscle cholesterol may be found in a quantity of about 6 % of the total lipids. This level is similar to that found in mammalian muscle.

As already explained, fish species may be categorized as lean or fatty depending on how they store lipids for energy. Lean fish use the liver as their energy depot, and the fatty species store lipids in fat cells througout the body.

The fat cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail. In some species which store extraordinarily high amounts of lipids the fat may also be deposited in the belly cavity. Depending on the amount of polyunsaturated fatty acids, most fish fats are more or less liquid at low temperature.

Finally, fat depots are also typically found spread throughout the muscle

structure. The concentration of fat cells appears to be highest close to the myocommata and in the region between the light and dark muscle (Kiessling *et al.,* 1991). The dark muscle contains some triglycerides inside the muscle cells even in lean fish, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism.

In dark muscle the energy reserves are completely catabolized to CO_2 and water, whereas in light muscle lactic acid is formed. The mobilization of energy is much faster in light muscle than in dark muscle, but the formation of lactic acid creates fatigue, leaving the muscle unable to work for long periods at maximum speed. Thus, the dark muscle is used for continuous swimming activities and the light muscle for quick bursts, such as when the fish is about to catch a prey or to escape a predator.

An example of the seasonal variation in fat deposition in mackerel and capelin is shown in Figure 4.3, where it is seen that the lipid content in the different tissues varies considerably. The lipid stores are typically used for long spawning migrations and when building up gonads (Ando *et al.,* 1985 a). When the lipids are mobilized for these purposes there are questions as to whether the different fatty acids present in the triglyceride are utilized selectively. This is apparently not the case in salmon, but in cod a selective utilization of $C_{22:6}$ has been observed (Takama *et al.,* 1985).

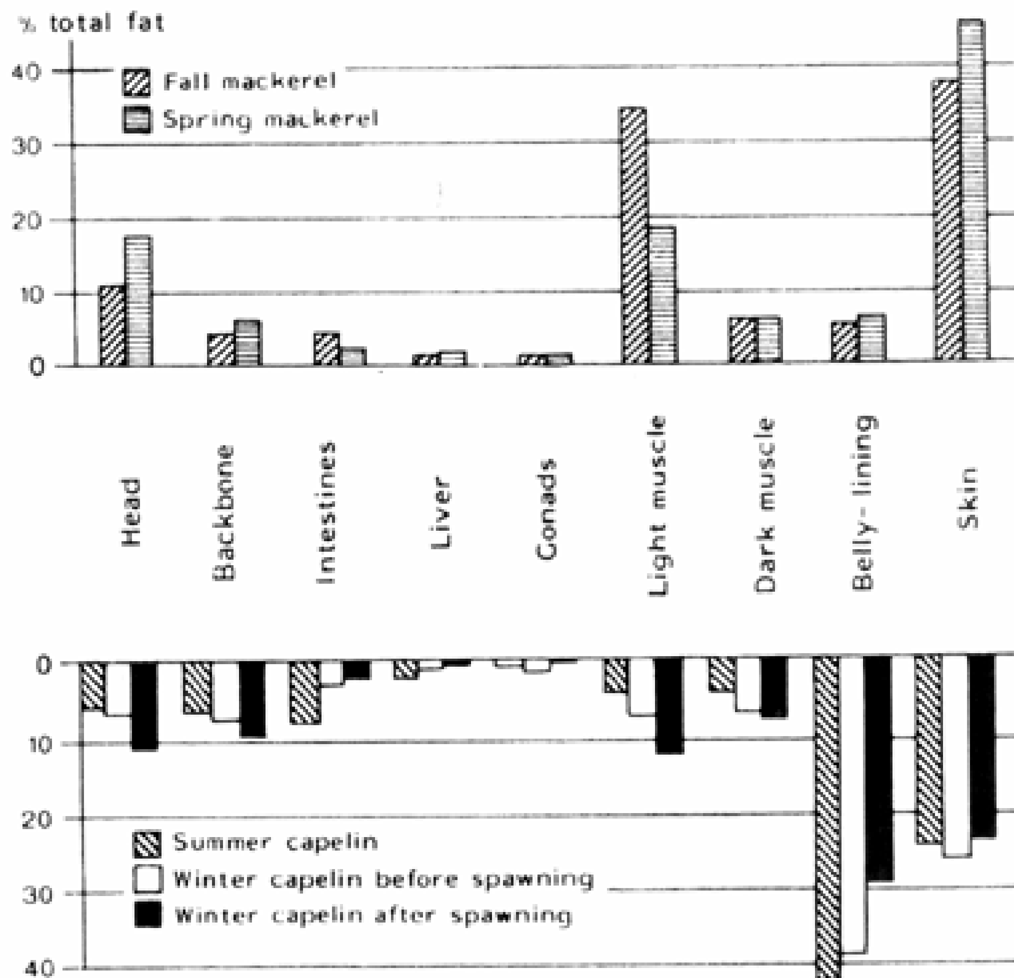


Figure 4.3 Distribution of the total fatin various parts of the body of mackerel (top) and capelin (bottom) of Norwegian origin (Lohne, 1976)

The phospholipids may also be mobilized to a certain extent during sustained migrations (Love, 1970), although this lipid fraction is considered to be conserved much more than the triglycerides.

In elasmobranchs, such as sharks, a significant quantity of the lipid is stored in the liver and may consist of fats like diacyl-alkyl-glyceryl esters or squalene. Some sharks may have liver oils with a minimum of 80 % of the lipid as unsaponifiable substance, mostly in the form of squalene (Buranudeen and Richards-Rajadurai, 1986).

Fish lipids differ from mammalian lipids. The main difference is that fish lipids include up to 40% of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two double bonds per fatty acid molecule while the depot fats of fish contain several fatty acids with five or six double bonds (Stansby and Hall, 1967).

The percentage of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in the polyunsaturated fatty acids of lipids from freshwater fish (approximately 70 %) than in the corresponding lipids from marine fish (approximately 88 %), (Stansby and Hall, 1967). However, the composition of the lipids is not completely fixed but can vary with the feed intake and season.

In human nutrition fatty acids such as linoleic and linolenic acid are regarded as essential since they cannot be synthesized by the organism. In marine fish, these fatty acids constitute only around 2 % of the total lipids, which is a small percentage compared with many vegetable oils. However, fish oils contain other polyunsaturated fatty acids which are "essential" to prevent skin diseases in the same way as linoleic and arachidonic acid. As members of the linolenic acid family (first double bond in the third position, w-3 counted from the terminal methyl group), they will also have neurological benefits in growing children. One of these fatty acids, eicosapentaenoic acid (C20:5 w 3), has recently attracted considerable attention because Danish scientists have found this acid high in the diet of a group of Greenland Eskimos virtually free from arteriosclerosis. Investigations in the United Kingdom and elsewhere have documented that eicosapen-taenoic acid in the blood is an extremely potent antithrombotic factor (Simopoulos et al., 1991).

4.3 Proteins

The proteins in fish muscle tissue can be divided into the following three groups:

1. Structural proteins (actin, myosin, tropormyosin and actomyosin), which constitute 70-80 % of the total protein content (compared with 40 % in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength (³0.5 M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). This fraction constitutes 25-30 % of the protein.
3. Connective tissue proteins (collagen), which constitute approximately 3 % of the -protein in teleostei and about 10 % in elasmobranchii (compared with 17 % in mammals).

The structural proteins make up the contractile apparatus responsible for the muscle movement as explained in section 3.2. The amino-acid composition is approximately the same as for the corresponding proteins in mammaliam muscle, although the physical properties may be slightly different. The

isoelectric point (pI) is around pH 4.5-5.5. At the corresponding pH values the proteins have their lowest solublity, as illustrated in Figure 4.4.

The conformational structure of fish proteins is easily changed by changing the physical environment. Figure 4.4 shows how the solubility characteristics of the myofibrillar proteins are changed after freeze-drying. Treatment with high salt concentrations or heat may lead to denaturation, after which the native protein structure has been irreversibly changed.

When the proteins are denatured under controlled conditions their properties may be utilized for technological purposes. A good example is the production of surimi-based products, in which the gel forming ability of the myofibrillar proteins is used. After salt and stabilizers are added to a washed, minced preparation of muscle proteins, and after a controlled heating and cooling procedure the proteins form a very strong gel (Suzuki, 1981).

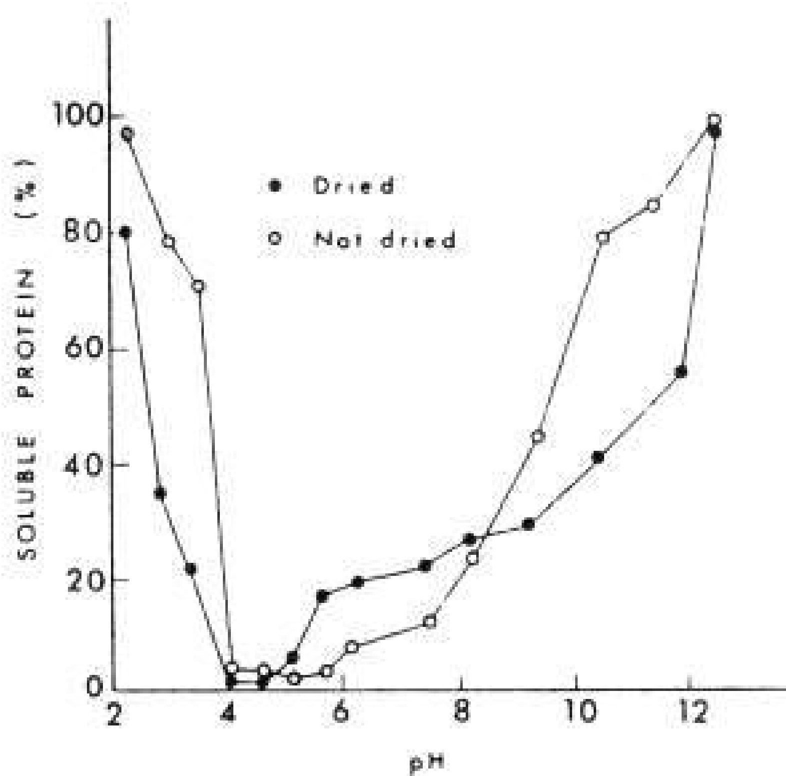


Figure 4.4 Solubility of myofibrillar protein before and after freeze drying at pH values ranging from 2 to 12 (Spinelli *et al.,*1972)

The majority of the sarcoplasmic proteins are enzymes participating in the cell metabolism, such as the anaerobic energy conversion from glycogen to ATP. If the organelles within the muscle cells are broken, this protein fraction may also contain the metabolic enzymes localized inside the endoplasmatic reticulum, mitochondria and lysosomes.

The fact that the composition of the sarcoplasmic protein fraction changes when the organelles are broken was suggested as a method for differentiating fresh from frozen fish, under the assumption that the organelles were intact until freezing (Rehbein *et al.,* 1978*,* Rehbein, 1979, Salfi *et al.,* 1985). However, it was later stated that these methods should be used with great caution, as some of the enzymes are liberated from the organelles also during iced storage of fish (Rehbein, 1992).

The proteins in the sarcoplasmic fraction are excellently suited to distinguishing between different fish species, as all the different species have their characteristic band pattern when separated by the isoelectric focusing method. The method was succesfully introduced by Lundstrom (1980) and has been used by many laboratories and for many fish species. A review of the literature is given by Rehbein (1990).

The chemical and physical properties of collagen proteins are different in tissues such as skin, swim bladder and the myocommata in muscle (Mohr, 1971). In general, collagen fibrils form a delicate network structure with varying complexity in the different connective tissues in a pattern similar to that found in mammals. However, the collagen in fish is much more thermolabile and contains fewer but more labile cross-links than collagen from warm-blooded vertebrates. The hydroxyprolin content is in general lower in fish than in mammals, although a total variation between 4.7 and 10 % of the collagen has been observed (Sato *et at,* 1989).

Different fish species contain varying amounts of collagen in the body tissues. This has led to a theory that the distribution of collagen may reflect the swimming behaviour of the species (Yoshinaka *et at, 1988).* Further, the varying amounts and varying types of collagen in different fishes may also have an influence on the textural properties of fish muscle (Montero and Borderias, 1989). Borresen (1976) developed a method for isolation of the collagenous network surrounding each individual muscle cell. The structure and composition of these structures has been further characterized in cod by Almaas (1982).

The role of collagen in fish was reviewed by Sikorsky *et al.* (1984). An excellent, more recent review is given by Bremner (1992), in which the most recent literature of the different types of collagen found in fish is presented.

Fish proteins contain all the essential amino-acids and, like milk, eggs and mammalian meat proteins, have a very high biological value (Table 4.3).

Table 4.3 Essential amino-acids (percentage) in various proteins

Amino-acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

SOURCES: Braekkan, 1976; Moustgard, 1957

Cereal grains are ususally low in lysine and/or the sulphur-containing amino-acids (methionine and cysteine), whereas fish protein is an excellent source of these aminoacids. In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly.

In addition to the fish proteins already mentioned there is a renewed interest in specific protein fractions that may be recovered from by-products, particularly in the viscera. One such example is the basic protein or protamines found in the milt of the male fish. The molecular weight is usually below 10 000 kD and the pI is higher than 10. This is a result of the extreme amino-acid composition that may show as much as 65 % arginine.

The presence of the basic proteins has long been known, and it is also known that they are not present in all fish species (Kossel, 1928). The best sources are salmonids and herring, whereas ground fish like cod are not found to contain protamines.

The extreme basic character of protamines makes them interesting for several reasons. They will adhere to most other proteins less basic. Thus they have the effect of enhancing functional properties of other food proteins (Poole *et al.,* 1987; Phillips *et al.,* 1989). However, there is a problem in removing all lipids present in the milt from the protein preparation, as this results in an off-flavour in the concentrations to be used in foods.

Another interesting feature of the basic proteins is their ability to prevent growth of microorganisms (Braekkan and Boge, 1964; Kamal *et al.,* 1986). This appears to be the most promising use of these basic proteins in the future.

4.4 N-containing extractives

The N-containing extractives can be defined as the water-soluble, low molecular weight, nitrogen- containing compounds of non-protein nature. This NPN-fraction (non-protein nitrogen) constitutes from 9 to 18 % of the total nitrogen in teleosts.

The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino-acids, nucleotides and purine bases, and, in the case of cartilaginous fish, urea.

Table 4.4 lists some of the components in the NPN-fraction of various fish, poultry meat and mammalian meat.

Table 4.4 Major differences in muscle extractives

Compound in mg/100 wet weight[1)]	Fish Cod	Fish Herring	Fish Shark species	Crustaceans Lobster	Poutry Leg muscle	Mammalian muscle
1) Total extractives	1200	1200	3000	5500	1200	3500
2) Total free amino-acids:	75	300	100	3000	440	350

Arginine	<10	<10	<10	750	<20	<10
Glycine	20	20	20	100-1000	<20	<10
Glutamic acid	<10	<10	<10	270	55	36
Histidine	<1.0	86	<1.0	-	<10	<10
Proline	<1.0	<1.0	<1.0	750	<10	<10
3) Creatine	400	400	300	0	-	550
4) Betaine	0	0	150	100	-	-
5) Trimethylamine oxide	350	250	500-1000	100	0	0
6) Anserine	150	0	0	0	280	150
7) Carnosine	0	0	0	0	180	200
8) Urea	0	0	2000	-	-	35

[1] It should be noted that the unit in this table refers to the total molecular weight of the compound

SOURCE: Shewan, 1974

An example of the distribution of the different compounds in the NPN-fraction in freshwater and marine fish is shown in Figure 4.5. It should be noted that the composition varies not only from species to species, but also within the species depending on size, season, muscle sample, etc.

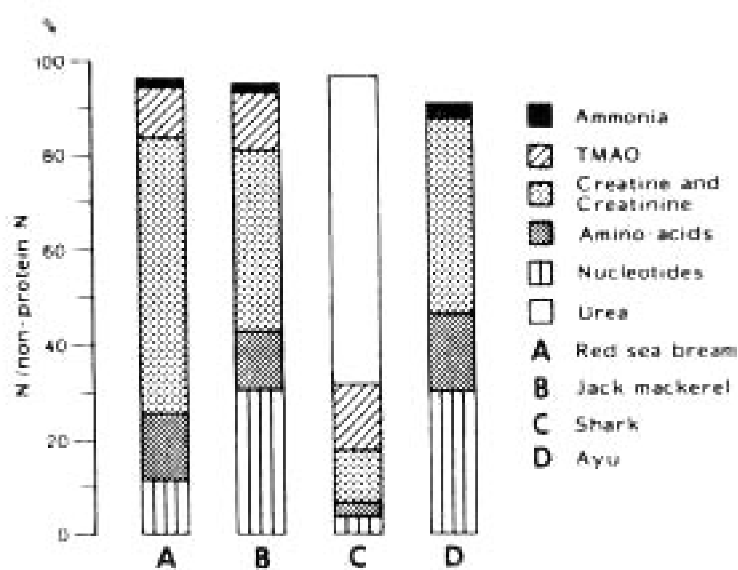


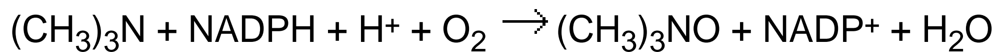
Figure 4.5 Distribution of non-protein nitrogen in fish muscles of two marine bonyfish (A,B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama *et al.,* 1977)

TMAO constitutes a characteristic and important part of the NPN-fraction in marine species and deserves further mention. This component is found in all marine fish species in quantities from 1 to 5 % of the muscle tissue (dry weight) but is virtually absent from freshwater species and from terrestrial organisms (Anderson and Fellers, 1952; Hebard *et al.,* 1982).

One exception was recently found in a study of Nile perch and tilapia from Lake Victoria, where as much as 150-200 mg TMAO/100 g of fresh fish was found (Gram *et al.,* 1989).

Although much work has been conducted on the origin and role of TMAO, there is still much to be clarified. Stroem *et al.* (1979) have shown that TMAO is formed by biosynthesis in certain zooplankton species. These organisms possess an enzyme (TMA mono-oxygenase) which oxidizes TMA to TMAO. TMA is commonly found in marine plants as are many other methylated amines (monomethylamine and dimethylamine). Plankton-eating fish may obtain their TMAO from feeding on these zooplankton (exogenous origin). Belinski (1964) and Agustsson and Stroem (1981) have shown that certain fish species are able to synthesize TMAO from TMA, but this synthesis is regarded as being of minor importance.

The TMA-oxidase system is found in the microsomes of the cells and is dependent on the presence of Nicotinamide ademine denucleotide phosphate (NADPH):



It is puzzling that this mono-oxygenase can be widely found in mammals (where it is thought to function as a detoxifier), while most fish have low or no detectable actitity of this enzyme.

Japanese research (Kawabata, 1953) indicates that there is a TMAO-reducing system present in the dark muscle of certain pelagic fishes.

The amount of TMAO in the muscle tissue depends on the species, season, fishing ground, etc. In general, the highest amount is found in elasmobranchs and squid (75-250 mg N/100 g); cod have somewhat less (60-120 mg N/100 g) while flatfish and pelagic fish have the least. An extensive compilation of data is given by Hebard *et* al. (1982). According to Tokunaga (1970), pelagic fish (sardines, tuna, mackerel) have their highest concentration of TMAO in the dark muscle while demersal, white-fleshed fish have a much higher content in the white muscle.

In elasmobranchs, TMAO seems to play a role in osmoregulation, and it has been shown that a transfer of small rays to a mixture of fresh and sea water (1: 1) will result in a 50 % reduction of intracellular TMAO. The role of TMAO in teleosts is more uncertain.

Several hypotheses for the role of TMAO have been proposed:

- TMAO is essentially a waste product, a detoxified form of TMA
- TMAO is an osmoregulator
- TMAO functions as an "anti-freeze"
- TMAO has no significant function. It is accumulated in the muscle when the fish is fed a TMAO-containing diet

According to Stroem (1984), it is now generally believed that TMAO has an osmoregulatory role.

As the occurrence of TMAO had previously been found virtually only in marine species until the observation published by Gram *et al.* (1989), it was speculated that TMAO together with high amounts of taurine could have additional effects, at least in fresh water fish (Anthoni *et al.,* 1990 a).

Quantitatively, the main component of the NPN-fraction is creatine. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction.

The NPN-fraction also contains a fair amount of free amino-acids. These constitute 630 mg/ 100 g light muscle in mackerel *(Scomber scombrus),* 350-420 mg/ 100 g in herring *(Clupea harengus)* and 310-370 mg/100 g in capelin *(Mallotus villosus).* The relative importance of the different amino- acids varies with species. Taurine, alanine, glycine and imidazole-containing amino-acids seem to dominate in most fish. Of the imidazole-containing amino-acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine.

The amount of nucleotides and nucleotide fragments in dead fish depends on the state of the fish and is discussed in section 5.

4.5 Vitamins and minerals

The amount of vitamins and minerals is species-specific and can furthermore vary with season. In general, fish meat is a good source of the B vitamins and, in the case of fatty species, also of the A and D vitamins. Some freshwater species such as carp have high thiaminase actitity so the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular but also of iron, copper and selenium. Saltwater fish have a high content of iodine. In Tables 4.5 and 4.6 some of the vitamin and mineral contents are listed. Because of the natural variation of these constituents, it is impossible to give accurate figures.

Table 4.5 Vitamins in fish

Fish	**A (IU/g)**	**D (IU/g)**	**B_1 (thiamine) (µ/g)**	**B_2 (riboflavin) (µ/g)**	**Niacin (µ/g)**	**Pantothenic acid (µ/g)**	**B_6 (µ/g)**
Cod fillet	0-50	0	0.7	0.8	20	1.7	1.7
Herring fillet	20-400	300-1000	0.4	3.0	40	10	4.5
Cod-liver oil	200-10.000	20-300	-	[1] 3.4	[1] 15	[1] 4.3	-

1) Whole liver

SOURCE: Murray and Burt, 1969

Table 4.6 Some mineral constituents of fish muscle

Element	Average value (mg/100 g)	Range (mg/100 g)
Sodium	72	30 -134
Potassium	278	19 -502
Calcium	79	19 -881
Magnesium	38	4.5-452
Phosphorus	190	68-550

SOURCE: Murray and Burt, 1969

The vitamin content is comparable to that of mammals except in the case of the A and D vitamins which are found in large amounts in the meat of fatty species and in abundance in the liver of species such as cod and halibut. It should be noted that the sodium content of fish meat is relatively low which makes it suitable for low-sodium diets.

In aquacultured fish, the contents of vitamins and minerals are considered to reflect the composition of the corresponding components in the fish feed, although the observed data should be interpreted with great caution (Maage *et al.,* 1991). In order to protect the n-3 polyunsaturated fatty acids, considered of great importance both for fish and human health, vitamin E may be added to the fish feed as an antioxidant. It has been shown that the resulting level of vitamin E in the fish tissue corresponds to the concentration in the feed (Waagbo *et al.,* 1991).

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4. CHEMICAL COMPOSITION

4.1. Principal constituents
4.2. Lipids
4.3. Proteins
4.4. N-containing extractives
4.5. Vitamins and minerals

4.1 Principal constituents

The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment and season.

The principal constituents of fish and mammals may be divided into the same categories, and examples of the variation between the constituents in fish are shown in Table 4.1. The composition of beef muscle has been included for comparison.

Table 4.1 Principal constituents (percentage) of fish and beef muscle

Constituent	Fish (fillet)			Beef (isolated muscle)
	Min.	Normal variation	Max.	
Protein	6	16-21	28	20

Lipid	0.1	0.2-25	67	3
carboydrate		<0.5		1
Ash	0.4	1.2-1.5	105	1
Water	28	66-81	96	75

SOURCES: Stansby, 1962; Love, 1970

As can be seen from Table 4.1, a substantial normal variation is observed for the constituents of fish muscle. The minimum and maximum values listed are rather extreme and encountered more rarely.

The variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Fish will have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Usually spawning, whether occurring after long migrations or not, calls for higher levels of energy. Fish having energy depots in the form of lipids will rely on this. Species performing long migrations before they reach specific spawning grounds or rivers may utilize protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish. Most species, in addition, do usually not ingest much food during spawning migration and are therefore not able to supply energy through feeding.

During periods of heavy feeding, at first the protein content of the muscle tissue will increase to an extent depending upon how much it has been depleted, e.g., in relation to spawning migration. Then the lipid content will show a marked and rapid increase. After spawning the fish resumes feeding behaviour and often migrates to find suitable sources of food. Plankton-eating species such as herring will then naturally experience another seasonal variation than that caused by spawning, since plankton production depends on the season and various physical parameters in the oceans.

The lipid fraction is the component showing the greatest variation. Often, the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of spawning. Figure 4.1 shows the characteristic variations in the North Sea herring (4.1a) and mackerel (4.1b).

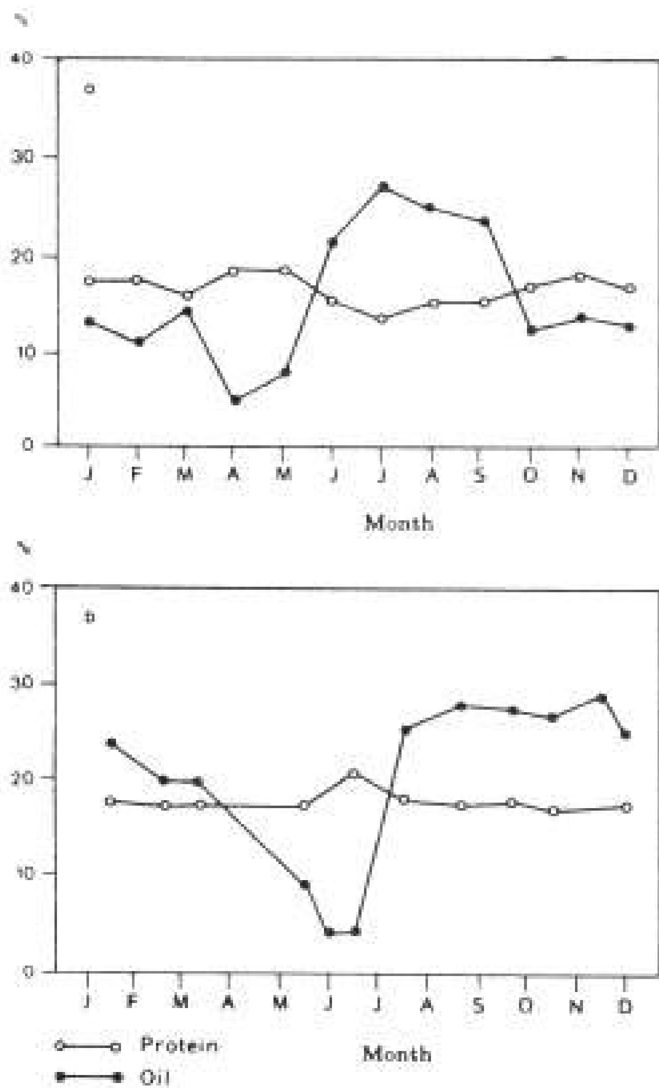


Figure 4.1 Seasonal variation in the chemical composition of (a) herring fillets *(Clupea harengus)* and (b) mackerel fillets *(Scomber scombrus).* Each point indicates the mean value of eight fillets

Although the protein fraction is rather constant in most species, variations have been observed such as protein reduction occuring in salmon during long spawning migrations (Ando *et al.,* 1985 b; Ando and Hatano, 1986) and in Baltic cod during the spawning season, which for this species extends from January to June/July (Borresen, 1992). The latter variation is illustrated in Figure 4.2.

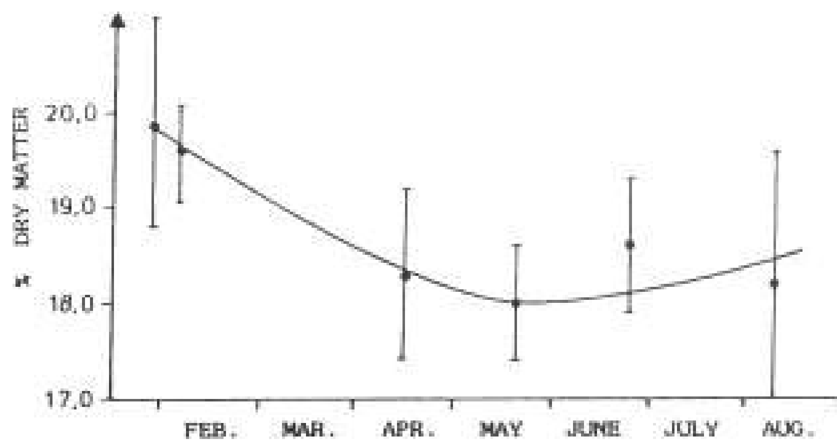


Figure 4.2 Variation in percentage dry matter in muscle of Baltic cod Vertical bars represent standard deviation of the mean value. (Borresen, 1992)

Some tropical fish also show a marked seasonal I variation in chemical composition. West African shad *(Ethmalosa dorsalis)* shows a range in fat content of 2-7 % (wet weight) over the year with a maximum in July (Watts, 1957). Corvina *(Micropogon furnieri)* and pescada-foguete*(Marodon ancylodon)* captured off the Brazilian coast had a fat content range of 0.2-8.7 % and 0.1-5.4 % respectively (Ito and Watanabe, 1968). It has also been observed that the oil content of these species varies with size, larger fish containing about 1 % more oil than smaller ones. Watanabe (1971) examined freshwater fish from Zambia and found a variation from 0.1 to 5.0 % in oil content of four species including both pelagics and demersals.

A possible method for discriminating lean from fatty fish species is to term fish that store lipids only in the liver as lean, and fish storing lipids in fat cells distributed in other body tissues as fatty fish. Typical lean species are the bottom-dwelling ground fish like cod, saithe and hake. Fatty species include the pelagics like herring, mackerel and sprat. Some species store lipids in limited parts of their body tissues only, or in lower quantities than typical fatty species, and are consequently termed semi- fatty species (e.g., barracuda, mullet and shark).

The lipid content of fillets from lean fish is low and stable whereas the lipid content in fillets from fatty species varies considerably. However, the variation in the percentage of fat is reflected in the percentage of water, since fat and water normally constitute around 80 % of the fillet. As a rule of thumb, this can be used to estimate the fat content from an analysis of the amount of water in the fillet. In fact, this principle is being utilized with success in a fat-analysing instrument called the Torry Fish Fat Meter, where it is the water content that is actually being measured (Kent et *al.,* 1992).

Whether a fish is lean or fatty the actual fat content has consequences for the technological characteristics postmortem. The changes taking place in fresh lean fish may be predicted from knowledge of biochemical reactions in the protein fraction, whereas in fatty species changes in the lipid fractions have to be included. The implication may be that the storage time is reduced due to lipid oxidation, or special precautions have to be taken to avoid this.

The variations in water, lipid and protein contents in various fish species are shown in Table 4.2.

Table 4.2 Chemical composition of the fillets of various fish species

Species	**Scientific name**	**Water %**	**Lipid %**	**Protein %**	**Energy value(kJ/ 100 g)**
Blue whiting a)	Micromesistius poutassou	79-80	1.9-3.0	13.8-15.9	314-388
Cod a)	Gadus morhua	78-83	0.1-0.9	15.0-19.0	295-332
Eel a)	Anguilla anguilla	60-71	8.0-31.0	14.4	
Herring a)	Clupea harengus	60-80	0.4-22.0	16.0-19.0	
Plaice a)	Pleuronectes platessa	81	1.1-3.6	15.7-17.8	332-452
Salmon a)	Salmo salar	67-77	0.3-14.0	21.5	
Trout a)	Salmo trutta	70-79	1.2-10.8	18.8-19.1	
Tuna a)	Thunnus spp.	71	4.1	25.2	581
Norway lobster a)	Nephrops norvegicus	77	0.6-2.0	19.5	369
Pejerrey b)	Basilichthys bornariensis	80	0.7-3.6	17.3-17.9	
Carp b)	Cyprinus carpio	81.6	2.1	16.0	
Sabalo c)	Prochilodus platensis	67.0	4.3	23.4	
Pacu c)	Colossoma macropomum	67.1	18.0	14.1	
Tambaqui c)	Colossoma brachypomum	69.3	15.6	15.8	
Chincuiña c)	Pseudoplatystoma tigrinum	70.8	8.9	15.8	
Corvina c)	Plagioscion squamosissimus	67.9	5.9	21.7	
Bagré c)	Ageneiosus spp.	79.0	3.7	14.8	

SOURCES: a) Murray and Burt, 1969, b)Poulter and Nicolaides, 1995 a. c) Poulter and Nicolaides, 1985 b

The carbohydrate content in fish muscle is very low, usually below 0.5 %. This

is typical for striated muscle, where carbohydrate occurs in glycogen and as part of the chemical constituents of nucleotides. The latter is the Source of ribose liberated as a consequence of the autolytic changes *post mortem*.

As demonstrated above, the chemical composition of the different fish species will show variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. These factors are observed in wild, free-living fishes in the open sea and inland waters. Fish raised in aquaculture may also show variation in chemical composition, but in this case several factors are controlled, thus the chemical composition may be predicted. To a certain extent the fish farmer is able to design the composition of the fish by selecting the farming conditions. It has been reported that factors such as feed composition, environment, fish size, and genetic traits all have an impact on the composition and quality of the aquacultured fish (Reinitz et al., 1979).

The single factors having the most pronounced Impact on the chemical composition is considered to be the feed composition. The fish farmer is interested in making the fish grow as fast as possible on a minimum amount of feed, as the feed is the major cost component in aquaculture. The growth potential is highest when the fish is fed a diet with a high lipid content for energy purposes and a high amount of protein containing a well balanced composition of amino acids.

However, the basic metabolic pattern of the fish sets some limits as to how much lipid can be metabolized relative to protein. Because protein is a much more expensive feed ingredient than lipid, numerous experiments have been performed in order to substitute as much protein as possible with lipids. Among the literature that may be consulted is the following: Watanabe *et al., 1979;* Watanabe, *1982;* Wilson and Halver, *1986;* and Watanabe *et al., 1987.*

Usually most fish species will use some of the protein for energy purposes regardless of the lipid content. When the lipid content exceeds the maximum that can be metabolized for energy purposes, the remainder will be deposited in the tissues, resulting in a fish with very high fat content. Apart from having a negative impact on the overall quality, it may also decrease the yield, as most surplus fat will be stored in depots in the belly cavity, thus being discarded as waste after evisceration and filleting.

A normal way of reducing the fat content of aquacultured fish before harvesting is to starve the fish for a period. It has been demonstrated for both fatty and lean fish species that this affects the lipid content (see, e.g., Reinitz, 1983; Johansson and Kiessling, 1991*;* Lie and Huse, 1992).

It should be mentioned that in addition to allowing for the possibility of, within certain limits, predetermining the fish composition in aquaculture operations, keeping fish in captivity under controlled conditions also offers the possibility of conducting experiments in which variation in chemical composition observed in wild fish may be provoked. The experiments may be designed such that the mechanisms behind the variations observed in wild fish may be elucidated.

4.2 Lipids

The lipids present in teleost fish species may be divided into two major groups: the phospholipids and the triglycerides. The phospholipids make up the integral structure of the unit membranes in the cells; thus, they are often called structural lipids. The triglycerides are lipids used for storage of energy in fat depots, usually within special fat cells surrounded by a phospholipid membrane and a rather weak collagen network. The triglycerides are often termed depot fat. A few fish have wax esters as part of their depot fats.

The white muscle of a typical lean fish such as cod contains less than 1 % lipids. Of this, the phospholipids make up about 90 % *(Ackman, 1980).* The phospholipid fraction in a lean fish muscle consists of about 69 % phosphatidyl-choline, 19 % *phosphatidyl-ethanolamine* and 5 % phosphatidyl-serine. In addition, there are several other phospholipids occurring in minor quantities.

The phospholipids are all contained in membrane structures, including the outer cell membrane, the endoplasmic reticulum and other intracellular tubule systems, as well as membranes of the organelles like mitochondria. In addition to phospholipids, the membranes also contain cholesterol, contributing to the membrane rigidity. In lean fish muscle cholesterol may be found in a quantity of about 6 % of the total lipids. This level is similar to that found in mammalian muscle.

As already explained, fish species may be categorized as lean or fatty depending on how they store lipids for energy. Lean fish use the liver as their energy depot, and the fatty species store lipids in fat cells througout the body.

The fat cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail. In some species which store extraordinarily high amounts of lipids the fat may also be deposited in the belly cavity. Depending on the amount of polyunsaturated fatty acids, most fish fats are more or less liquid at low temperature.

Finally, fat depots are also typically found spread throughout the muscle

structure. The concentration of fat cells appears to be highest close to the myocommata and in the region between the light and dark muscle (Kiessling *et al.,* 1991). The dark muscle contains some triglycerides inside the muscle cells even in lean fish, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism.

In dark muscle the energy reserves are completely catabolized to CO_2 and water, whereas in light muscle lactic acid is formed. The mobilization of energy is much faster in light muscle than in dark muscle, but the formation of lactic acid creates fatigue, leaving the muscle unable to work for long periods at maximum speed. Thus, the dark muscle is used for continuous swimming activities and the light muscle for quick bursts, such as when the fish is about to catch a prey or to escape a predator.

An example of the seasonal variation in fat deposition in mackerel and capelin is shown in Figure 4.3, where it is seen that the lipid content in the different tissues varies considerably. The lipid stores are typically used for long spawning migrations and when building up gonads (Ando *et al.,* 1985 a). When the lipids are mobilized for these purposes there are questions as to whether the different fatty acids present in the triglyceride are utilized selectively. This is apparently not the case in salmon, but in cod a selective utilization of $C_{22:6}$ has been observed (Takama *et al.,* 1985).

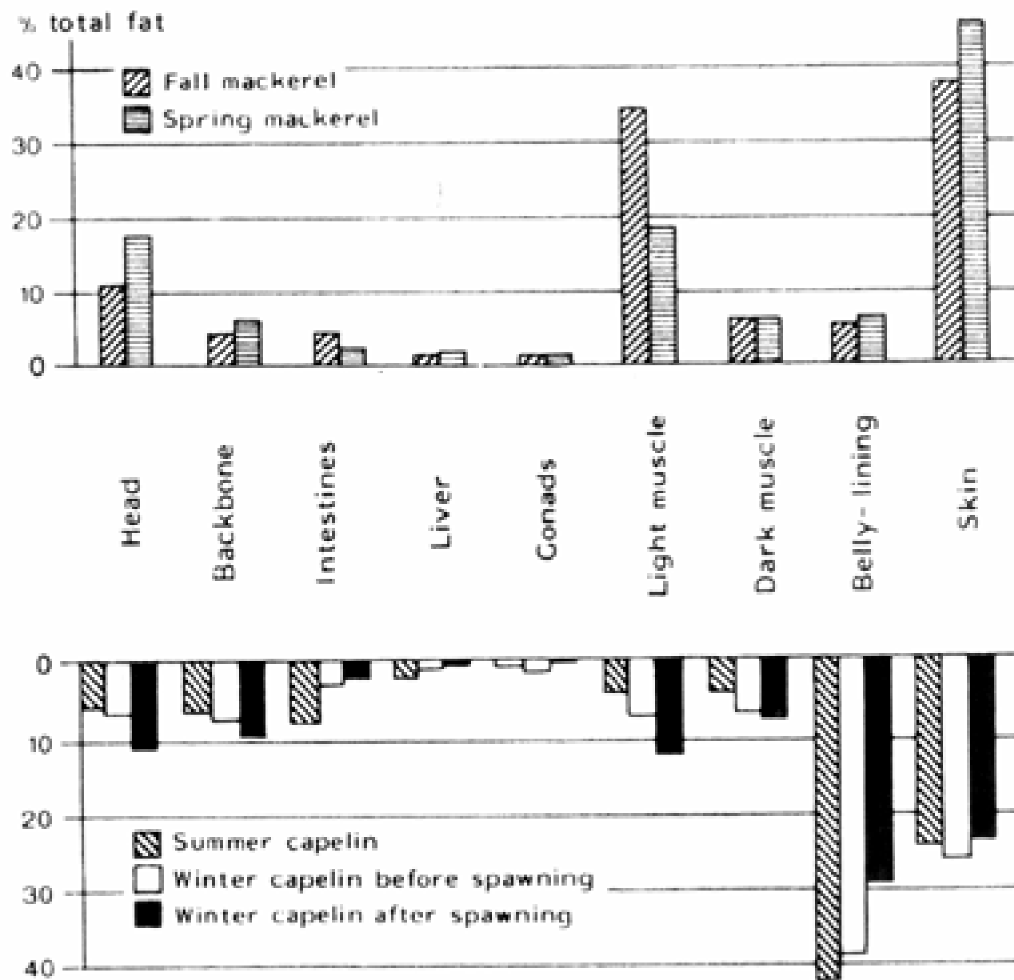


Figure 4.3 Distribution of the total fatin various parts of the body of mackerel (top) and capelin (bottom) of Norwegian origin (Lohne, 1976)

The phospholipids may also be mobilized to a certain extent during sustained migrations (Love, 1970), although this lipid fraction is considered to be conserved much more than the triglycerides.

In elasmobranchs, such as sharks, a significant quantity of the lipid is stored in the liver and may consist of fats like diacyl-alkyl-glyceryl esters or squalene. Some sharks may have liver oils with a minimum of 80 % of the lipid as unsaponifiable substance, mostly in the form of squalene (Buranudeen and Richards-Rajadurai, 1986).

Fish lipids differ from mammalian lipids. The main difference is that fish lipids include up to 40% of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two double bonds per fatty acid molecule while the depot fats of fish contain several fatty acids with five or six double bonds (Stansby and Hall, 1967).

The percentage of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in the polyunsaturated fatty acids of lipids from freshwater fish (approximately 70 %) than in the corresponding lipids from marine fish (approximately 88 %), (Stansby and Hall, 1967). However, the composition of the lipids is not completely fixed but can vary with the feed intake and season.

In human nutrition fatty acids such as linoleic and linolenic acid are regarded as essential since they cannot be synthesized by the organism. In marine fish, these fatty acids constitute only around 2 % of the total lipids, which is a small percentage compared with many vegetable oils. However, fish oils contain other polyunsaturated fatty acids which are "essential" to prevent skin diseases in the same way as linoleic and arachidonic acid. As members of the linolenic acid family (first double bond in the third position, w-3 counted from the terminal methyl group), they will also have neurological benefits in growing children. One of these fatty acids, eicosapentaenoic acid (C20:5 w 3), has recently attracted considerable attention because Danish scientists have found this acid high in the diet of a group of Greenland Eskimos virtually free from arteriosclerosis. Investigations in the United Kingdom and elsewhere have documented that eicosapen-taenoic acid in the blood is an extremely potent antithrombotic factor (Simopoulos et al., 1991).

4.3 Proteins

The proteins in fish muscle tissue can be divided into the following three groups:

1. Structural proteins (actin, myosin, tropormyosin and actomyosin), which constitute 70-80 % of the total protein content (compared with 40 % in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength (^{3}0.5 M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). This fraction constitutes 25-30 % of the protein.
3. Connective tissue proteins (collagen), which constitute approximately 3 % of the -protein in teleostei and about 10 % in elasmobranchii (compared with 17 % in mammals).

The structural proteins make up the contractile apparatus responsible for the muscle movement as explained in section 3.2. The amino-acid composition is approximately the same as for the corresponding proteins in mammaliam muscle, although the physical properties may be slightly different. The

isoelectric point (pI) is around pH 4.5-5.5. At the corresponding pH values the proteins have their lowest solublity, as illustrated in Figure 4.4.

The conformational structure of fish proteins is easily changed by changing the physical environment. Figure 4.4 shows how the solubility characteristics of the myofibrillar proteins are changed after freeze-drying. Treatment with high salt concentrations or heat may lead to denaturation, after which the native protein structure has been irreversibly changed.

When the proteins are denatured under controlled conditions their properties may be utilized for technological purposes. A good example is the production of surimi-based products, in which the gel forming ability of the myofibrillar proteins is used. After salt and stabilizers are added to a washed, minced preparation of muscle proteins, and after a controlled heating and cooling procedure the proteins form a very strong gel (Suzuki, 1981).

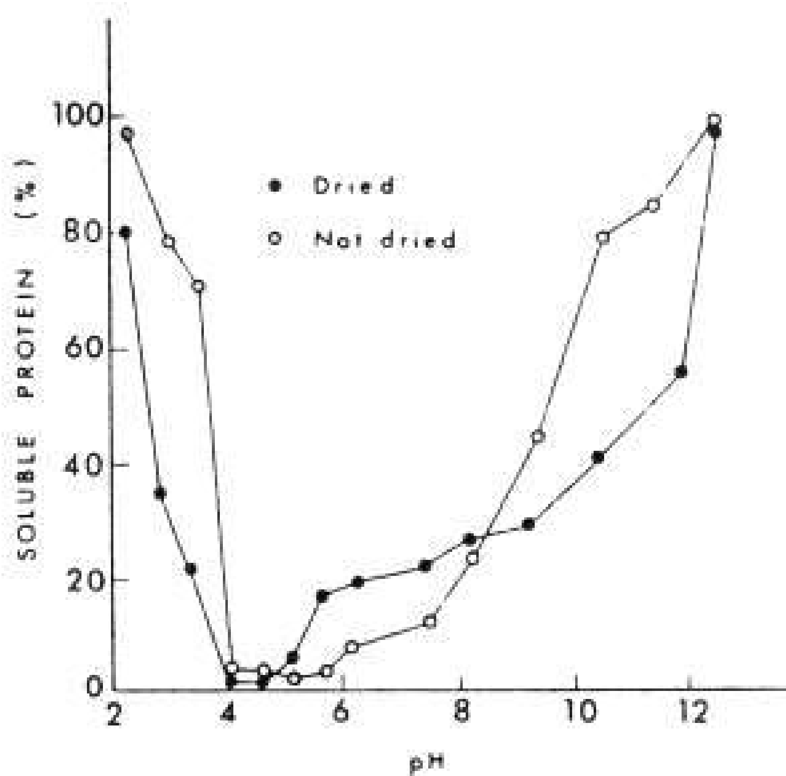


Figure 4.4 Solubility of myofibrillar protein before and after freeze drying at pH values ranging from 2 to 12 (Spinelli *et al.,*1972)

The majority of the sarcoplasmic proteins are enzymes participating in the cell metabolism, such as the anaerobic energy conversion from glycogen to ATP. If the organelles within the muscle cells are broken, this protein fraction may also contain the metabolic enzymes localized inside the endoplasmatic reticulum, mitochondria and lysosomes.

The fact that the composition of the sarcoplasmic protein fraction changes when the organelles are broken was suggested as a method for differentiating fresh from frozen fish, under the assumption that the organelles were intact until freezing (Rehbein *et al.,* 1978*,* Rehbein, 1979, Salfi *et al.,* 1985). However, it was later stated that these methods should be used with great caution, as some of the enzymes are liberated from the organelles also during iced storage of fish (Rehbein, 1992).

The proteins in the sarcoplasmic fraction are excellently suited to distinguishing between different fish species, as all the different species have their characteristic band pattern when separated by the isoelectric focusing method. The method was succesfully introduced by Lundstrom (1980) and has been used by many laboratories and for many fish species. A review of the literature is given by Rehbein (1990)*.*

The chemical and physical properties of collagen proteins are different in tissues such as skin, swim bladder and the myocommata in muscle (Mohr, 1971)*.* In general, collagen fibrils form a delicate network structure with varying complexity in the different connective tissues in a pattern similar to that found in mammals. However, the collagen in fish is much more thermolabile and contains fewer but more labile cross-links than collagen from warm-blooded vertebrates. The hydroxyprolin content is in general lower in fish than in mammals, although a total variation between 4.7 and 10 % of the collagen has been observed (Sato *et at,* 1989).

Different fish species contain varying amounts of collagen in the body tissues. This has led to a theory that the distribution of collagen may reflect the swimming behaviour of the species (Yoshinaka *et at, 1988).* Further, the varying amounts and varying types of collagen in different fishes may also have an influence on the textural properties of fish muscle (Montero and Borderias, 1989). Borresen (1976) developed a method for isolation of the collagenous network surrounding each individual muscle cell. The structure and composition of these structures has been further characterized in cod by Almaas (1982).

The role of collagen in fish was reviewed by Sikorsky *et al.* (1984). An excellent, more recent review is given by Bremner (1992), in which the most recent literature of the different types of collagen found in fish is presented.

Fish proteins contain all the essential amino-acids and, like milk, eggs and mammalian meat proteins, have a very high biological value (Table 4.3).

Table 4.3 Essential amino-acids (percentage) in various proteins

Amino-acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

SOURCES: Braekkan, 1976; Moustgard, 1957

Cereal grains are ususally low in lysine and/or the sulphur-containing amino-acids (methionine and cysteine), whereas fish protein is an excellent source of these aminoacids. In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly.

In addition to the fish proteins already mentioned there is a renewed interest in specific protein fractions that may be recovered from by-products, particularly in the viscera. One such example is the basic protein or protamines found in the milt of the male fish. The molecular weight is usually below 10 000 kD and the pI is higher than 10. This is a result of the extreme amino-acid composition that may show as much as 65 % arginine.

The presence of the basic proteins has long been known, and it is also known that they are not present in all fish species (Kossel, 1928). The best sources are salmonids and herring, whereas ground fish like cod are not found to contain protamines.

The extreme basic character of protamines makes them interesting for several reasons. They will adhere to most other proteins less basic. Thus they have the effect of enhancing functional properties of other food proteins (Poole *et al.,* 1987; Phillips *et al.,* 1989). However, there is a problem in removing all lipids present in the milt from the protein preparation, as this results in an off-flavour in the concentrations to be used in foods.

Another interesting feature of the basic proteins is their ability to prevent growth of microorganisms (Braekkan and Boge, 1964; Kamal *et al.,* 1986). This appears to be the most promising use of these basic proteins in the future.

4.4 N-containing extractives

The N-containing extractives can be defined as the water-soluble, low molecular weight, nitrogen- containing compounds of non-protein nature. This NPN-fraction (non-protein nitrogen) constitutes from 9 to 18 % of the total nitrogen in teleosts.

The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino-acids, nucleotides and purine bases, and, in the case of cartilaginous fish, urea.

Table 4.4 lists some of the components in the NPN-fraction of various fish, poultry meat and mammalian meat.

Table 4.4 Major differences in muscle extractives

Compound in mg/100 wet weight[1)]	Fish Cod	Fish Herring	Fish Shark species	Crustaceans Lobster	Poutry Leg muscle	Mammalian muscle
1) Total extractives	1200	1200	3000	5500	1200	3500
2) Total free amino-acids:	75	300	100	3000	440	350

Arginine	<10	<10	<10	750	<20	<10
Glycine	20	20	20	100-1000	<20	<10
Glutamic acid	<10	<10	<10	270	55	36
Histidine	<1.0	86	<1.0	-	<10	<10
Proline	<1.0	<1.0	<1.0	750	<10	<10
3) Creatine	400	400	300	0	-	550
4) Betaine	0	0	150	100	-	-
5) Trimethylamine oxide	350	250	500-1000	100	0	0
6) Anserine	150	0	0	0	280	150
7) Carnosine	0	0	0	0	180	200
8) Urea	0	0	2000	-	-	35

[1] It should be noted that the unit in this table refers to the total molecular weight of the compound

SOURCE: Shewan, 1974

An example of the distribution of the different compounds in the NPN-fraction in freshwater and marine fish is shown in Figure 4.5. It should be noted that the composition varies not only from species to species, but also within the species depending on size, season, muscle sample, etc.

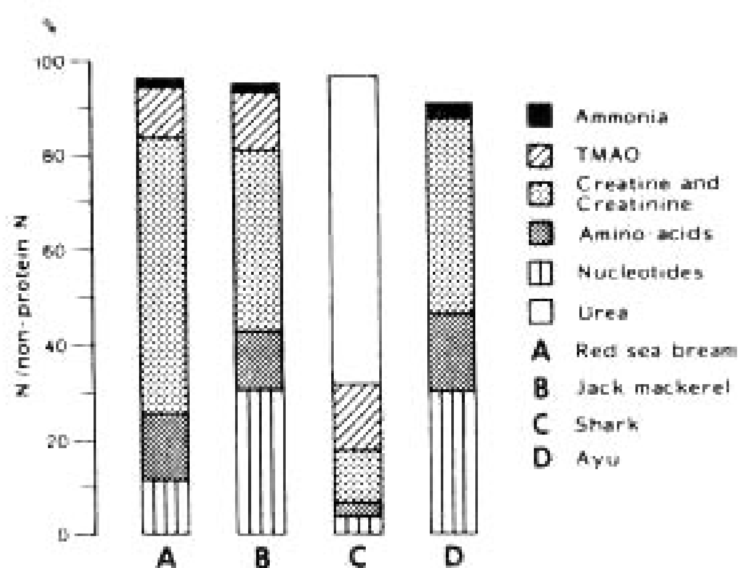


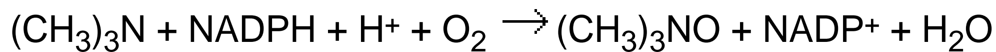
Figure 4.5 Distribution of non-protein nitrogen in fish muscles of two marine bonyfish (A,B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama *et al.,* 1977)

TMAO constitutes a characteristic and important part of the NPN-fraction in marine species and deserves further mention. This component is found in all marine fish species in quantities from 1 to 5 % of the muscle tissue (dry weight) but is virtually absent from freshwater species and from terrestrial organisms (Anderson and Fellers, 1952; Hebard *et al.,* 1982).

One exception was recently found in a study of Nile perch and tilapia from Lake Victoria, where as much as 150-200 mg TMAO/100 g of fresh fish was found (Gram *et al.,* 1989).

Although much work has been conducted on the origin and role of TMAO, there is still much to be clarified. Stroem *et al.* (1979) have shown that TMAO is formed by biosynthesis in certain zooplankton species. These organisms possess an enzyme (TMA mono-oxygenase) which oxidizes TMA to TMAO. TMA is commonly found in marine plants as are many other methylated amines (monomethylamine and dimethylamine). Plankton-eating fish may obtain their TMAO from feeding on these zooplankton (exogenous origin). Belinski (1964) and Agustsson and Stroem (1981) have shown that certain fish species are able to synthesize TMAO from TMA, but this synthesis is regarded as being of minor importance.

The TMA-oxidase system is found in the microsomes of the cells and is dependent on the presence of Nicotinamide ademine denucleotide phosphate (NADPH):



It is puzzling that this mono-oxygenase can be widely found in mammals (where it is thought to function as a detoxifier), while most fish have low or no detectable actitity of this enzyme.

Japanese research (Kawabata, 1953) indicates that there is a TMAO-reducing system present in the dark muscle of certain pelagic fishes.

The amount of TMAO in the muscle tissue depends on the species, season, fishing ground, etc. In general, the highest amount is found in elasmobranchs and squid (75-250 mg N/100 g); cod have somewhat less (60-120 mg N/100 g) while flatfish and pelagic fish have the least. An extensive compilation of data is given by Hebard *et* al. (1982). According to Tokunaga (1970), pelagic fish (sardines, tuna, mackerel) have their highest concentration of TMAO in the dark muscle while demersal, white-fleshed fish have a much higher content in the white muscle.

In elasmobranchs, TMAO seems to play a role in osmoregulation, and it has been shown that a transfer of small rays to a mixture of fresh and sea water (1: 1) will result in a 50 % reduction of intracellular TMAO. The role of TMAO in teleosts is more uncertain.

Several hypotheses for the role of TMAO have been proposed:

- TMAO is essentially a waste product, a detoxified form of TMA
- TMAO is an osmoregulator
- TMAO functions as an "anti-freeze"
- TMAO has no significant function. It is accumulated in the muscle when the fish is fed a TMAO-containing diet

According to Stroem (1984), it is now generally believed that TMAO has an osmoregulatory role.

As the occurrence of TMAO had previously been found virtually only in marine species until the observation published by Gram *et al.* (1989), it was speculated that TMAO together with high amounts of taurine could have additional effects, at least in fresh water fish (Anthoni *et al.,* 1990 a).

Quantitatively, the main component of the NPN-fraction is creatine. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction.

The NPN-fraction also contains a fair amount of free amino-acids. These constitute 630 mg/ 100 g light muscle in mackerel *(Scomber scombrus),* 350-420 mg/ 100 g in herring *(Clupea harengus)* and 310-370 mg/100 g in capelin *(Mallotus villosus).* The relative importance of the different amino- acids varies with species. Taurine, alanine, glycine and imidazole-containing amino-acids seem to dominate in most fish. Of the imidazole-containing amino-acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine.

The amount of nucleotides and nucleotide fragments in dead fish depends on the state of the fish and is discussed in section 5.

4.5 Vitamins and minerals

The amount of vitamins and minerals is species-specific and can furthermore vary with season. In general, fish meat is a good source of the B vitamins and, in the case of fatty species, also of the A and D vitamins. Some freshwater species such as carp have high thiaminase actitity so the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular but also of iron, copper and selenium. Saltwater fish have a high content of iodine. In Tables 4.5 and 4.6 some of the vitamin and mineral contents are listed. Because of the natural variation of these constituents, it is impossible to give accurate figures.

Table 4.5 Vitamins in fish

Fish	A (IU/g)	D (IU/g)	B_1 (thiamine) (µ/g)	B_2 (riboflavin) (µ/g)	Niacin (µ/g)	Pantothenic acid (µ/g)	B_6 (µ/g)
Cod fillet	0-50	0	0.7	0.8	20	1.7	1.7
Herring fillet	20-400	300-1000	0.4	3.0	40	10	4.5
Cod-liver oil	200-10.000	20-300	-	[1] 3.4	[1] 15	[1] 4.3	-

1) Whole liver

SOURCE: Murray and Burt, 1969

Table 4.6 Some mineral constituents of fish muscle

Element	Average value (mg/100 g)	Range (mg/100 g)
Sodium	72	30 -134
Potassium	278	19 -502
Calcium	79	19 -881
Magnesium	38	4.5-452
Phosphorus	190	68-550

SOURCE: Murray and Burt, 1969

The vitamin content is comparable to that of mammals except in the case of the A and D vitamins which are found in large amounts in the meat of fatty species and in abundance in the liver of species such as cod and halibut. It should be noted that the sodium content of fish meat is relatively low which makes it suitable for low-sodium diets.

In aquacultured fish, the contents of vitamins and minerals are considered to reflect the composition of the corresponding components in the fish feed, although the observed data should be interpreted with great caution (Maage *et al.,* 1991). In order to protect the n-3 polyunsaturated fatty acids, considered of great importance both for fish and human health, vitamin E may be added to the fish feed as an antioxidant. It has been shown that the resulting level of vitamin E in the fish tissue corresponds to the concentration in the feed (Waagbo *et al.,* 1991).

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5. POSTMORTEM CHANGES IN FISH

5.1. Sensory changes
5.2. Autolytic changes
5.3. Bacteriological changes
5.4. Lipid oxidation and hydrolysis

5.1 Sensory changes

Sensory changes are those perceived with the senses, i.e., appearance, odour, texture and taste.

Changes in raw fresh fish

The first sensory changes of fish during storage are concerned with appearance and texture. The characteristic taste of the species is normally developed the first couple of days during storage in ice.

The most dramatic change is onset of rigor mortis. Immediately after death the muscle is totally relaxed and the limp elastic texture usually persists for some hours, whereafter the muscle will contract. When it becomes hard and stiff the whole body becomes inflexible and the fish is in rigor mortis This condition usually lasts for a day or more and then rigor resolves. The resolution of rigor mortis makes the muscle relax again and it becomes limp, but no longer as elastic as before rigor. The rate in onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish (Table 5.1).

The effect of temperature on rigor is not uniform. In the case of cod, high temperatures give a fast onset and a very strong rigor mortis. This should be avoided as strong rigor tensions may cause gaping, i.e., weakening of the connective tissue and rupture of the fillet.

It has generally been accepted that the onset and duration of rigor mortis are more rapid at high temperatures, but observations, especially on tropical fish show the opposite effect of temperature with regard to the onset of rigor. It is evident that in these species

the onset of rigor is accelerated at 0°C compared to 10°C, which is in good correlation with a stimulation of biochemical changes at 0°C (Poulter *et al.,* 1982; Iwamoto *et al.,* 1987). However, an explanation for this has been suggested by Abe and Okuma (1991) who have shown that onset of rigor mortis in carp (Cyprinus *carpio)* depends on the difference in sea temperature and storage temperature. When the difference is large the time from death to onset of rigor is short and *vice versa.*

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 hours (Azam *et al.,* 1990; Proctor *et al.,* 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor the fish body will be completely stiff; the filleting yield will be very poor, and rough handling can cause gaping. If the fillets are removed from the bone pre-rigor the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink up to 52 % and white muscle up to 15 % of the original length (Buttkus, 1963). If the fish is cooked pre-rigor the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Post-rigor the flesh will become firm, succulent and elastic.

Table 5.1 Onset and duration of rigor mortis in various fish species

Species	**Condition**	**Temperature °C**	**Time from death to onset of *rigor* (hours)**	**Time from death to end of *rigor* (hours)**
Cod *(Gadus morhua)*	Stressed	0	2-8	20-65
	Stressed	10-12	1	20-30
	Stressed	30	0.5	1-2
	Unstressed	0	14-15	72-96
Grouper *(Epinephelus malabaricus)*	Unstressed	2	2	18
Blue Tilapia *(Areochromis aureus)*	Stressed	0	1	
	Unstressed	0	6	
Tilapia *(Tilapia mossanibica)* small 60g	Unstressed	0-2	2-9	26.5

Grenadier *(Macrourus whitson)*	Stressed	0	<1	35-55
Anchovy *(Engraulis anchoita)*	Stressed	0	20-30	18
Plaice *(Pleuronectes platessa)*	Stressed	0	7-11	54-55
Coalfish *(Pollachius virens)*	Stressed	0	18	110
Redfish *(Sebastes* spp.)	Stressed	0	22	120
Japanese flounder *(Paralichthys olivaceus)*		0	3	>72
		5	12	>72
		10	6	72
		15	6	48
		20	6	24
Carp *(Cyprinus carpio)*		0	8	
		10	60	
		20	16	
	Stressed	0	1	
	Unstressed	0	6	

SOURCES: Hwang *et al.,* 1991; Iwamoto *et al., 1987;* Korhonen *et al.,* 1990;Nakayama *et al.,* 1992; Nazir and Magar, 1963; Partmann, 1965; Pawar and Magar, 1965; Stroud, 196; Trucco *et al.,* 1982

Whole fish and fillets frozen pre-rigor can give good products if they are carefully thawed at a low temperature in order to give *rigor mortis* time to pass while the muscle is still frozen.

The sensory evaluation of raw fish in markets and landing sites is done by assessing the appearance, texture and odour. The sensory attributes for fish are listed in Table 5.2. Most scoring systems are based upon changes taking place during storage in melting

ice. It should be remembered that the characteristic changes vary depending on the storage method. The appearance of fish stored under chilled condition without ice does not change as much as for iced fish, but the fish spoil more rapidly and an evaluation of cooked flavour will be necessary. A knowledge of the time /temperature history of the fish should therefore be essential at landing.

The characteristic sensory changes in fish post mortem vary considerably depending on fish species and storage method. A general description has been provided by the EEC in the guidelines for quality assessment of fish as shown in Table 5.2. The suggested scale is numbered from 0 to 3, where 3 is the best quality.

The West European Fish Technologists' Association has compiled a multilingual glossary of odours and flavours which also can be very useful when looking for descriptive words for sensory evaluation of freshness of fish (Howgate et al., 1992 (Appendix C).

Changes in eating quality

If quality criteria of chilled fish during storing are needed, sensory assessment of the cooked fish can be conducted. Some of the attributes for cooked fish and shellfish are mentioned in Table 5.2. A characteristic pattern of the deterioration of fish stored in ice can be found and divided into the following four phases:

- **Phase 1** The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic. In cod, haddock, whiting and flounder, the sweet taste is maximized 2-3 days after catching.
- **Phase 2** There is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours. The texture is still pleasant.
- **Phase 3** There is sign of spoilage and a range of volatile, unpleasant-smelling substances is produced depending on the fish species and type of spoilage (aerobic, anaerobic). One of the volatile compounds may be trimethylamine (TMA) derived from the bacterial reduction of trimethyl-aminoxide (TMAO). TMA has a very characteristic "fishy" smell. At the beginning of the phase the off-flavour may be slightly sour, fruity and slightly bitter, especially in fatty fish. During the later stages sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smells develop. The texture becomes either soft and watery or tough and dry.
- **Phase 4** The fish can be characterized as spoiled and putrid.

Table 5.2 Freshness ratings: Council Regulation (EEC) No. 103/76 OJ No. L20 (28 January 1976) (EEC, 1976)

Criteria	
	Marks

Part of fish inspected	3	2	1	0
Appearance				
Skin	Bright, iridescent pigmentation, no discoloration Aqueous, transparent, mucus	Pigmentation bright but not lustrous Slightly cloudy mucus	Pigmentation in the process of becoming discoloured and dull Milky mucus	[1]Dull pigmentation Opaque mucus
Eye	Convex (bulging) Transparent cornea Black, bright pupil	Convex and slightly sunken Slightly opalescent cornea Black, dull pupil	Flat Opalescent cornea Opaque pupil	[1]Concave in the centre Milky cornea Grey pupil
Gills	Bright colour No mucus	Less coloured Slight traces of clear mucus	Becoming discoloured Opaque mucus	[1]Yellowish Milky mucus
Flesh (cut from abdomen)	Bluish, translucent, smooth, shining No change in original colour	Velvety, waxy, dull Colour slightly changed	Slightly opaque	[1]Opaque
Colour (along vertebral column)	Uncoloured	Slightly pink	Pink	[1]Red
Organs	Kidneys and residues of other organs should be bright red, as should the blood inside the aorta	Kidneys and residues of other organs should be dull red; blood becoming discoloured	Kidneys and residues of other organs and blood should be pale red	Kidneys and residues of other organs and should be brownish in colour
Condition				

Flesh	Firm and elastic Smooth surface	Less elastic	Slightly soft (flaccid), less elastic Waxy (velvety) and dull surface	[1]Soft (flaccid) Scales easily detached from skin, surface rather wrinkled, inclining to mealy
Vertebral column	Breaks instead of coming away	Sticks	Sticks slightly	[1]Does not stick
Peritoneum	Sticks completely to flesh	Sticks	Sticks slightly	[1]Does not stick
		Smell		
Gills, skin abdominal cavity	Seaweed	No smell of seaweed or any bad smell	Slightly sour	[1]Sour

[1] Or in a more advanced state of decay.

A numbered scale may be used for the sensory evaluation of cooked fish as shown in Figure 5.1. The scale is numbered from 0 to 10, 10 indicating absolute freshness, 8 good quality and 6 a neutral tasteless fish. The rejection level is 4. Using the scale in this way the graph becomes S-shaped indicating a fast degradation of the fish during the first phase, a slower rate in phase 2 and 3 and finally a high rate when the fish is spoiled.

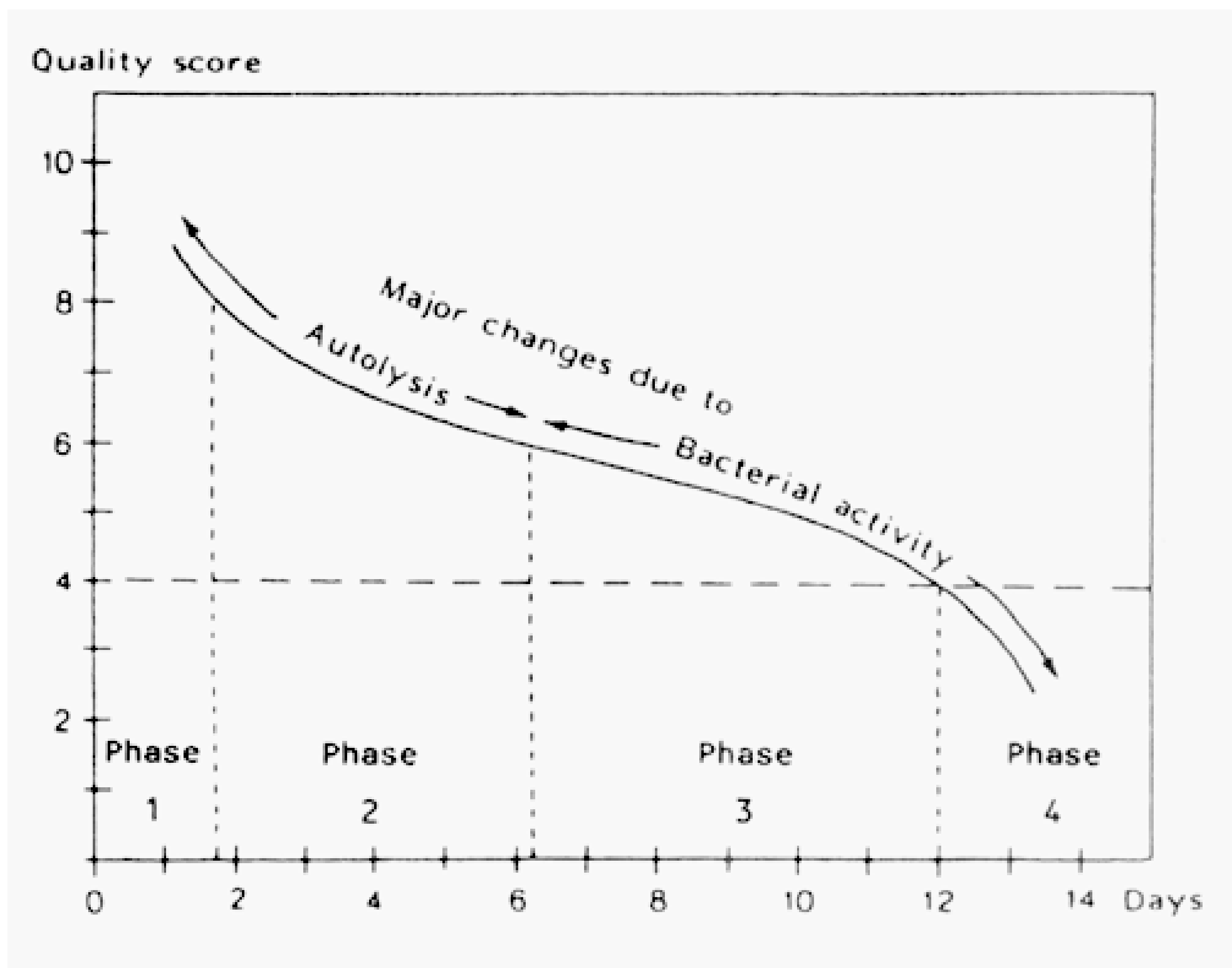


Figure 5.1 Changes in the eating quality of iced (0°C) cod (Huss, 1976)

Other scales can well be used and can change the shape of the graph. It is, however, important to understand the kind of results desired from the sensory analysis in order to ask the right questions to the sensory assessors.

5.2 Autolytic Changes

Autolysis means "self-digestion". It has been known for many years that there are at least two types of fish spoilage: bacterial and enzymatic. Uchyama and Ehira (1974) showed that for cod and yellowtail tuna, enzymatic changes related to fish freshness preceded and were unrelated to changes in the microbiological quality. In some species (squid, herring), the enzymatic changes precede and therefore predominate the spoilage of chilled fish. In others, autolysis contributes to varying degrees to the overall quality loss in addition to microbially-mediated processes.

Production of energy in post mortem muscle

At the point of death, the supply of oxygen to the muscle tissue is interrupted because the blood is no longer pumped by the heart and is not circulated through the gills where, in the living fish, it becomes enriched with oxygen. Since no oxygen is available for normal respiration, the production of energy from ingested nutrients is greatly restricted.

Figure 5.2 illustrates the normal pathway for the production of muscle energy in most living teleost fish (bony finfish). Glycogen (stored carbohydrate) or fat is oxidized or "burned" by the tissue enzymes in a series of reactions which ultimately produce carbon dioxide (CO_2), water and the energy-rich organic compound adenosine triphosphate (ATP). This type of respiration takes place in two stages: an anaerobic and an aerobic stage. The latter depends on the continued presence of oxygen (O_2) which is only available from the circulatory system. Most crustaceans are capable of respiring outside the aquatic environment by absorption of atmospheric oxygen for limited periods.

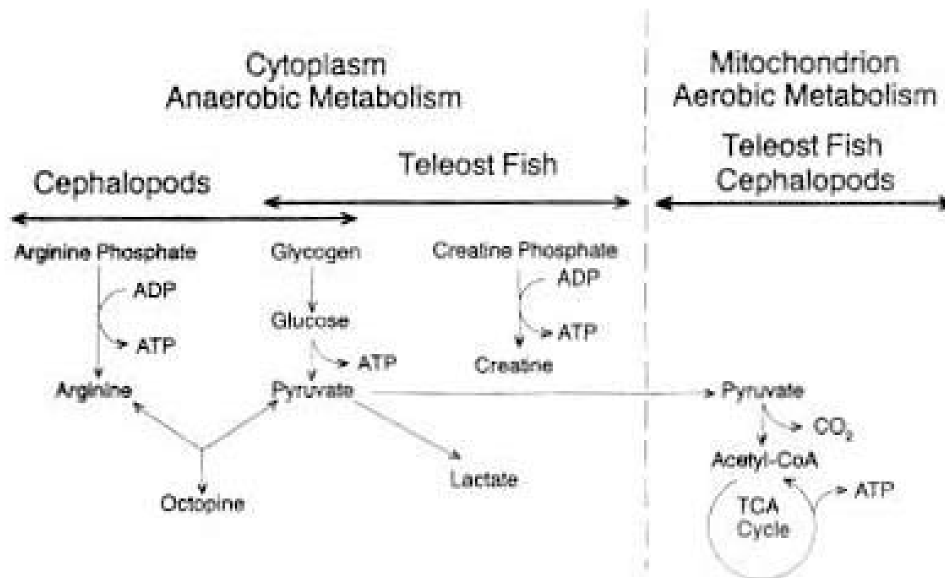


Figure 5.2 Aerobic and anaerobic breakdown of glycogen in fish muscle

Figure 5.2 also illustrates that, under anaerobic conditions, ATP may be synthesized by two other important pathways from creatine phosphate or from arginine phosphate. The former source of energy is restricted to vertebrate muscle (teleost fish) while the latter is characteristic of some invertebrates such as the cephalopods (squid and octopus). In either case, ATP production ceases when the creatine or arginine phosphates are depleted. It is interesting to note that octopine is the end-product from the anaerobic metabolism of cephalopods and is not acidic (unlike lactate), thus any changes in post mortem pH in such animals are not related to the production of lactic acid from glycogen.

For most teleost fish, glycolysis is the only possible pathway for the production of energy once the heart stops beating. This more inefficient process has principally lactic and pyruvic acids as its end-products. In addition, ATP is produced in glycolysis, but only 2 moles for each mole of glucose oxidized as compared to 36 moles ATP produced for each mole of glucose if the glycolytic end products are oxidized aerobically in the mitochondrion in the living animal. Thus, after death, the anaerobic muscle cannot maintain its normal level of ATP, and when the intracellular level declines from 7-10 µmoles/g to £ 1.0 µmoles/g tissue, the muscle enters rigor mortis. Post mortem glycolysis results in the accumulation of lactic acid which in turn lowers the pH of the muscle. In cod, the pH drops from 6.8 to an ultimate pH of 6.1-6.5. In some species of fish, the final pH may be lower: in large mackerel, the ultimate rigor pH may be as low as

5.8-6.0 and as low as 5.4-5.6 in tuna and halibut, however such low pH levels are unusual in marine teleosts. These pHs are seldom as low as those observed for post mortem mammalian muscle. For example, beef muscle often drops to pH levels of 5.1 in rigor mortis. The amount of lactic acid produced is related to the amount of stored carbohydrate (glycogen) in the living tissue. In general, fish muscle contains a relatively low level of glycogen compared to mammals, thus far less lactic acid is generated after death. Also, the nutritional status of the fish and the amount of stress and exercise encountered before death will have a dramatic effect on the levels of stored glycogen and consequently on the ultimate post mortem pH. As a rule, well-rested, well-fed fish contain more glycogen than exhausted fish. In a recent study of Japanese loach (Chiba et al., 1991), it was shown that only minutes of pre-capture stress resulted in a decrease of 0.50 pH units in 3 hours as compared to non-struggling fish whose pH dropped only 0.10 units in the same time period. In addition, the same authors showed that bleeding of fish significantly reduced the post mortem production of lactic acid.

The post mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water-holding capacity. Muscle tissue in the state of rigor mortis loses its moisture when cooked and is particularly unsuitable for further processing which involves heating, since heat denaturation enhances the water loss. Loss of water has a detrimental effect on the texture of fish muscle and it has been shown by Love (1975) that there is an inverse relationship between muscle toughness and pH, unacceptable levels of toughness (and water-loss on cooking) occurring at lower pH levels (Figure 5.3).

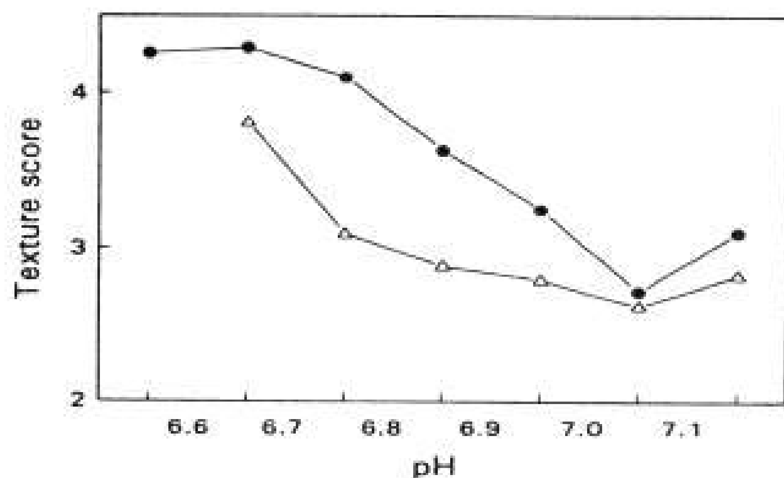


Figure 5.3.Relationship between cod muscle texture and pH, adapted from Love (1975). Black spots refer to fish caught from St. Kilda, Atlantic Ocean, whereas triangles refer to fish caught on Fells Bank, Davis Strait

Autolysis and nucleotide catabolism

As mentioned earlier, rigor mortis sets in when the muscle ATP level drops to £ 1.0 µmoles/g. ATP is not only a source of high energy which is required for muscle contraction in the living animal, but also acts as a muscle plasticizer. Muscle contraction

per se is controlled by calcium and an enzyme, ATP-ase which is found in every muscle cell. When intracellular Ca^{+2} levels are 1 μM, Ca^{+2} - activated ATP-ase reduces the amount of free muscle ATP which results in the interaction between the major contractile proteins, actin and myosin. This ultimately results in the shortening of the muscle, making it stiff and inextensible. A fish in rigor mortis cannot normally be filleted or processed because the carcass is too stiff to be manipulated and is often contorted, making machine-handling impossible (see also section 3.2 on bleeding and section 5.1 on sensory changes).

The **resolution** of rigor is a process still not completely understood but always results in the subsequent softening (relaxation) of the muscle tissue and is thought to be related to the activation of one or more of the naturally-occurring muscle enzymes, digesting away certain components of the rigor mortis complex. The softening of the muscle during resolution of rigor (and eventually spoilage processes) is coincidental with the autolytic changes. Among the changes, one of the first to be recognized was the degradation of ATP-related compounds in a more-or-less predictable manner after death. Figure 5.4 illustrates the degradation of ATP to form adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx). The degradation of ATP catabolites proceeds in the same manner with most fish but the speed of each individual reaction (from one catabolite to another) greatly varies from one species to another and often progresses coincidentally with the perceived level of spoilage as determined by trained analysts. Saito et al. (1959) were the first to observe this pattern and to develop a formula for fish freshness based on these autolytic changes:

$$K\% = \frac{[Ino] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [Ino] + [Hx]} \times 100$$

where [ATP], [ADP], [AMP], [IMP], [Ino] and [Hx] represent the relative concentrations of these compounds in fish muscle measured at various times during chilled storage.

The K or "freshness" index gives a relative freshness rating based primarily on the autolytic changes which take place during post mortem storage of the muscle. Thus, the higher the K value, the lower the freshness level. Unfortunately, some fish species such as Atlantic cod reach a maximum K value well in advance of the shelf life as determined by trained judges, and K is therefore not considered reliable as a freshness index for all marine finfish. Also, the degradation of nucleotide catabolites is only coincidental with perceived changes in freshness and not necessarily related to the cause of freshness deterioration since only Hx is considered to have a direct effect on the perceived bitter off-flavour of spoiled fish (Hughes and Jones, 1966). It is now widely accepted that IMP is responsible for the desirable fresh fish flavour which is only present in top quality seafood. None of the nucleotide catabolites are considered to be related to the perceived changes in texture during the autolytic process except of course ATP whose loss is associated with *rigor mortis.*

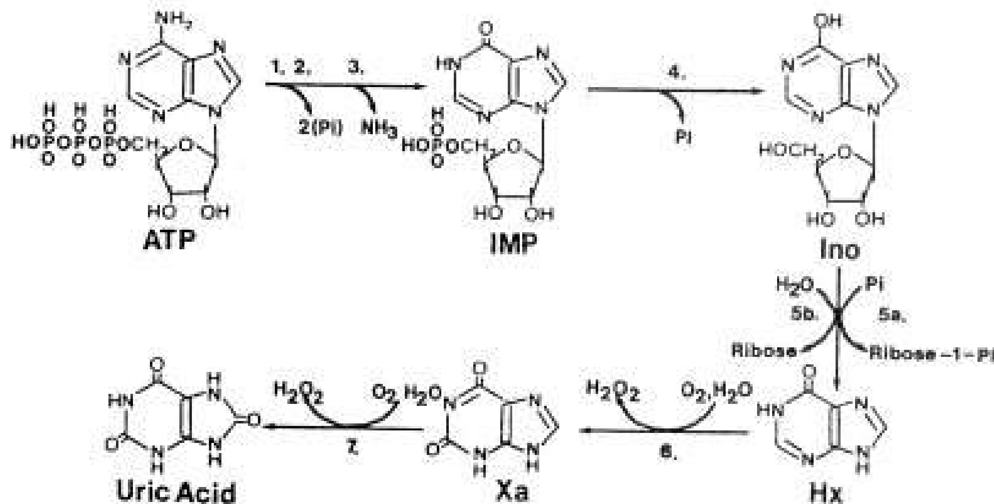


Figure 5.4 *Postmortem* ATP degradation in fish muscle. Enzymes include: 1. ATP-ase; 2. myokinase; 3. AMP deaminase; 4.IMP phosphohydrolase; 5a. nucleoside phosphorylase; 5b. inosine nucleosidase; 6,7. xanthine oxidase. Source: Gill (1992)

Surette *et al.* (1988) followed the autolysis of sterile and non-sterile cod as indicated by the ATP catabolites. The rates of formation and breakdown of IMP were the same in both sterile and non- sterile samples of cod tissue (Figures 5.5a and 5.5b), indicating that the catabolic pathway for the degradation of ATP through to inosine is entirely due to autolytic enzymes.

The conversion of Iino to Hex was accelerated by about 2 days for the non-sterile samples, suggesting that bacterial nucleoside phosphorylase (enzyme 5.a in Figure 5.4) plays a major role in the *postmortem* production of Hx in refrigerated cod (see also section 5.3). It is interesting to note that Surette *et al.* (1988) were not able to recover nucleoside phosphorylase from freshly killed cod, but Surette *et al.* (1990) later went on to isolate and purify this enzyme from a *Proteus* bacterium recovered from spoiled cod fillets. As mentioned earlier, large variations can be expected in the patterns of nucleotide degradation from one species to another. The variations in Hx among various types of fish are shown in Figure 5.6. It is clear therefore that Hx determination would likely not be useful for such species as swordfish and redfish.

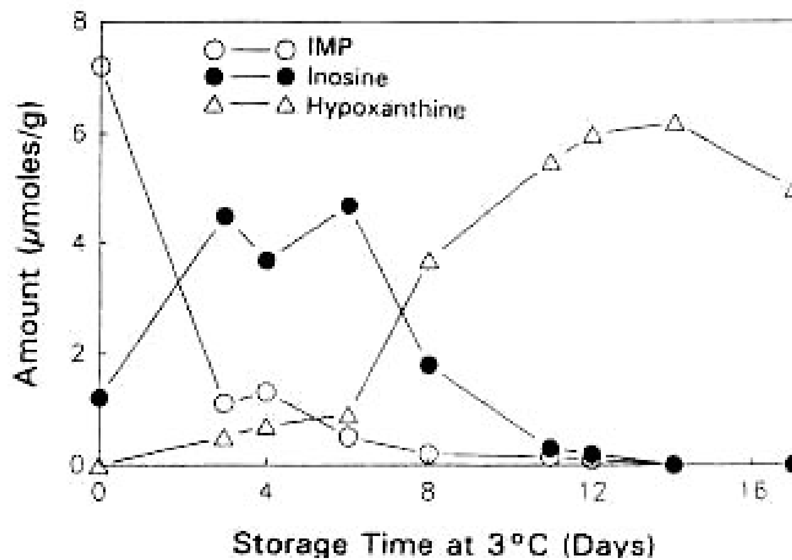


Figure 5.5a Changes in IMP, Ino and Hx in sterile cod fillets at 3°C adapted from Gill (1990)

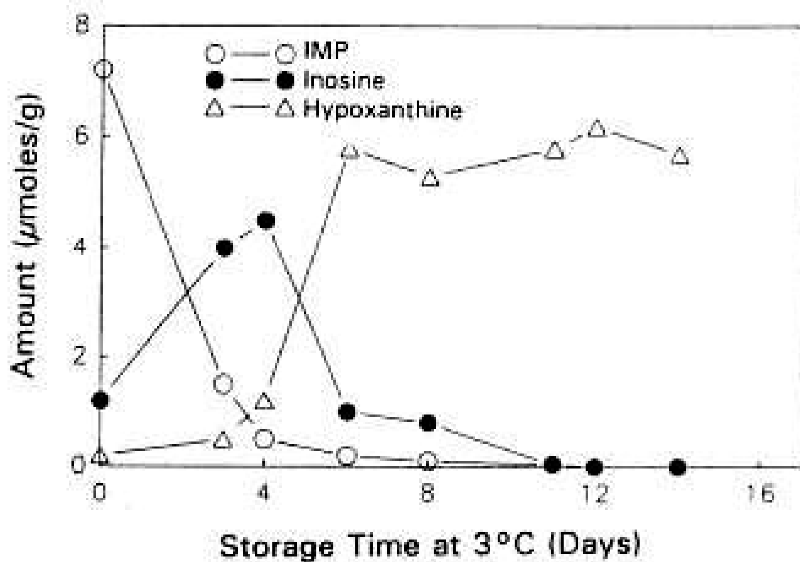


Figure 5.5b Changes in IMP, Ino and Hx in non-sterile cod fillets at 3°C adapted from Gill (1990)

There is little doubt that physical handling accelerates the autolytic changes in chilled fish. Surette et al. (1988) reported that the breakdown rate of the nucleotide catabolites was greater in sterile fillets than in non-sterile gutted whole cod. This is perhaps not surprising since many of the autolytic enzymes have been shown to be compartmentalized in discrete membrane-bound packages which become broken when subjected to physical abuse and result in the intimate mixing of enzyme and substrate. Crushing of the fish by ice or other fish can seriously affect the edibility and filleting yields even for fish which have a relatively low bacterial load, demonstrating the importance of autolytic processes. Iced fish should never be stored in boxes deeper than 30 cm and it is equally important to be sure that fish boxes are not permitted to "nest" one on top of the other if autolysis is to be minimized. Systems for conveying fish and for

discharge from the vessels must be designed so as to avoid physical damage to the delicate tissues.

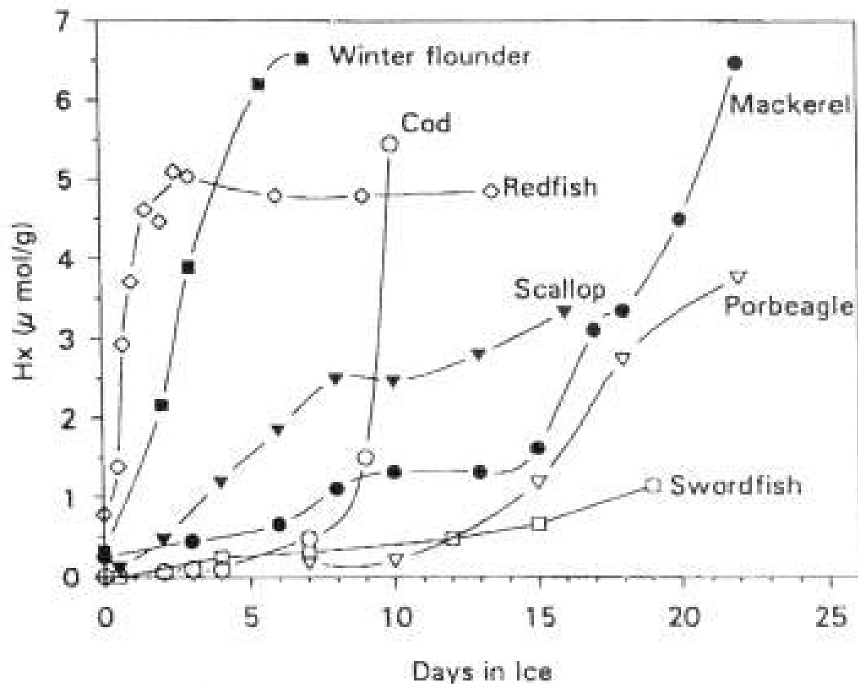


Figure 5.6 Variation in the rate of Hx accumulation of several species during storage in ice. Adapted from Fraser *et al.* (1967)

Several rapid methods have been developed for the determination of individual nucleotide catabolites or combinations including the freshness index. Two recent reviews should be consulted (Gill, 1990, 1992).

Autolytic changes involving proteolytic enzymes

Many proteases have been isolated from fish muscle and the effects of proteolytic breakdown are often related to extensive softening of the tissue. Perhaps one of the most notable examples of autolytic proteolysis is the incidence of belly-bursting in pelagic (fatty fish) species such as herring and capelin. This type of tissue softening is most predominant in summer months when pelagics are feeding heavily, particularly on "red feed" consisting of copepods and euphausiids. The low molecular weight peptides and free amino-acids produced by the autolysis of proteins not only lower the commercial acceptability of pelagics, but in bulk-stored capelin, autolysis has been shown to accelerate the growth of spoilage bacteria by providing a superior growth environment for such organisms (Aksnes and Brekken, 1988). The induction of bacterial spoilage in capelin by autolysis also resulted in the decarboxylation of amino-acids, producing biogenic amines and lowered the nutritive value of the fish significantly. This is particularly important since autolysis and bacterial growth greatly lower the commercial value of pelagics used for the production of fishmeal.

Similarly, bulk-stored herring used for fishmeal has been found to contain carboxy-peptidases A and B, chymotrypsin, and trypsin; and preliminary studies have shown that

proteolysis can be inhibited by the addition of potato extracts which not only slowed the proteolysis but resulted in lower microbial growth and preservation of the nutritional value of the meal (Aksnes, 1989).

More recently, Botta *et al.* (1992) found that autolysis of the visceral cavity (belly-bursting) of herring was related more to physical handling practices than to biological factors such as fish size, amount of red feed in the gut or roe content. In particular, it was shown that for herring, freezing/thawing, thawing time at 15°C and time of iced storage, had a far greater influence on belly- bursting than biological factors.

Cathepsins

Although several proteolytic enzymes have been discovered in the fish tissues, it has perhaps been the cathepsins which have been described most often. The cathepsins are "acid" proteases usually found packaged in tiny, submicroscopic organelles called lysozomes. In living tissue, lysozomal proteases are believed to be responsible for protein breakdown at sites of injury. Thus cathepsins are for the most part inactive in living tissue but become released into the cell juices upon physical abuse or upon freezing and thawing of *post mortem* muscle.

Cathepsins D and L are believed to play a major role in the autolytic degradation of fish tissue since most of the other cathepsins have a relatively narrow pH range of activity far too low to be of physiological significance. Reddi *et al.* (1972) demonstrated that an enzyme believed to be cathepsin D from winter flounder was active over a pH range of 3-8 with a maximum near pH 4.0, although no attempt was made to confirm the identity of the enzyme using synthetic substrates or specific inhibitors. Nevertheless, the enzyme was far less active in the presence of ATP, suggesting that such an enzyme would only be active in *post mortem* fish muscle. Also, the enzyme activity was inhibited strongly by the presence of salt (Figure 5.7) with virtually no activity remaining after a 25-hour incubation in the presence of 5% sodium chloride. It is therefore unlikely that Reddi's enzyme was active in salted fish products.

Cathepsin L has been implicated in the softening of salmon muscle during spawning migration. It is likely that this enzyme contributes more to autolysis of fish muscle than cathepsin D since it is far more active at neutral pH, and has been shown to digest both myofibrillar proteins (actomyosin) as well as connective tissue. Yamashita and Konogaya (1990) produced strong evidence implicating cathepsin L rather than other cathepsins in the softening of salmon during spawning. They demonstrated that electrophoresis of purified myofibrils treated with cathepsin L resulted in patterns which were almost identical to patterns of proteins recovered from muscle from spawning fish. Furthermore, the cathepsin L autolytic activity correlated well with the texture of the muscle as measured instrumentally. The linear correlation between cathepsin L activity and breaking strength of the muscle was excellent; r = 0.86 and -0.95 for fresh and frozen/thawed tissue, respectively. It is interesting that, in all cases, the autolytic ability as measured by cathepsin L activity was higher in frozen/thawed tissue than in fresh tissue. Freezing and thawing often break down cell membranes allowing autolytic membrane-bound enzymes to react with their natural substrates. The enzyme and its

naturally occurring inhibitor were further studied by the same authors (Yamashita and Konogaya, 1992). Cathepsin L has also been associated with the production of a jelly-like softening of flounder (Toyohara *et l.,* 1993 a) and the uncontrollable softening of Pacific hake muscle which has been parasitized by Myxosporidia (Toyohara *et al.,* 1993 b).

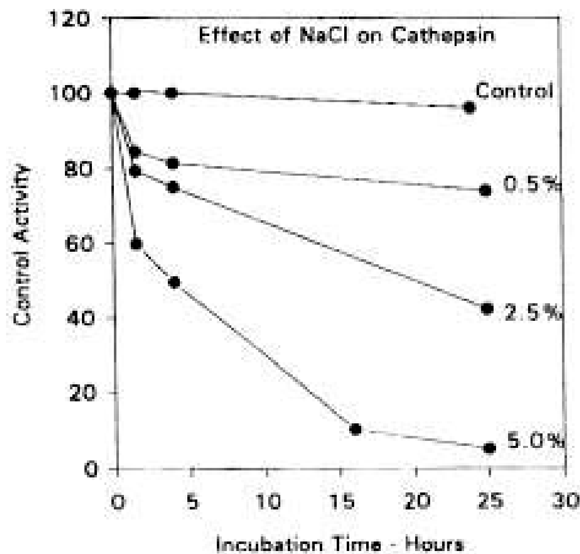


Figure 5.7 Effect of NaCl on the catheptic activity. Adapted from Reddi *et al.* (1972)

The tissues of such infected fish have little commercial value, but at present it is not known if it is the parasite or the host which secretes the proteolytic enzymes which autolyze the muscle.

In addition to their detrimental effect on texture, catheptic enzymes induce intentional autolytic changes in fermented fish products. For example, cathepsins are believed to be responsible for major textural changes during the fermentation of salted preserved Japanese squid and Crucian carp (Makinodan *et al.,* 1991, 1993).

Calpains

A second group of intracellular proteases called "calpains" or "calcium activated factor" (CAF) has recently been associated with fish muscle autolysis and is found in meats, finfish and crustaceans. Tenderness is probably the most important quality characteristic of red meat. It has been known for nearly a century that *post mortem* aging of red meat results in the tenderization process. Calpains have been found primarily responsible for the *post mortem* autolysis of meat through digestion of the z- line proteins of the myofibril. Although toughness is seldom a problem with unfrozen fish muscle, softening through autolysis is a serious problem limiting the commercial value. The calpains are intra-cellular endopeptidases requiring cysteine and calcium; μ-calpain requiring 5-50 μM Ca^{2+} , m-calpain requiring 150-1000 μM Ca^{2+} . Most calpains are active at physiological pH, making it reasonable to suspect their importance in fish-softening during chilled storage.

Studies have shown that in crustacean muscle, calpains are associated with moltinduced textural changes to the muscle and carry out non-specific generalized digestion of the myofibrillar proteins. However, vertebrate muscle calpains have been shown to be very specific, digesting primarily tropinin- T, desmin, titin and nebulin, attacking neither vertebrate actin or myosin (Koohmaraie, 1992). In contrast, fish calpains digest myosin (specifically the myosin heavy chain) to form an initial fragment with approximate molecular weight of 150 000 Da (Muramoto *et al.,* 1989). The same authors demonstrated that fish calpains were far more active at low temperatures than were mammalian calpains and that the rates of cleavage were species-specific, being most active against myosins with lowest heat stabilities. Thus, fish species adapted to colder environmental temperatures are more susceptible to calpain autolysis than those from tropical waters. Although calpain has been identified in several fish species including carp (Toyohara *et al.,* 1985), tilapia and shrimp (Wang *et al.,* 1993), as well as tuna, croaker, red seabream and trout (Muramoto *et al.,* 1989) to name a few, little work has to date demonstrated a "cause and effect" relationship between calpain activity and instrumental measurements of texture.

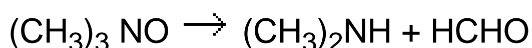
Collagenases

To this point, all of the *post mortem* autolytic changes described have involved changes within the muscle cell *per se.* However, the flesh of teleost fish is divided into blocks of muscle cells separated into "flakes" or myotomes by connective tissue called myocommata (Figure 3.3). Each muscle cell or fibre is surrounded with connective tissue which attaches to the myocommata at the ends of the cells by means of fine collagenous fibrils. During chilled storage, these fibrils deteriorate (Bremner and Hallett, 1985). More recently, it was shown that instrumental measurements of texture of chilled trout muscle decreased as the amount of type V collagen was solubilized, presumably due to the action of autolytic collagenase enzymes (Sato *et al.,* 1991). It is these enzymes which presumably cause "gaping" or breakdown of the myotome during long-term storage on ice or short term storage at high temperature. For Atlantic cod, it has been shown that upon reaching 17°C, gaping is inevitable presumably because of degradation of the connective tissue and rapid shortening of the muscle due to high temperature *rigor.*

The relatively short shelf life of chilled prawns due to softening of the tissue has also been shown to be due to the presence of collagenase enzymes (Nip *et al.,* 1985). The source of the collagenase enzymes in prawn is thought to be the hepatopancreas (digestive organ).

Autolytic changes during frozen storage

The reduction of trimethylamine oxide (TMAO), an osmoregulatory compound in many marine teleost fish, is usually due to bacterial action (section 5.3) but in some species an enzyme is present in the muscle tissue which is able to break down TMAO into dimethylamine (DMA) and formaldehyde (FA):



$$(CH_3)_3NO \rightarrow (CH_3)_2NH + HCHO$$

It is important to note that the amount of formaldehyde produced is equivalent to the dimethylamine formed but is of far greater commercial significance. Formaldehyde induces cross- linking of the muscle proteins making the muscle tough and readily lose its water holding capacity. The enzyme responsible for formal dehyde-induced toughening is called TMAO-ase or TMAO demethylase and is most commonly found in the gadoid fishes (cod family). Most of the TMAO demethylase enzymes reported to date weremembrane-bound and become most active when the tissue membranes are disrupted by freezing or artificially by detergent solubilization. Dark (red) muscle has a higher rate of activity than white muscle whereas other tissues such as kidney, spleen and gall bladder are extremely rich in the enzyme. Thus, it is important that minced fish is completely free of organ tissue such as kidney from gadoid species if toughening in frozen storage is to be avoided. It is often difficult to ensure that the kidney is removed prior to mechanical deboning since this particular organ runs the full length of the backbone and is adherent to it. The TMAO-ase enzyme has been isolated from the microsomal fraction in hake muscle (Parkin and Hultin, 1986) and the lysosomal membrane in kidney tissue (Gill *et al.,* 1992). It has been shown that the toughening of frozen hake muscle is correlated to the amount of formaldehyde produced, and that the rate of FA production is greatest at high frozen-storage temperatures (Gill *et al., 1979).* In addition, it has been shown that the amount of FA-induced toughening is enhanced by physical abuse to the catch prior to freezing and by temperature fluctuations during frozen storage. The most practical means of preventing the autolytic production of FA is to store fish at temperatures < -30°C to minimize temperature fluctuations in the cold store and to avoid rough handling or the application of physical pressure on the fish prior to freezing. The autolytic changes affecting the edibility of fresh and frozen fish are summarized in Table 5.3. Generally, the most important single factor affecting autolysis is physical disruption of the muscle cells. No attempt has been made here to deal with the alkaline proteases associated with the softening of cooked surimi products. An article by Kinoshita *et al.* (1990) deals with the heat-activated alkaline proteases associated with the softening in surimi-based products.

Table 5.3 Summary of Autolytic Changes in Chilled Fish

Enzyme(s)	Substrate	Changes Encountered	Prevention/Inhibition
glycolytic enzymes	glycogen	production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle high temperature rigor may result in gaping	fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible pre-rigor stress must be avoided

autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	loss of fresh fish flavour, gradual production of bitternes with Hx (later stages)	same as above rough handling or crushing accelerates breakdown
cathepsins	proteins, peptides	softening of tissue making processing difficult or impossible	rough handling during storage and discharge
chymotrypsin, trypsin, carboxy-peptidases	proteins, peptides	autolysis of visceral cavity in pelagics (belly-bursting)	problem increased with freezing/thawing or long- term chill storage
calpain	myofibrillar proteins	softening, molt-induced softening in crustaceans	removal of calcium thus preventing activation?
collagenases	connective tissue	gaping" of fillets softening	connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	formaldehyde-induced toughening of frozen gadoid fish	store fish at temperature <= -30°C physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

5.3 Bacteriological changes

The bacterial flora on live fish

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously and Liston (1980) states a normal range of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g (Shewan, 1962).

The bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1977). Fish caught in very cold, clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e., 10^7 cfu/cm^2 are found on fish from polluted warm waters. Many different bacterial species can be found on the fish surfaces. The bacteria on temperate water fish are all classified according to their growth temperature range as either psychrotrophs or psychrophiles. Psychrotrophs (cold-tolerant) are bacteria capable of growth at 0°C but with optimum around 25°C. Psychrophiles (cold-loving) are

bacteria with maximum growth temperature around 20°C and optimum temperature at 15°C (Morita, 1975). In warmer waters, higher numbers of mesophiles can be isolated. The microflora on temperate water fish is dominated by psychrotrophic Gram-negative rodshaped bacteria belonging to the genera *Pseudomonas, Moraxella, Acinetobacter, Shewanella* and *Flavobacterium.* Members of the *Vibrionaceae (Vibrio* and *Photobacterium)* and the *Aeromonadaceae (Aeromonas* spp.) are also common aquatic bacteria and typical of the fish flora (Table 5.4). Gram-positive organisms as *Bacillus, Micrococcus, Clostridium, Lactobacillus* and coryneforms can also be found in varying proportions, but in general, Gram-negative bacteria dominate the microflora. Shewan (1977) concluded that Gram-positive *Bacillus* and Micrococcus dominate on fish from tropical waters. However, this conclusion has later been challenged by several studies which have found that the microflora on tropical fish species is very similar to the flora on temperate species (Acuff *et al.,*1984; Gram *et al.,* 1990; Lima dos Santos 1978; Surendran *et al.,* 1989). A microflora consisting of *Pseudomonas, Acinetobacter, Moraxella* and *Vibrio* has been found on newly-caught fish in several Indian studies (Surendran *et al.,* 1989). Several authors conclude, as Liston (1980), that the microflora on tropical fish often carry a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish.

Aeromonas spp. are typical of freshwater fish, whereas a number of bacteria require sodium for growth and are thus typical of marine waters. These include *Vibrio*, *Photobacterium* and *Shewanella*. However, although *Shewanella putrefaciens* is characterized as sodium-requiring, strains of *S. putrefaciens* can also be isolated from freshwater environments (DiChristina and DeLong, 1993; Gram et al., 1990; Spanggaard et al., 1993). Although *S. putrefaciens* has been isolated from tropical freshwaters, it is not important in the spoilage of freshwater fish (Lima dos Santos, 1978; Gram, 1990).

Table 5.4 Bacterial flora on fish caught in clean, unpolluted waters

Gram-negative	Gram-positive	Comments
Pseudomonas	*Bacillus*	
Moraxella	*Clostridium*	
Acinetobacter	*Micrococcus*	
Shewanella putrefaciens	*Lactobacillus*	
Flavobacterium	Coryneforms	
Cytophaga		
Vibrio *Photobacterium* *Aeromonas*		*Vibrio* and *Photobacterium* are typical of marine waters; *Aeromonas* is typical of freshwater

In polluted waters, high numbers of *Enterobacteriaceae* may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Fujioka *et al.,* 1988).

The taxonomy of S. *putrefaciens* has been rather confused. The organism was originally associated with the *Achromobacter* group but was later placed in the Shewan *Pseudomonas* group IV. Based on percentage of guanine+ cytosine (GC%) it was transferred to the genus *Alteromonas,* but on the basis of 5SRNA homology it was reclassified to a new genus, *Shewanella* (MacDonnell and Colwell, 1985). It has recently been suggested that the genus *Aeromonas* spp. which was a member of the *Vibrionaceae* family be transferred to its own family, the *Aeromonadaceae* (Colwell *et al.,* 1986).

Japanese studies have shown very high numbers of microorganisms in the gastrointestinal tract of fish, and as such numbers are much higher than in the surrounding water, this indicates the presence of a favourable ecological niche for the microorganisms. Similarly, Larsen *et al.* (1978) reported up to 10^7 cfu/g of vibrio-like organisms in the intestinal tract of cod and Westerdahl *et al.* (1991) also isolated high numbers of vibrio-like organisms from the intestines of turbot. *Photobacterium phosphoreum* which can be isolated from the surface can also be isolated in high numbers from the intestinal tract of some fish species (Dalgaard, 1993). On the contrary, some authors believe that the microflora of the gastrointestinal tract is merely a reflection of the environment and the food intake.

Microbial invasion

The flesh of healthy live or newly-caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh (Figure 5.8 a). When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibres. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during iced storage. Ruskol and Bendsen (1992) showed that bacteria can be detected by microscope in the flesh when the number of organisms on the skin surface increases above 10^6 cfu/cm^2 (Figure 5.6 b). This was seen at both iced and ambient temperatures. No difference was found in the invasive patterns of specific spoilage bacteria (e.g., S. *putrefaciens)* and non-spoilage bacteria.

Since only a limited number of organisms actually invade the flesh and microbial growth mainly takes place at the surface, spoilage is probably to a large extent a consequence of bacterial enzymes diffusing into the flesh and nutrients diffusing to the outside.

Fish spoil at very different rates (see also section 6.5), and differences in surface properties of fish have been proposed to explain this. Skins of fish have very different textures. Thus whiting *(Merlangius merlangus)* and cod *(Gadus morhua)* which have a very fragile integument spoil rapidly compared to several flatfish such as plaice that has a very robust dermis and epidermis. Furthermore, the latter group has a very thick slime layer, which includes several antibacterial components, such as antibodies, complement and bacteriolytic enzymes (Murray and Fletcher, 1976; Hjelmland *et al.,* 1983).

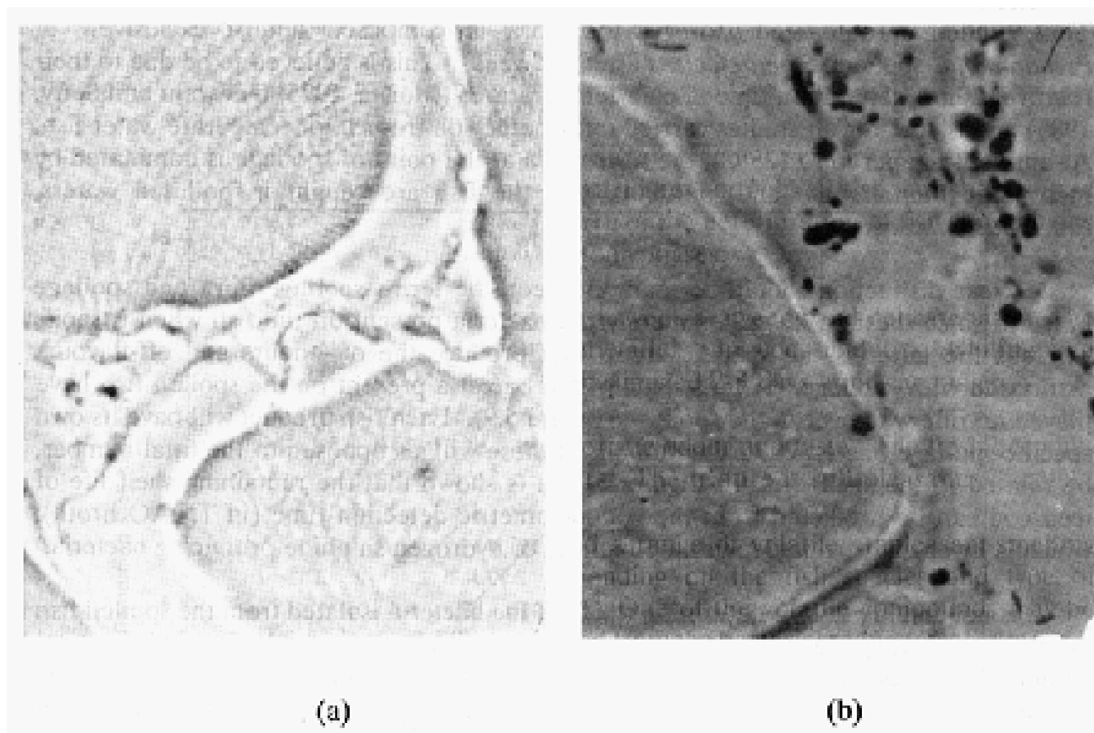


Figure 5.8 Histological section of (a) newly-caught cod and (b) cod fillets stored 12 days in ice. The section has been Giemsa-stained (Ruskol and Bendsen, 1992)

Changes in the microflora during storage and spoilage/Specific spoilage organisms

Bacteria on fish caught in temperate waters will enter the exponential growth phase almost immediately after the fish have died. This is also true when the fish are iced, probably because the microflora is already adapted to the chill temperatures. During ice storage, the bacteria will grow with a doubling time of approximately 1 day and will, after 2-3 weeks, reach numbers of 10^8-10^9 cfu/g flesh or cm^2 skin. During ambient storage, a slightly lower level of 10^7-10^8 cfu/g is reached in 24 hours. The bacteria on fish caught in tropical waters will often pass through a lag-phase of 1-2 weeks if the fish are stored in ice, whereafter exponential growth begins. At spoilage, the bacterial level on tropical fish is similar to the levels found on temperate fish species (Gram, 1990; Gram *et al.,* 1990).

If iced fish are stored under anaerobic conditions or if stored in CO_2 containing atmosphere, the number of the normal psychrotrophic bacteria such as S. *putrefaciens* and *Pseudomonas* is often much lower, i.e., 10^6-10^7 cfu/g than on the aerobically stored fish. However, the level of bacteria of psychrophilic character such as P. *phosphoreum* reaches a level of 10^7-10^8 cfu/g when the fish spoil (Dalgaard *et al.,* 1993).

The composition of the microflora also changes quite dramatically during storage. Thus, under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas* spp. and S. *putrefaciens* after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985) and is true for all studies carried out whether on tropical or temperate-water fish. At ambient temperature (25°C), the microflora at the point of spoilage is dominated by mesophilic

Vibrionaceae and, particularly if the fish are caught in polluted waters, *Enterobacteriaceae.*

A clear distinction should be made between the terms **spoilage flora** and **spoilage bacteria** since the first describes merely the bacteria present on the fish when it spoils whereas the latter is the specific group that produce the off-odours and off-flavours associated with spoilage. A large part of the bacteria present on the spoiled fish have played no role whatever in the spoilage (Figure 5.9). Each fish product will have its own specific spoilage bacteria and the number of these will, as opposed to the total number, be related to the shelf life. In Figure 5.10, it is shown that the remaining shelf life of iced cod can be predicted from the conductometric detection time (in TMAO broth), which is inversely correlated with the number of hydrogen sulphide-producing bacteria.

It is not an easy task to determine which of the bacteria isolated from the spoiled fish are those causing spoilage, and it requires extensive sensory, microbiological and chemical studies. First, the sensory, microbiological and chemical changes during storage must be studied and quantified, including a determination of the level of a given chemical compound that correlates with spoilage (the chemical spoilage indicator). Second, bacteria are isolated at the point of sensory rejection. Pure and mixed cultures of bacteria are screened in sterile fish substrates for their **spoilage potential**, i.e., their ability to produce sensory (off-odours) and chemical changes typical of the spoiling product. Finally, the selected strains are tested to evaluate their **spoilage activity**, i.e., if their growth rate and their qualitative and quantitative production of off-odours are similar to the measurements in the spoiled product (Dalgaard, 1993).

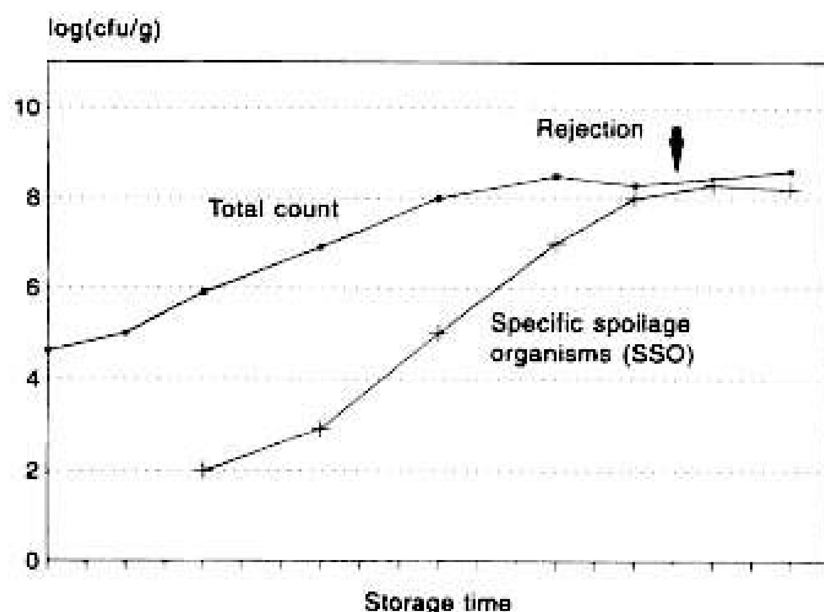


Figure 5.9 Changes in total counts and specific spoilage bacteria during storage (modified after Dalgaard (1993)

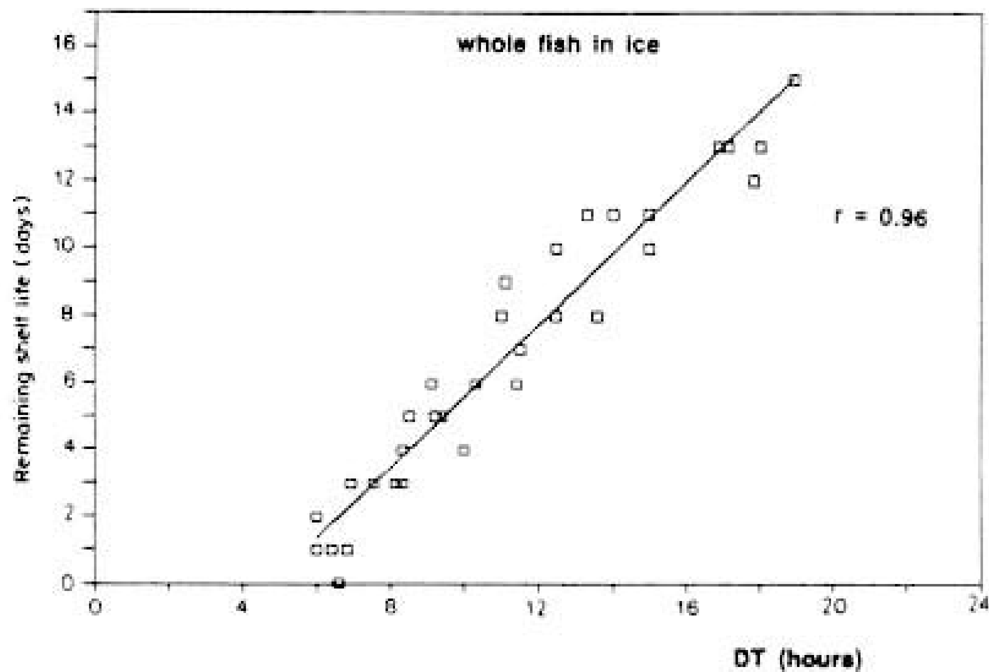


Figure 5.10 Comparison of remaining shelf life of iced cod and detection time in a TMAO broth (Jorgensen et al., 1988)

The latter step is particularly important, as some bacteria may produce the chemical compounds associated with spoilage but are unable to do so in significant amounts, and they are thus not the specific spoilage bacteria. When stored aerobically, levels of 10^8-10^9 cfu/g of specific spoilage bacteria are required to cause spoilage. The spoilage of packed fish is seen at a much lower level of 10^7 cfu P. *phosphoreum* per gramme. This relatively low level is probably due to the very large size (5 µm) of the bacterium resulting in a much higher yield of for example, TMA per cell (Dalgaard, 1993).

Spoilage potential and activity can be assessed in several fish substrates as sterile, raw fish juice (Lerke *et al.,* 1963), heat-sterilized fish juice (Castell and Greenough, 1957; Gram *et al. , 1987*; Dalgaard, 1993) or on sterile muscle blocks (Herbert *et al.,* 1971). The latter is the most complicated but is also that yielding results comparable to the product. If any of the fish juices are chosen, it is important that the growth rate of the spoilage bacteria in the model system is equal to the growth rate in the product.

A qualitative test for the ability of the bacteria to produce H_2S and/or reduce TMAO may also be used when the spoilage flora is screened for potential spoilage bacteria. A medium where the reduction of TMAO to TMA is seen as a redox indicator changes colour, and the formation of H_2S is evident from a black precipitation of FeS which has been developed for this purpose (Gram *et al.,*1987).

Shewanella putrefaciens has been identified as the specific spoilage bacteria of marine temperate- water fish stored aerobically in ice. If the product is vacuum-packed, P. phosphoreum participates in the spoilage and it becomes the specific spoilage bacteria of CO_2 packed fish (see section 6.3). The spoilage flora on iced tropical fish from marine

waters is composed almost exclusively of *Pseudomonas* spp. and *S. putrefaciens*. Some *Pseudomonas* spp. are the specific spoilers of iced stored tropical freshwater fish (Lima dos Santos, 1978; Gram et al., 1990) and are also, together with *S. putrefaciens*, spoilers of marine tropical fish stored in ice (Gillespie and MacRae, 1975; Gram, 1990).

At ambient temperature, motile aeromonads are the specific spoilers of aerobically stored freshwater fish (Gorzyka and Pek Poh Len, 1985; Gram *et al.,* 1990). Barile *et al.* (1985) showed that a large proportion of the flora on ambient-stored mackerel consisted of *S. putrefaciens,* indicating that this bacterium may also take part in the spoilage.

Table 5.5 gives an overview of the specific spoilage bacteria of fresh fish products stored in ice and at ambient temperature.

Table 5.5 Dominating microflora and specific spoilage bacteria at spoilage of fresh, white fish (cod) (from Huss, 1994)

Storage temperature	Packaging atmosphere	Dominating microflora	Specific spoilage organisms (SSO)	References
0°C	Aerobic	Gram-negative psychrotrophic, non-fermentative rods *(Pseudomonas spp., S. putrefaciens, Moraxella, Acinetobacter)*	S. *putrefaciens* *Pseudomonas* [3]	2,3,4,9
0°C	Vacuum	Gram-negative rods; psychrotrophic or with psychrophilic character (S. *putrefaciens, Photobacterium)*	S. *putrefaciens P. phosphoreum*	1,9
0°C	MAP[1]	Gram-negative fermentative rods with psychrophilic character *(Photobacterium)* Gram-negative non-fermentative psychrotrophic rods (1-10% of flora; *Pseudomonas, S. putrefaciens)* Gram-positive rods (LAB 2)	*P. phosphoreum*	1,7
5°C	Aerobic	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10

5°C	Vacuum	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10
5°C	MAP	Gram-negative psychrotrophic rods *(Vibrionaceae)*	*Aeromonas spp.*	6
20-30°C	Aerobic	Gram-negative mesophilic fermentative rods *(Vibrionaceae, Enterobacteriaceae)*	Motile *Aeromonas* spp. *(A. hydrophila)*	2,4,5,8

1) Modified Atmosphere Packaging (CO_2 containing)
2) LAB: Lactic Acid Bacteria
3) Fish caught in tropical waters or freshwaters tend to have a spoilage dominated by *Pseudomonas spp.*

References: 1) Battle *et al.* (1985); 2) Dalgaard *et al.* (1993); 3) Donald and Gibson (1992); 4) Gorczyca and Pek Poh Len (1985); 5) Gram *et al.* (1987); 6) Gram *et al.* (1990); 7) Gram and Dalgaard (pers. comm.); 8) Jorgensen and Huss (1989); 9) Lima dos Santos (1978); 10) van Spreekens (1977)

Biochemical changes induced by bacterial growth during storage and spoilage

Comparison of the chemical compounds developing in naturally spoiling fish and sterile fish has shown that most of the volatile compounds are produced by bacteria (Shewan, 1962) as shown in Figure 5.11. These include trimethylamine, volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecularweight compounds.

The substrates for the production of volatiles are the carbohydrates (e.g., lactate and ribose), nucleotides (e.g., inosine mono-phosphate and inosine) and other NPN molecules. The amino-acids are particularly important substrates for formation of sulphides and ammonia.

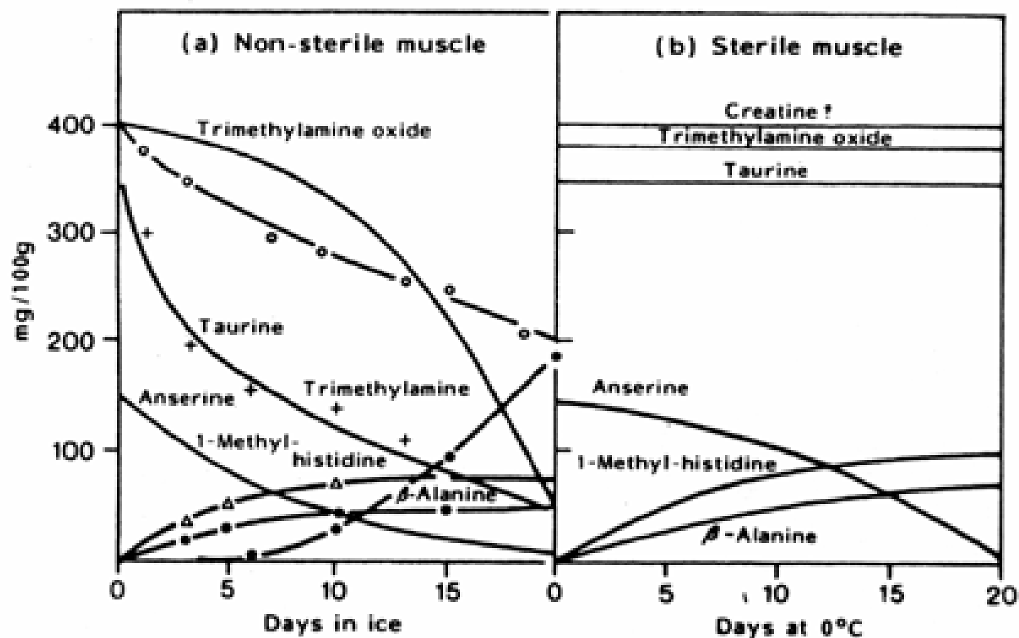


Figure 5.11 Changes in the nitrogenous extractives in a) spoiling and b) autolysing cod muscle (Shewan, 1962)

Microorganisms obtain far more energy from aerobic oxidation than from an anaerobic fermentation; thus the complete oxidation of 1 mole glucose (or other hexose) via Kreb's cycles yields 6 moles of CO_2 and 36 moles of ATP. On the contrary, the fermentation of 1 mole glucose gives only 2 moles of ATP and two moles of lactic acid. The initial aerobic growth on fish is dominated by bacteria using carbohydrates as substrate and oxygen as terminal electron-acceptor with the concurrent production of CO_2 and H_2O.

Reduction of Trimethylarnine Oxide (TMAO)

The growth of oxygen-consuming bacteria results in the formation of anaerobic or microaerophilic niches on the fish. This does, however, not necessarily favour the growth of anaerobic bacteria. Some of the bacteria present on fish are able to carry out a respiration (with the ATP advantage) by using other molecules as electron acceptor. It is typical of many of the specific spoilage bacteria on fish that they can use TMAO as electron acceptorin an anaerobic respiration. The reduced component, TMA, which is one of the dominant components of spoiling fish, has a typical fishy odour. The level of TMA found in fresh fish rejected by sensory panels varies between fish species, but is typically around 10-15 mg TMA-N/100 g in aerobically stored fish and at a level of 30 mg TMA-N/100 g in packed cod (Dalgaard *et al.,* 1993).

The TMAO reduction is mainly associated with the genera of bacteria typical of the marine environment *(Alteromonas, Photobactetium, Vibrio* and S. *putrefaciens),* but is also carried out by *Aeromonas* and intestinal bacteria of the *Enterobacteriaceae.* TMAO reduction has been studied in fermentative, facultative anaerobic bacteria like *E. coli* (Sakaguchi *et al.,* 1980) and *Proteus* spp. (Stenberg *et al.,* 1982) as well as in the non-

fermentative S. *putrefaciens* (Easter *et al,* 1983; Ringo *et al,* 1984). During aerobic growth, S. *putrefaciens* uses the Kreb's cycle to produce the electrons that are later channelled through the respiratory chain. Ringo *et al.* (1984) suggested that during anaerobic respiration S. *putrefaciens* also uses the complete Kreb's cycle (Figure 5.12), whereas it has recently been shown that in the anaerobic respiration in S. *putrefaciens,* only part of the Kreb's cycle is used (Figure 5.13) and electrons are also generated by another metabolic pathway, namely the serine pathway (Scott and Nealson, 1994). S. *putrefaciens* can use a variety of carbon sources as substrate in its TMAO-dependent anaerobic respiration, including formate and lactate. Compounds like acetate and succinate that are used in the oxygen respiration cannot be used when TMAO is terminal electron acceptor (DiChristina and DeLong, 1994) and on the contrary, acetate is a product of the anaerobic TMAO reduction (Ringo *et al.,* 1984; Scott and Nealson, 1994).

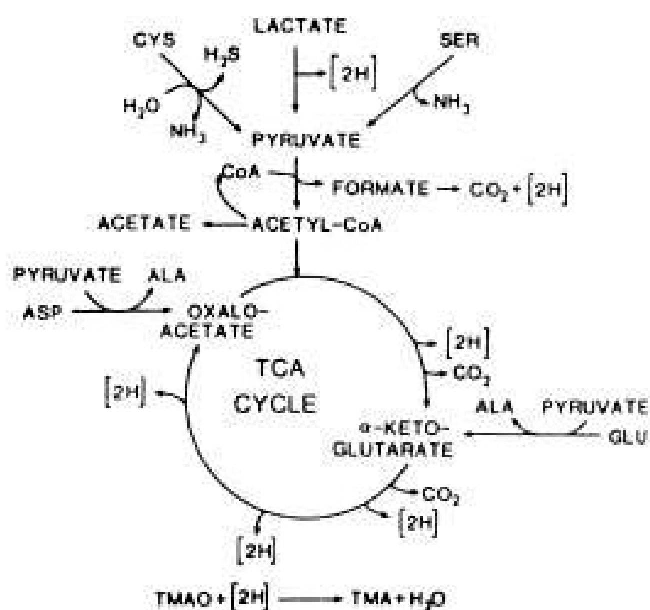


Figure 5.12 Anaerobic reduction of TMAO by *Shewanella putrefaciens* (formerly *Alteromonas)* as suggested by Ringo *et al.* (1984)

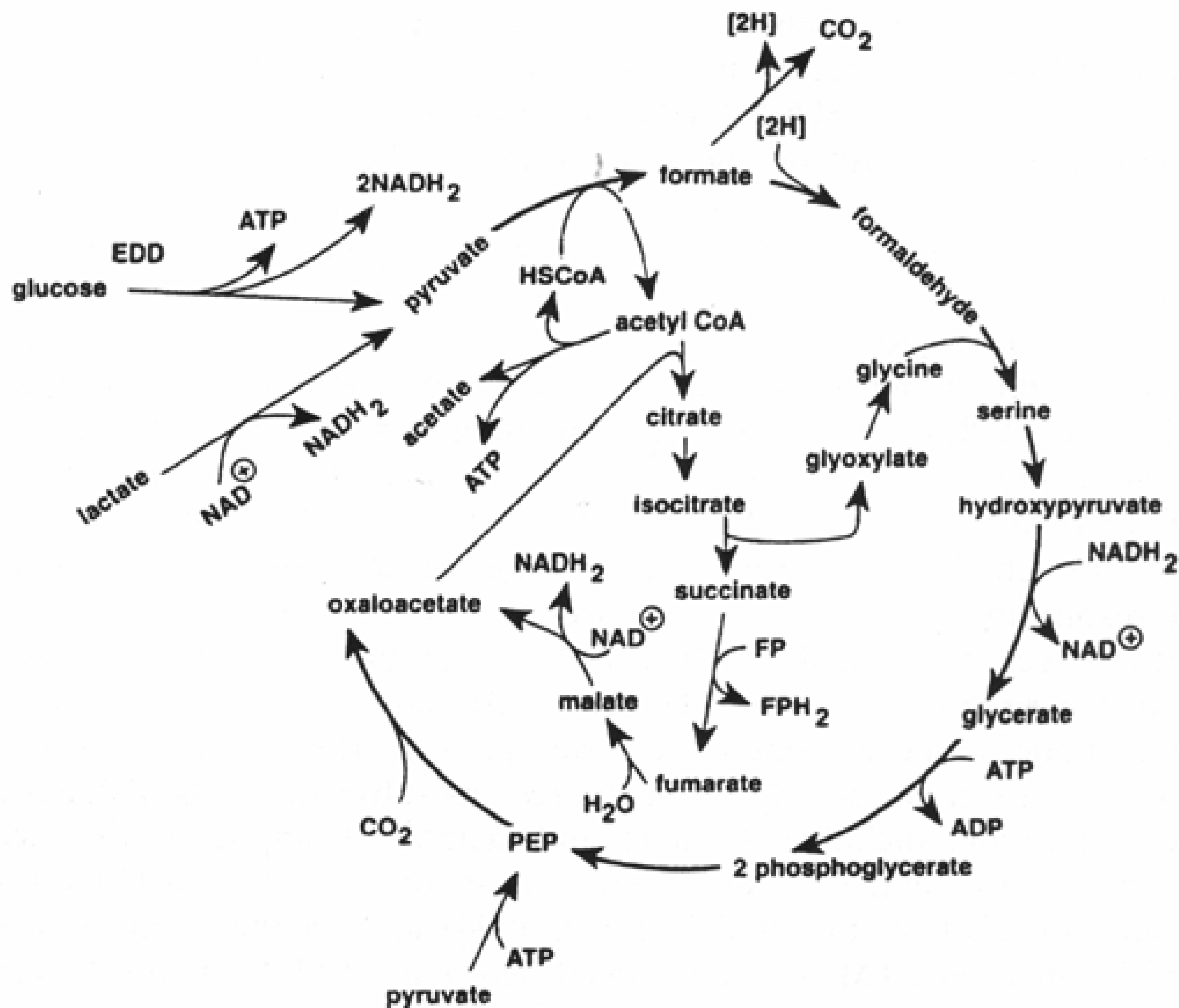


Figure 5.13 Proposed route of carbon during anaerobiosis for S. *putrefaciens* (Scott and Nealson, 1994)

Contrary to this, sugars and lactate are the main substrates generating electrons when *Proteus* spp. reduces TMAO. The reduction is accompanied by a production of acetate as the main product (Kjosbakken and Larsen, 1974).

TMAO is, as mentioned in section 4.4, a typical component of marine fish, and it has recently been reported that also some tropical freshwater fish contain high amounts of TMAO (Anthoni *et al.,* 1990). However, TMA is not necessarily a characteristic component during spoilage of such fish because spoilage is due to *Pseudomonas spp.* (Gram *et al.,* 1990).

The development of TMA is in many fish species paralleled by a production of hypoxanthine. Hypoxanthine can, as described in section 5.2. be formed by the autolytic decomposition of nucleotides, but it can also be formed by bacteria; and the rate of bacterial formation is higher than the autolytic. Both Jorgensen *et al.* (1988) and

Dalgaard (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod (Figure 5.14). Several of the spoilage bacteria produce hypoxanthine from inosine or inosine mono-phosphate, including *Pseudomonas* spp. (Surette *et al.,* 1988) S. *putrefaciens* (van Spreekens, 1977; Jorgensen and Huss, 1989; Gram, 1989) and P. *phosphoreum* (van Spreekens, 1977).

In cod and other gadoid fishes, TMA constitutes most of the so-called total volatile bases, TVB (also called total volatile nitrogen, TVN) until spoilage. However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB levels still rise due to formation of NH3 and other volatile amines. A little ammonia is also formed in the first weeks of iced storage due to autolysis. In some fish that do not contain TMAO or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVB is seen during storage, probably resulting from the deamination of amino-acids.

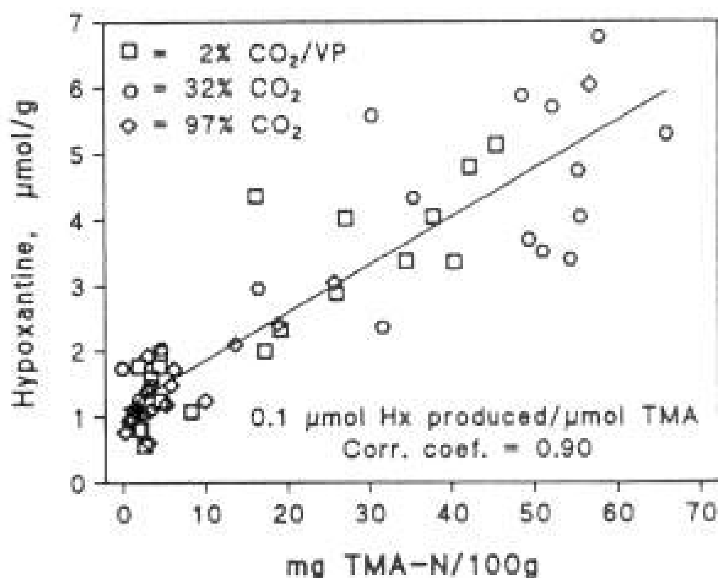


Figure 5.14 Relationship between contents of TMA and Hx during storage of packed cod in ice (Dalgaard *et al.,* 1993)

Volatile sulphur-compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. S. *putrefaciens* and some *Vibrionaceae* produce H_2S from the sulphur containing amino-acid 1-cysteine (Stenstroem and Molin, 1990; Gram *et al.,* 1987). On the contrary, neither *Pseudomonas* nor P. *phosphoreum* produce significant amounts of H_2S. Thus, hydrogen sulphide, which is typical of spoiling iced cod stored aerobically, is not produced in spoiling CO_2 packed fish (Dalgaard *et al.,* 1993). Methylmercaptan (CH_3SH) and dimethylsulphide ($(CH_3)_2S$) are both formed from the other sulphur-containing amino-acid, methionine. Taurine, which is also sulphur-containing, occurs as free amino-acid in very high concentrations in fish muscle. It disappears from the fish flesh during storage (Figure 5.11) but this is because of leakage rather than because of bacterial attack (Herbert and Shewan, 1975). The formation of compounds in naturally-spoiling cod as compared to sterile muscle is shown in Figure 5.15.

The volatile sulphur-compounds are very foul-smelling and can be detected even at ppb levels, so even minimal quantities have a considerable effect on quality. Ringo *et al. (1984)* have shown that cysteine is used as substrate in the Kreb's cycle when electrons are transferred to TMAO, and the formation of H_2S and TMA is thus to some extent a linked reaction (Figure 5.12).

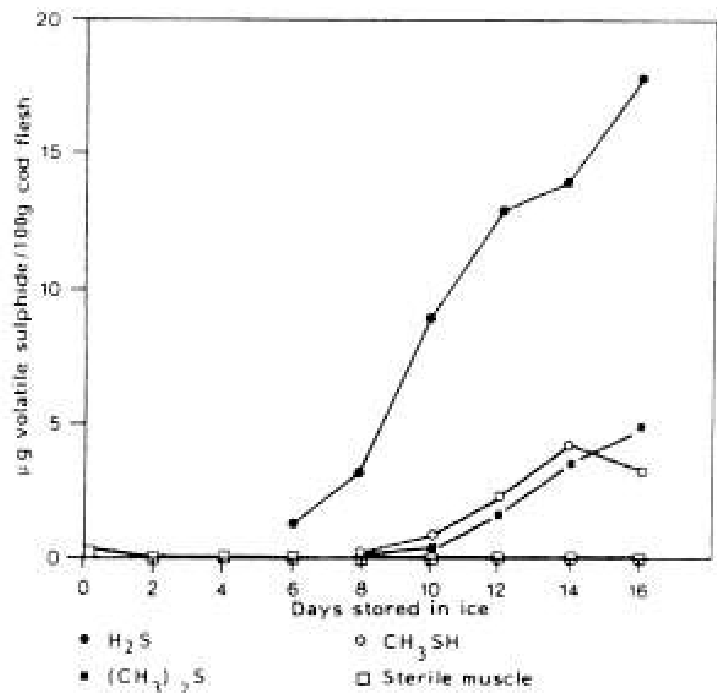


Figure 5.15 Production of HA CH_3SH and $(CH_3)_2S$ in naturally spoiling cod fillets and sterile muscle blocks (Herbert and Shewan, 1976)

Contrary to the iced spoilage by S. *putrefaciens* and the ambient spoilage by *Vibrionaceae* which is dominated by H_2S and TMA, the spoilage caused by *Pseudomonas* spp. is characterized by absence of these compounds (Gram *et al.,* 1989, Gram *et al.,* 1990). Fruity, rotten, sulphydryl odours and flavours are typical of the Pseudomonas spoilage of iced fish. *Pseudomonas* spp. produce a number of volatile aldehydes, ketones, esters and sulphides (Edwards *et al.,* 1987; Miller *et al.,* 1973 a, 1973 b). However, it is not known which specific compounds are responsible for the typical off-odours (Table 5.6). The fruity off-odours produced by *Pseudomonas fragi* originate from monoaminomonocarboxylic amino-acids.

Table 5.6 Typical spoilage compounds during spoilage of fresh fish stored aerobically or packed in ice or at ambient temperature

Specific spoilage organism	Typical spoilage compounds
Shewanella putrefaciens	*TMA, H_2S, CH_3SH, $(CH_3)_2S$, Hx*
Photobacterium phosphoreum	TMA, Hx
Pseudomonas spp.	ketones, aldehydes, esters, non-H_2S sulphides

Vibrionaceae	TMA, H_2S
anaerobic spoilers	NH_3, acetic, butyric and propionic acid

As mentioned above, TVB will continue to rise even after TMA has reached its maximum. This latter rise is due to proteolysis commencing when several of the free amino-acids have been used. Lerke *et al.* (1967) separated fish juice into a protein and a non-protein fraction and inoculated spoilage bacteria in each fraction and in the whole juice. The non-protein fraction of a fish juice spoiled as the whole juice whereas only faint off-odours were detected in the protein fraction of the juice. Although some authors have used the number of proteolytic bacteria as indicators of spoilage, it must be concluded that the turnover of the protein fraction is not of major importance in spoilage of fresh fish.

Some of the compounds typically formed by bacteria during spoilage of fish are shown in Table 5.7 together with the substrate used for the formation.

Table 5.7 Substrate and off-odour/off-flavour compounds produced by bacteria during spoilage of fish

Substrate	**Compounds produced by bacterial action**
TMAO	TMA
cysteine	H_2S
methionine	CH_3SH, $(CH_3)_2S$
carbohydrates and lactate	acetate, CO_2, H_2O
inosine, IMP	hypoxanthine
amino-acid s (glycine, serine, leucine)	esters, ketones, aldehydes
amino-acids, urea	NH_3

The formation of TMA is accompanied by a formation of ammonia during anaerobic storage of herring and mackerel (Haaland and Njaa, 1988). Prolonged anaerobic storage of fish results in vigorous production of NH_3 owing to further degradation of the amino-acids, and in the accumulation of lower fatty acids as acetic, butyric and propionic acid. The very strong NH_3-producers were found to be obligate anaerobes belonging to the family Bacteroidaceae genus *Fusobacterium* (Kjosbakken and Larsen, 1974; Storroe *et al.,* 1975, 1977). These organisms grow only in the spoiled fish extract and have little or no proteolytic activity relying on already hydrolysed proteins.

During iced storage of fresh fatty fish, changes in the lipid fraction is caused almost exclusively by chemical action, e.g., oxidation, whereas bacterial attack on the lipid fraction contributes little to the spoilage profile. During storage of lightly preserved fish, lipid hydrolysis caused by bacteria may be part of the spoilage profile.

5.4 Lipid oxidation and hydrolysis

The two distinct reactions in fish lipids of importance for quality deterioration are:

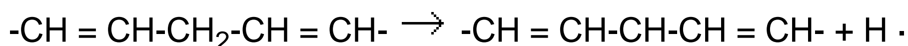
- oxidation
- hydrolysis

They result in production of a range of substances among which some have unpleasant (rancid) taste and smell. Some may also contribute to texture changes by binding covalently to fish muscle proteins. The various reactions are either *nonenzymatic* or catalyzed by *microbial* enzymes or by *intracellular* or *digestive* enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature.

Fatty fish are, of course, particularly susceptible to lipid degradation which can create severe quality problems even on storage at subzero temperatures.

Oxidation

The large amount of polyunsaturated fatty acid moieties found in fish lipids (see section 4.2) makes them highly susceptible to oxidation by an autocatalytic mechanism (Figure 5.16). The process is initiated as described below by abstraction of a hydrogen atom from the central carbon of the *pentadiene structure* found in most fatty acid acyl chains containing more than one double bond:



$$-CH = CH\text{-}CH_2\text{-}CH = CH- \rightarrow -CH = CH\text{-}CH\text{-}CH = CH- + H\cdot$$

Contrary to the native molecule, the lipid radical (L) reacts very quickly with atmospheric oxygen making a peroxy-radical (LOO) which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical L. This propagation continues until one of the radicals is removed by reaction with another radical or with an *antioxidant* (AH) whose resulting radical (A) is much less reactive. The hydroperoxides produced in relatively large amounts during propagation are tasteless, and it is therefore perhaps not surprising that the widely used "peroxide value" (section 8.2) usually correlates rather poorly to sensorial properties.

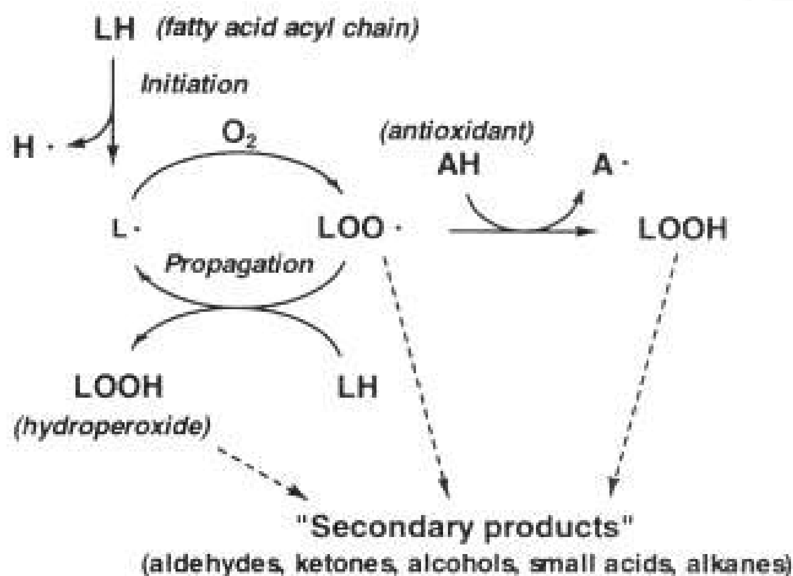


Figure 5.16 Autoxidation of polyunsaturated lipid

The hydroperoxides are readily broken down, catalyzed by heavy metal ions, to secondary autoxidation products of shorter carbon chain-length. These secondary products - mostly aldehydes, ketones, alcohols, small carboxylic acids and alkanes - give rise to a very broad odour spectrum and in some cases to a yellowish discoloration. Several of the aldehydes can be determined as "thiobarbituric acid-reactive substances" (section 8.2).

Metal ions are very important in the first step of lipid autoxidation - the initiation process - in catalyzing the formation of reactive oxygen species as for example the hydroxyl radical (OH). This radical immediately reacts with lipids or other molecules at the site where it is generated. The high reactivity may explain that free fatty acids have been found to be more susceptible to oxidation than the corresponding bound ones, because the amount of iron in the aqueous phase is probably greater than the amount bound to the surface of cellular membranes and lipid droplets.

Fatty acid hydroperoxides may also be formed enzymatically, catalyzed by *lipoxygenase* which is present in variable amounts in different fish tissues. A relatively high activity has been found in the gills and under the skin of many species. The enzyme is unstable and is probably important for lipid oxidation only in fresh fish. Cooking or freezing/thawing rather effectively destroys the enzyme activity.

The living cells possess several protection mechanisms directed against lipid oxidation products. An enzyme, glutathione peroxidase, exists which reduces hydroperoxides in the cellular membranes to the corresponding hydroxy-compounds. This reaction demands supply of reduced glutathione and will therefore cease post mortem when the cell is depleted of that substance. The membranes also contain the phenolic compound a-tocopherol (Vitamin E) which is considered the most important natural antioxidant. Tocopherol can donate a hydrogen atom to the radicals L- or LOO- functioning as the molecule AH in Figure 5.16. It is generally assumed, that the resulting tocopheryl radical

reacts with ascorbic acid (Vitamin C) at the lipid/water interface regenerating the tocopherol molecule. Other compounds, for example the carotenoids, may also function as antioxidants. Wood smoke contains phenols which may penetrate the fish surface during smoking and thereby provide some protection against lipid oxidation.

Hydrolysis

During storage, a considerable amount of free fatty acids (FFA) appears (Figure 5.17). The phenomenon is more profound in ungutted than in gutted fish probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase (TL in Figure 5.18) originating from the digestive tract or excreted by certain microorganisms. Cellular lipases may also play a minor role.

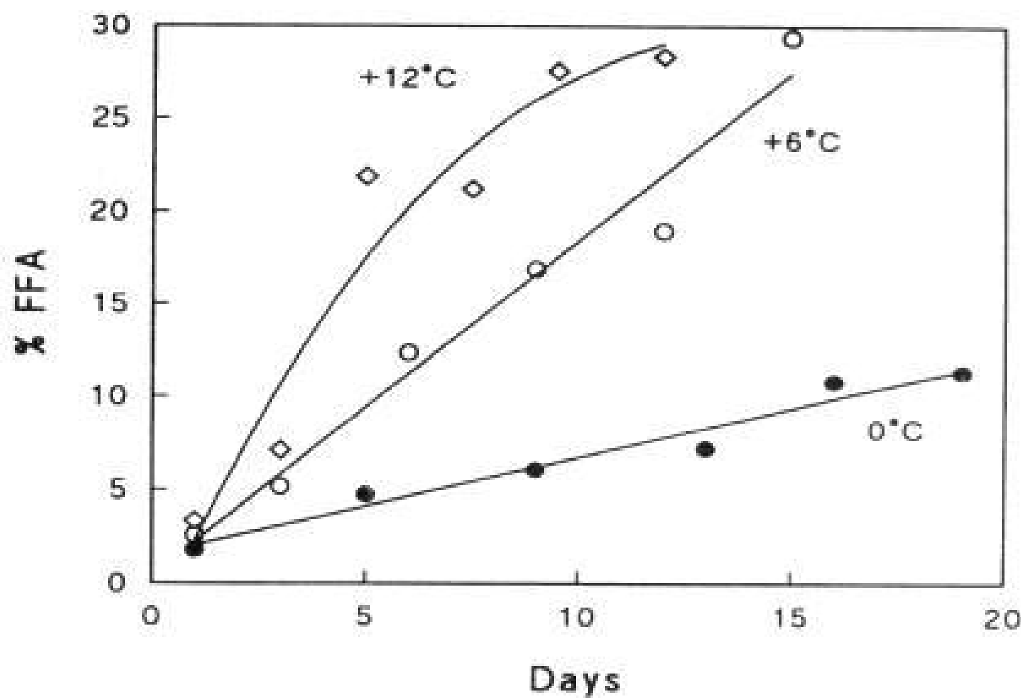


Figure 5.17 The development of free fatty acids in herring stored at different temperatures (Technological Laboratory, Danish Ministry of Fisheries, Annual Report, 1971)

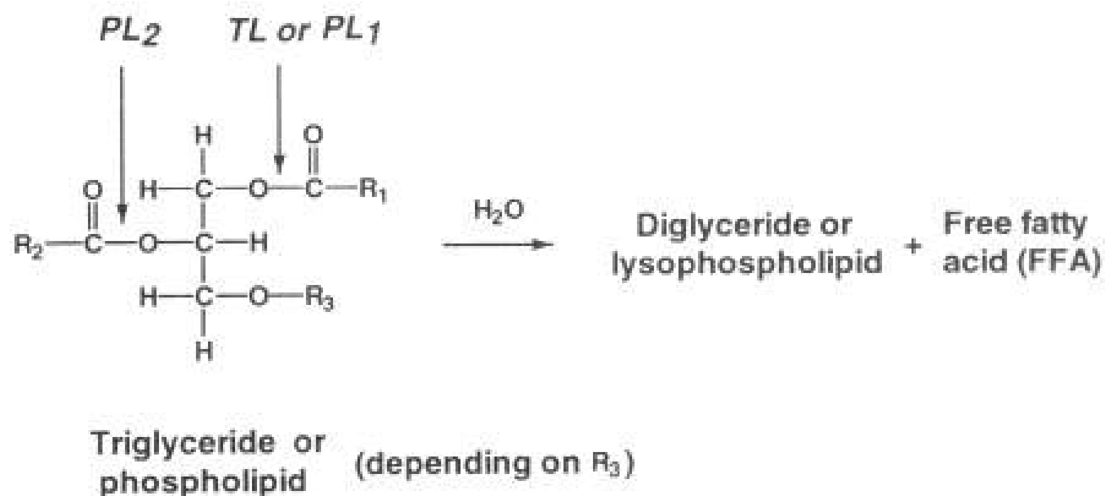
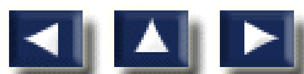


Figure 5.18 Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: PL_1 & PL_2 phospholipases;TL, triglyceride lipase

In lean fish, for example Atlantic cod, production of free fatty acids also occurs, even at low temperatures. The enzymes responsible are believed to be cellular phospholipases - in particular phospholipase A_2 (PL2 in Figure 5.18) - although a correlation between activity of these enzymes and the rate of appearance of FFA has as yet not been firmly established. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely of the polyunsaturated type, and hydrolysis therefore often leads to increased oxidation as well. Furthermore, the fatty acids themselves may cause a "soapy" off-flavour.



5. POSTMORTEM CHANGES IN FISH

5.1. Sensory changes
5.2. Autolytic changes
5.3. Bacteriological changes
5.4. Lipid oxidation and hydrolysis

5.1 Sensory changes

Sensory changes are those perceived with the senses, i.e., appearance, odour, texture and taste.

Changes in raw fresh fish

The first sensory changes of fish during storage are concerned with appearance and texture. The characteristic taste of the species is normally developed the first couple of days during storage in ice.

The most dramatic change is onset of rigor mortis. Immediately after death the muscle is totally relaxed and the limp elastic texture usually persists for some hours, whereafter the muscle will contract. When it becomes hard and stiff the whole body becomes inflexible and the fish is in rigor mortis This condition usually lasts for a day or more and then rigor resolves. The resolution of rigor mortis makes the muscle relax again and it becomes limp, but no longer as elastic as before rigor. The rate in onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish (Table 5.1).

The effect of temperature on rigor is not uniform. In the case of cod, high temperatures give a fast onset and a very strong rigor mortis. This should be avoided as strong rigor tensions may cause gaping, i.e., weakening of the connective tissue and rupture of the fillet.

It has generally been accepted that the onset and duration of rigor mortis are more rapid at high temperatures, but observations, especially on tropical fish show the opposite effect of temperature with regard to the onset of rigor. It is evident that in these species

the onset of rigor is accelerated at 0°C compared to 10°C, which is in good correlation with a stimulation of biochemical changes at 0°C (Poulter *et al.,* 1982; Iwamoto *et al.,* 1987). However, an explanation for this has been suggested by Abe and Okuma (1991) who have shown that onset of rigor mortis in carp (Cyprinus *carpio)* depends on the difference in sea temperature and storage temperature. When the difference is large the time from death to onset of rigor is short and *vice versa.*

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 hours (Azam *et al.,* 1990; Proctor *et al.,* 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor the fish body will be completely stiff; the filleting yield will be very poor, and rough handling can cause gaping. If the fillets are removed from the bone pre-rigor the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink up to 52 % and white muscle up to 15 % of the original length (Buttkus, 1963). If the fish is cooked pre-rigor the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Post-rigor the flesh will become firm, succulent and elastic.

Table 5.1 Onset and duration of rigor mortis in various fish species

Species	**Condition**	**Temperature °C**	**Time from death to onset of *rigor* (hours)**	**Time from death to end of *rigor* (hours)**
Cod *(Gadus morhua)*	Stressed	0	2-8	20-65
	Stressed	10-12	1	20-30
	Stressed	30	0.5	1-2
	Unstressed	0	14-15	72-96
Grouper *(Epinephelus malabaricus)*	Unstressed	2	2	18
Blue Tilapia *(Areochromis aureus)*	Stressed	0	1	
	Unstressed	0	6	
Tilapia *(Tilapia mossanibica)* small 60g	Unstressed	0-2	2-9	26.5

Grenadier *(Macrourus whitson)*	Stressed	0	<1	35-55
Anchovy *(Engraulis anchoita)*	Stressed	0	20-30	18
Plaice *(Pleuronectes platessa)*	Stressed	0	7-11	54-55
Coalfish *(Pollachius virens)*	Stressed	0	18	110
Redfish *(Sebastes* spp.)	Stressed	0	22	120
Japanese flounder *(Paralichthys olivaceus)*		0	3	>72
		5	12	>72
		10	6	72
		15	6	48
		20	6	24
Carp *(Cyprinus carpio)*		0	8	
		10	60	
		20	16	
	Stressed	0	1	
	Unstressed	0	6	

SOURCES: Hwang *et al.,* 1991; Iwamoto *et al., 1987;* Korhonen *et al.,* 1990;Nakayama *et al.,* 1992; Nazir and Magar, 1963; Partmann, 1965; Pawar and Magar, 1965; Stroud, 196; Trucco *et al.,* 1982

Whole fish and fillets frozen pre-rigor can give good products if they are carefully thawed at a low temperature in order to give *rigor mortis* time to pass while the muscle is still frozen.

The sensory evaluation of raw fish in markets and landing sites is done by assessing the appearance, texture and odour. The sensory attributes for fish are listed in Table 5.2. Most scoring systems are based upon changes taking place during storage in melting

ice. It should be remembered that the characteristic changes vary depending on the storage method. The appearance of fish stored under chilled condition without ice does not change as much as for iced fish, but the fish spoil more rapidly and an evaluation of cooked flavour will be necessary. A knowledge of the time /temperature history of the fish should therefore be essential at landing.

The characteristic sensory changes in fish post mortem vary considerably depending on fish species and storage method. A general description has been provided by the EEC in the guidelines for quality assessment of fish as shown in Table 5.2. The suggested scale is numbered from 0 to 3, where 3 is the best quality.

The West European Fish Technologists' Association has compiled a multilingual glossary of odours and flavours which also can be very useful when looking for descriptive words for sensory evaluation of freshness of fish (Howgate et al., 1992 (Appendix C).

Changes in eating quality

If quality criteria of chilled fish during storing are needed, sensory assessment of the cooked fish can be conducted. Some of the attributes for cooked fish and shellfish are mentioned in Table 5.2. A characteristic pattern of the deterioration of fish stored in ice can be found and divided into the following four phases:

- **Phase 1** The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic. In cod, haddock, whiting and flounder, the sweet taste is maximized 2-3 days after catching.
- **Phase 2** There is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours. The texture is still pleasant.
- **Phase 3** There is sign of spoilage and a range of volatile, unpleasant-smelling substances is produced depending on the fish species and type of spoilage (aerobic, anaerobic). One of the volatile compounds may be trimethylamine (TMA) derived from the bacterial reduction of trimethyl-aminoxide (TMAO). TMA has a very characteristic "fishy" smell. At the beginning of the phase the off-flavour may be slightly sour, fruity and slightly bitter, especially in fatty fish. During the later stages sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smells develop. The texture becomes either soft and watery or tough and dry.
- **Phase 4** The fish can be characterized as spoiled and putrid.

Table 5.2 Freshness ratings: Council Regulation (EEC) No. 103/76 OJ No. L20 (28 January 1976) (EEC, 1976)

Criteria	
	Marks

Part of fish inspected	3	2	1	0
Appearance				
Skin	Bright, iridescent pigmentation, no discoloration Aqueous, transparent, mucus	Pigmentation bright but not lustrous Slightly cloudy mucus	Pigmentation in the process of becoming discoloured and dull Milky mucus	[1]Dull pigmentation Opaque mucus
Eye	Convex (bulging) Transparent cornea Black, bright pupil	Convex and slightly sunken Slightly opalescent cornea Black, dull pupil	Flat Opalescent cornea Opaque pupil	[1]Concave in the centre Milky cornea Grey pupil
Gills	Bright colour No mucus	Less coloured Slight traces of clear mucus	Becoming discoloured Opaque mucus	[1]Yellowish Milky mucus
Flesh (cut from abdomen)	Bluish, translucent, smooth, shining No change in original colour	Velvety, waxy, dull Colour slightly changed	Slightly opaque	[1]Opaque
Colour (along vertebral column)	Uncoloured	Slightly pink	Pink	[1]Red
Organs	Kidneys and residues of other organs should be bright red, as should the blood inside the aorta	Kidneys and residues of other organs should be dull red; blood becoming discoloured	Kidneys and residues of other organs and blood should be pale red	Kidneys and residues of other organs and should be brownish in colour
Condition				

Flesh	Firm and elastic Smooth surface	Less elastic	Slightly soft (flaccid), less elastic Waxy (velvety) and dull surface	[1]Soft (flaccid) Scales easily detached from skin, surface rather wrinkled, inclining to mealy
Vertebral column	Breaks instead of coming away	Sticks	Sticks slightly	[1]Does not stick
Peritoneum	Sticks completely to flesh	Sticks	Sticks slightly	[1]Does not stick
		Smell		
Gills, skin abdominal cavity	Seaweed	No smell of seaweed or any bad smell	Slightly sour	[1]Sour

[1] Or in a more advanced state of decay.

A numbered scale may be used for the sensory evaluation of cooked fish as shown in Figure 5.1. The scale is numbered from 0 to 10, 10 indicating absolute freshness, 8 good quality and 6 a neutral tasteless fish. The rejection level is 4. Using the scale in this way the graph becomes S-shaped indicating a fast degradation of the fish during the first phase, a slower rate in phase 2 and 3 and finally a high rate when the fish is spoiled.

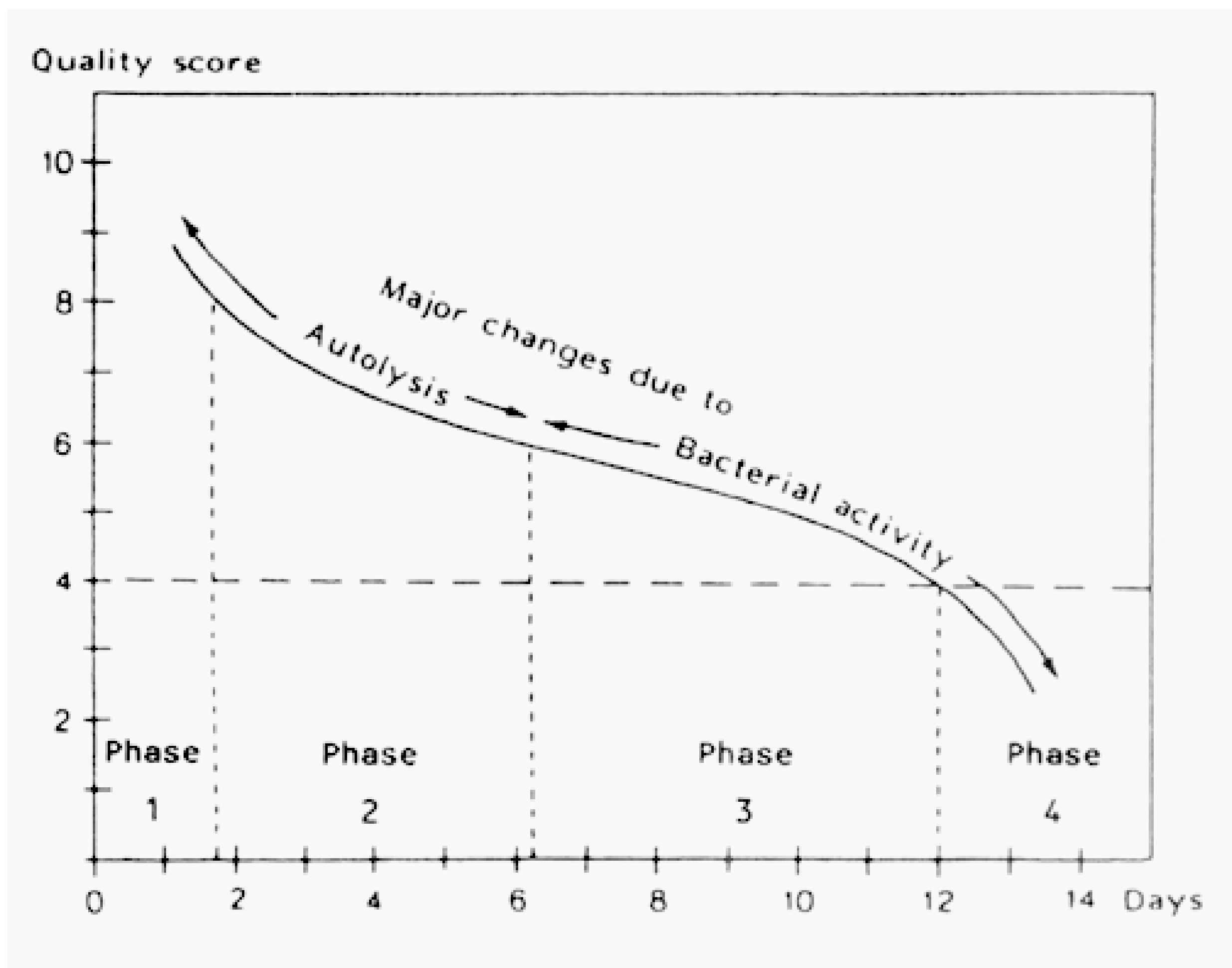


Figure 5.1 Changes in the eating quality of iced (0°C) cod (Huss, 1976)

Other scales can well be used and can change the shape of the graph. It is, however, important to understand the kind of results desired from the sensory analysis in order to ask the right questions to the sensory assessors.

5.2 Autolytic Changes

Autolysis means "self-digestion". It has been known for many years that there are at least two types of fish spoilage: bacterial and enzymatic. Uchyama and Ehira (1974) showed that for cod and yellowtail tuna, enzymatic changes related to fish freshness preceded and were unrelated to changes in the microbiological quality. In some species (squid, herring), the enzymatic changes precede and therefore predominate the spoilage of chilled fish. In others, autolysis contributes to varying degrees to the overall quality loss in addition to microbially-mediated processes.

Production of energy in post mortem muscle

At the point of death, the supply of oxygen to the muscle tissue is interrupted because the blood is no longer pumped by the heart and is not circulated through the gills where, in the living fish, it becomes enriched with oxygen. Since no oxygen is available for normal respiration, the production of energy from ingested nutrients is greatly restricted.

Figure 5.2 illustrates the normal pathway for the production of muscle energy in most living teleost fish (bony finfish). Glycogen (stored carbohydrate) or fat is oxidized or "burned" by the tissue enzymes in a series of reactions which ultimately produce carbon dioxide (CO_2), water and the energy-rich organic compound adenosine triphosphate (ATP). This type of respiration takes place in two stages: an anaerobic and an aerobic stage. The latter depends on the continued presence of oxygen (O_2) which is only available from the circulatory system. Most crustaceans are capable of respiring outside the aquatic environment by absorption of atmospheric oxygen for limited periods.

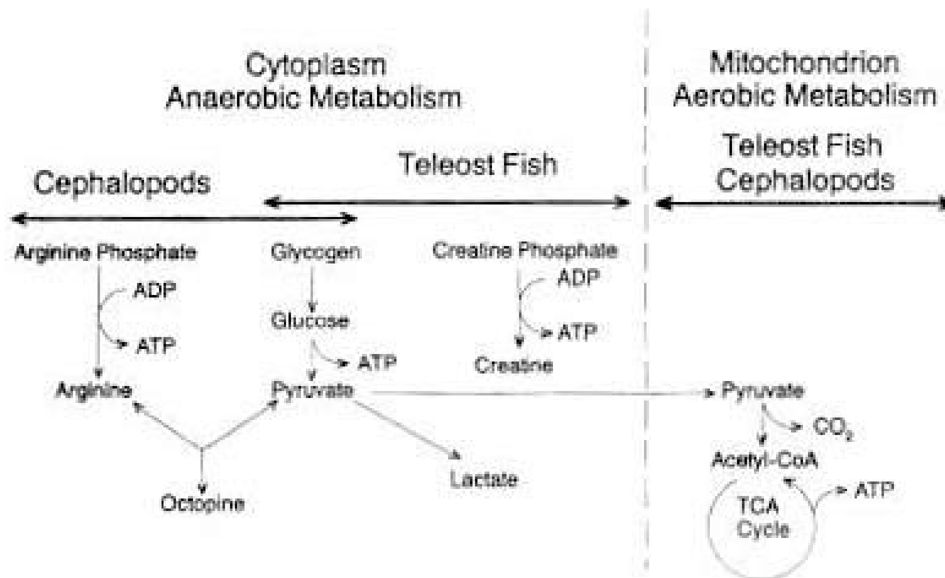


Figure 5.2 Aerobic and anaerobic breakdown of glycogen in fish muscle

Figure 5.2 also illustrates that, under anaerobic conditions, ATP may be synthesized by two other important pathways from creatine phosphate or from arginine phosphate. The former source of energy is restricted to vertebrate muscle (teleost fish) while the latter is characteristic of some invertebrates such as the cephalopods (squid and octopus). In either case, ATP production ceases when the creatine or arginine phosphates are depleted. It is interesting to note that octopine is the end-product from the anaerobic metabolism of cephalopods and is not acidic (unlike lactate), thus any changes in post mortem pH in such animals are not related to the production of lactic acid from glycogen.

For most teleost fish, glycolysis is the only possible pathway for the production of energy once the heart stops beating. This more inefficient process has principally lactic and pyruvic acids as its end-products. In addition, ATP is produced in glycolysis, but only 2 moles for each mole of glucose oxidized as compared to 36 moles ATP produced for each mole of glucose if the glycolytic end products are oxidized aerobically in the mitochondrion in the living animal. Thus, after death, the anaerobic muscle cannot maintain its normal level of ATP, and when the intracellular level declines from 7-10 µmoles/g to £ 1.0 µmoles/g tissue, the muscle enters rigor mortis. Post mortem glycolysis results in the accumulation of lactic acid which in turn lowers the pH of the muscle. In cod, the pH drops from 6.8 to an ultimate pH of 6.1-6.5. In some species of fish, the final pH may be lower: in large mackerel, the ultimate rigor pH may be as low as

5.8-6.0 and as low as 5.4-5.6 in tuna and halibut, however such low pH levels are unusual in marine teleosts. These pHs are seldom as low as those observed for post mortem mammalian muscle. For example, beef muscle often drops to pH levels of 5.1 in rigor mortis. The amount of lactic acid produced is related to the amount of stored carbohydrate (glycogen) in the living tissue. In general, fish muscle contains a relatively low level of glycogen compared to mammals, thus far less lactic acid is generated after death. Also, the nutritional status of the fish and the amount of stress and exercise encountered before death will have a dramatic effect on the levels of stored glycogen and consequently on the ultimate post mortem pH. As a rule, well-rested, well-fed fish contain more glycogen than exhausted fish. In a recent study of Japanese loach (Chiba et al., 1991), it was shown that only minutes of pre-capture stress resulted in a decrease of 0.50 pH units in 3 hours as compared to non-struggling fish whose pH dropped only 0.10 units in the same time period. In addition, the same authors showed that bleeding of fish significantly reduced the post mortem production of lactic acid.

The post mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water-holding capacity. Muscle tissue in the state of rigor mortis loses its moisture when cooked and is particularly unsuitable for further processing which involves heating, since heat denaturation enhances the water loss. Loss of water has a detrimental effect on the texture of fish muscle and it has been shown by Love (1975) that there is an inverse relationship between muscle toughness and pH, unacceptable levels of toughness (and water-loss on cooking) occurring at lower pH levels (Figure 5.3).

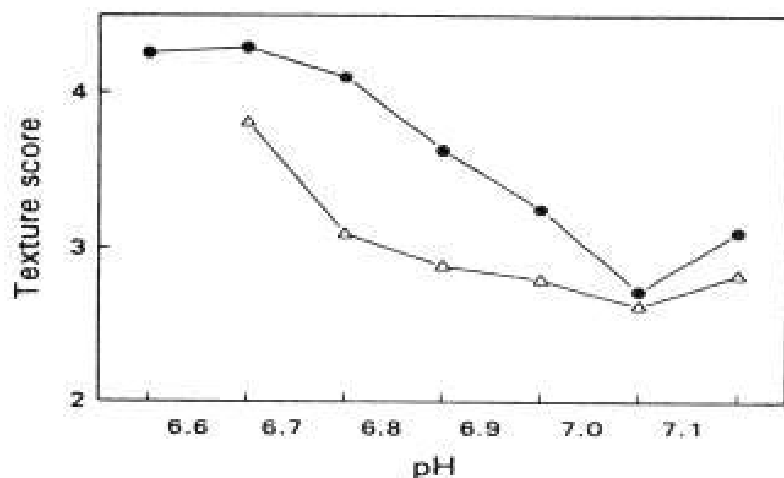


Figure 5.3.Relationship between cod muscle texture and pH, adapted from Love (1975). Black spots refer to fish caught from St. Kilda, Atlantic Ocean, whereas triangles refer to fish caught on Fells Bank, Davis Strait

Autolysis and nucleotide catabolism

As mentioned earlier, rigor mortis sets in when the muscle ATP level drops to £ 1.0 µmoles/g. ATP is not only a source of high energy which is required for muscle contraction in the living animal, but also acts as a muscle plasticizer. Muscle contraction

per se is controlled by calcium and an enzyme, ATP-ase which is found in every muscle cell. When intracellular Ca^{+2} levels are 1 μM, Ca^{+2} - activated ATP-ase reduces the amount of free muscle ATP which results in the interaction between the major contractile proteins, actin and myosin. This ultimately results in the shortening of the muscle, making it stiff and inextensible. A fish in rigor mortis cannot normally be filleted or processed because the carcass is too stiff to be manipulated and is often contorted, making machine-handling impossible (see also section 3.2 on bleeding and section 5.1 on sensory changes).

The **resolution** of rigor is a process still not completely understood but always results in the subsequent softening (relaxation) of the muscle tissue and is thought to be related to the activation of one or more of the naturally-occurring muscle enzymes, digesting away certain components of the rigor mortis complex. The softening of the muscle during resolution of rigor (and eventually spoilage processes) is coincidental with the autolytic changes. Among the changes, one of the first to be recognized was the degradation of ATP-related compounds in a more-or-less predictable manner after death. Figure 5.4 illustrates the degradation of ATP to form adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx). The degradation of ATP catabolites proceeds in the same manner with most fish but the speed of each individual reaction (from one catabolite to another) greatly varies from one species to another and often progresses coincidentally with the perceived level of spoilage as determined by trained analysts. Saito et al. (1959) were the first to observe this pattern and to develop a formula for fish freshness based on these autolytic changes:

$$K\% = \frac{[Ino] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [Ino] + [Hx]} \times 100$$

where [ATP], [ADP], [AMP], [IMP], [Ino] and [Hx] represent the relative concentrations of these compounds in fish muscle measured at various times during chilled storage.

The K or "freshness" index gives a relative freshness rating based primarily on the autolytic changes which take place during post mortem storage of the muscle. Thus, the higher the K value, the lower the freshness level. Unfortunately, some fish species such as Atlantic cod reach a maximum K value well in advance of the shelf life as determined by trained judges, and K is therefore not considered reliable as a freshness index for all marine finfish. Also, the degradation of nucleotide catabolites is only coincidental with perceived changes in freshness and not necessarily related to the cause of freshness deterioration since only Hx is considered to have a direct effect on the perceived bitter off-flavour of spoiled fish (Hughes and Jones, 1966). It is now widely accepted that IMP is responsible for the desirable fresh fish flavour which is only present in top quality seafood. None of the nucleotide catabolites are considered to be related to the perceived changes in texture during the autolytic process except of course ATP whose loss is associated with *rigor mortis.*

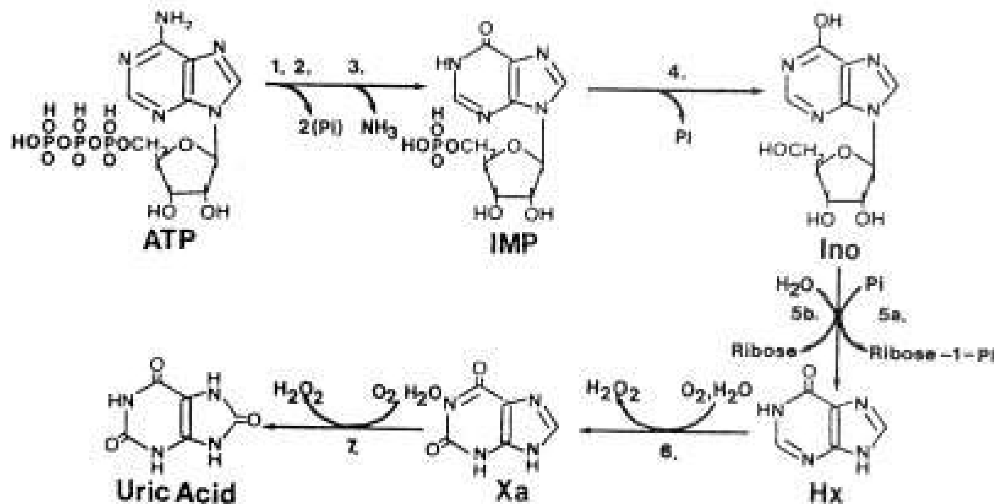


Figure 5.4 *Postmortem* ATP degradation in fish muscle. Enzymes include: 1. ATP-ase; 2. myokinase; 3. AMP deaminase; 4.IMP phosphohydrolase; 5a. nucleoside phosphorylase; 5b. inosine nucleosidase; 6,7. xanthine oxidase. Source: Gill (1992)

Surette *et al.* (1988) followed the autolysis of sterile and non-sterile cod as indicated by the ATP catabolites. The rates of formation and breakdown of IMP were the same in both sterile and non- sterile samples of cod tissue (Figures 5.5a and 5.5b), indicating that the catabolic pathway for the degradation of ATP through to inosine is entirely due to autolytic enzymes.

The conversion of Iino to Hex was accelerated by about 2 days for the non-sterile samples, suggesting that bacterial nucleoside phosphorylase (enzyme 5.a in Figure 5.4) plays a major role in the *postmortem* production of Hx in refrigerated cod (see also section 5.3). It is interesting to note that Surette *et al.* (1988) were not able to recover nucleoside phosphorylase from freshly killed cod, but Surette *et al.* (1990) later went on to isolate and purify this enzyme from a *Proteus* bacterium recovered from spoiled cod fillets. As mentioned earlier, large variations can be expected in the patterns of nucleotide degradation from one species to another. The variations in Hx among various types of fish are shown in Figure 5.6. It is clear therefore that Hx determination would likely not be useful for such species as swordfish and redfish.

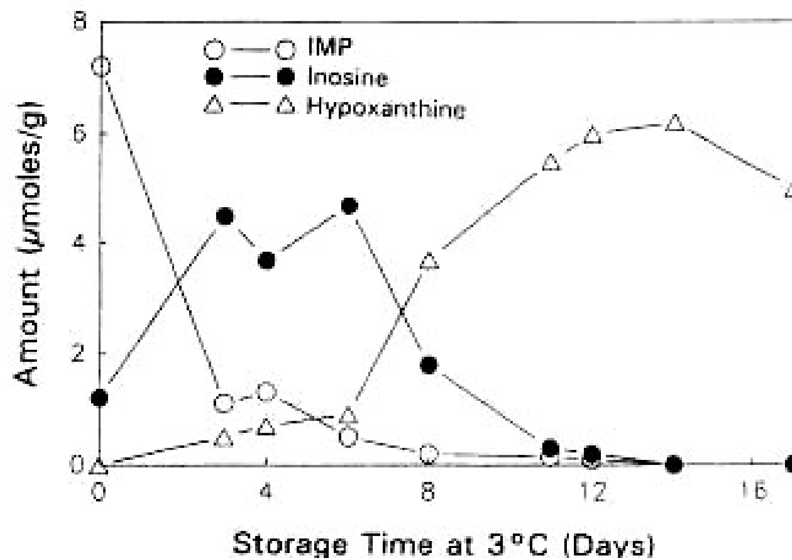


Figure 5.5a Changes in IMP, Ino and Hx in sterile cod fillets at 3°C adapted from Gill (1990)

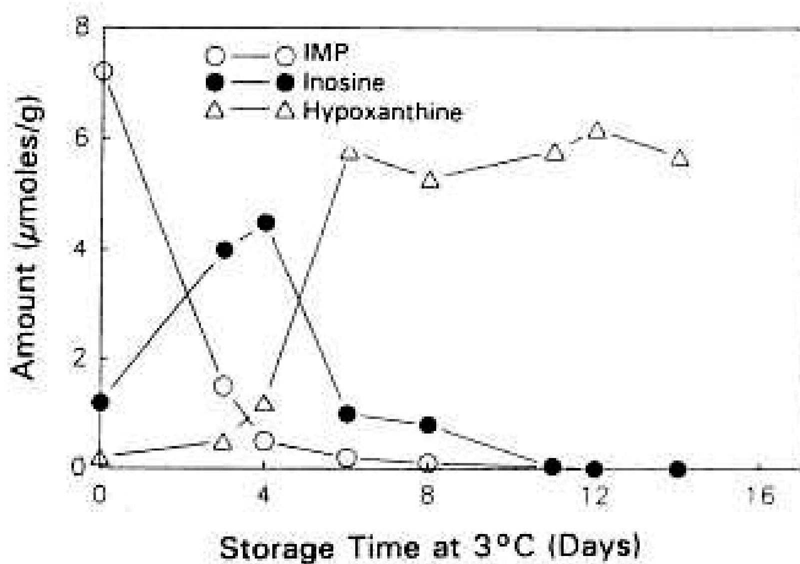


Figure 5.5b Changes in IMP, Ino and Hx in non-sterile cod fillets at 3°C adapted from Gill (1990)

There is little doubt that physical handling accelerates the autolytic changes in chilled fish. Surette et al. (1988) reported that the breakdown rate of the nucleotide catabolites was greater in sterile fillets than in non-sterile gutted whole cod. This is perhaps not surprising since many of the autolytic enzymes have been shown to be compartmentalized in discrete membrane-bound packages which become broken when subjected to physical abuse and result in the intimate mixing of enzyme and substrate. Crushing of the fish by ice or other fish can seriously affect the edibility and filleting yields even for fish which have a relatively low bacterial load, demonstrating the importance of autolytic processes. Iced fish should never be stored in boxes deeper than 30 cm and it is equally important to be sure that fish boxes are not permitted to "nest" one on top of the other if autolysis is to be minimized. Systems for conveying fish and for

discharge from the vessels must be designed so as to avoid physical damage to the delicate tissues.

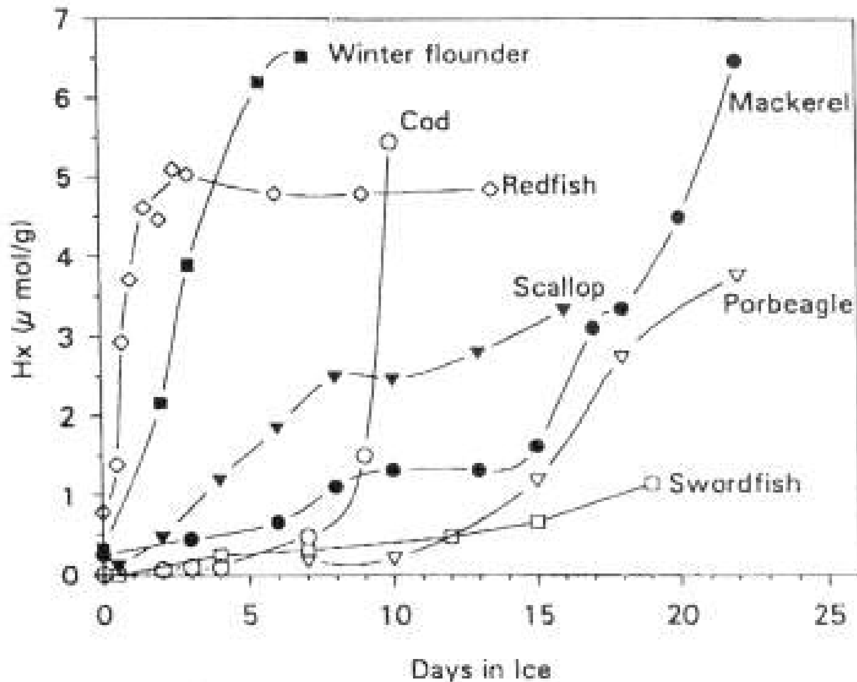


Figure 5.6 Variation in the rate of Hx accumulation of several species during storage in ice. Adapted from Fraser *et al.* (1967)

Several rapid methods have been developed for the determination of individual nucleotide catabolites or combinations including the freshness index. Two recent reviews should be consulted (Gill, 1990, 1992).

Autolytic changes involving proteolytic enzymes

Many proteases have been isolated from fish muscle and the effects of proteolytic breakdown are often related to extensive softening of the tissue. Perhaps one of the most notable examples of autolytic proteolysis is the incidence of belly-bursting in pelagic (fatty fish) species such as herring and capelin. This type of tissue softening is most predominant in summer months when pelagics are feeding heavily, particularly on "red feed" consisting of copepods and euphausiids. The low molecular weight peptides and free amino-acids produced by the autolysis of proteins not only lower the commercial acceptability of pelagics, but in bulk-stored capelin, autolysis has been shown to accelerate the growth of spoilage bacteria by providing a superior growth environment for such organisms (Aksnes and Brekken, 1988). The induction of bacterial spoilage in capelin by autolysis also resulted in the decarboxylation of amino-acids, producing biogenic amines and lowered the nutritive value of the fish significantly. This is particularly important since autolysis and bacterial growth greatly lower the commercial value of pelagics used for the production of fishmeal.

Similarly, bulk-stored herring used for fishmeal has been found to contain carboxy-peptidases A and B, chymotrypsin, and trypsin; and preliminary studies have shown that

proteolysis can be inhibited by the addition of potato extracts which not only slowed the proteolysis but resulted in lower microbial growth and preservation of the nutritional value of the meal (Aksnes, 1989).

More recently, Botta *et al.* (1992) found that autolysis of the visceral cavity (belly-bursting) of herring was related more to physical handling practices than to biological factors such as fish size, amount of red feed in the gut or roe content. In particular, it was shown that for herring, freezing/thawing, thawing time at 15°C and time of iced storage, had a far greater influence on belly- bursting than biological factors.

Cathepsins

Although several proteolytic enzymes have been discovered in the fish tissues, it has perhaps been the cathepsins which have been described most often. The cathepsins are "acid" proteases usually found packaged in tiny, submicroscopic organelles called lysozomes. In living tissue, lysozomal proteases are believed to be responsible for protein breakdown at sites of injury. Thus cathepsins are for the most part inactive in living tissue but become released into the cell juices upon physical abuse or upon freezing and thawing of *post mortem* muscle.

Cathepsins D and L are believed to play a major role in the autolytic degradation of fish tissue since most of the other cathepsins have a relatively narrow pH range of activity far too low to be of physiological significance. Reddi *et al.* (1972) demonstrated that an enzyme believed to be cathepsin D from winter flounder was active over a pH range of 3-8 with a maximum near pH 4.0, although no attempt was made to confirm the identity of the enzyme using synthetic substrates or specific inhibitors. Nevertheless, the enzyme was far less active in the presence of ATP, suggesting that such an enzyme would only be active in *post mortem* fish muscle. Also, the enzyme activity was inhibited strongly by the presence of salt (Figure 5.7) with virtually no activity remaining after a 25-hour incubation in the presence of 5% sodium chloride. It is therefore unlikely that Reddi's enzyme was active in salted fish products.

Cathepsin L has been implicated in the softening of salmon muscle during spawning migration. It is likely that this enzyme contributes more to autolysis of fish muscle than cathepsin D since it is far more active at neutral pH, and has been shown to digest both myofibrillar proteins (actomyosin) as well as connective tissue. Yamashita and Konogaya (1990) produced strong evidence implicating cathepsin L rather than other cathepsins in the softening of salmon during spawning. They demonstrated that electrophoresis of purified myofibrils treated with cathepsin L resulted in patterns which were almost identical to patterns of proteins recovered from muscle from spawning fish. Furthermore, the cathepsin L autolytic activity correlated well with the texture of the muscle as measured instrumentally. The linear correlation between cathepsin L activity and breaking strength of the muscle was excellent; $r = 0.86$ and -0.95 for fresh and frozen/thawed tissue, respectively. It is interesting that, in all cases, the autolytic ability as measured by cathepsin L activity was higher in frozen/thawed tissue than in fresh tissue. Freezing and thawing often break down cell membranes allowing autolytic membrane-bound enzymes to react with their natural substrates. The enzyme and its

naturally occurring inhibitor were further studied by the same authors (Yamashita and Konogaya, 1992). Cathepsin L has also been associated with the production of a jelly-like softening of flounder (Toyohara *et l.,* 1993 a) and the uncontrollable softening of Pacific hake muscle which has been parasitized by Myxosporidia (Toyohara *et al.,* 1993 b).

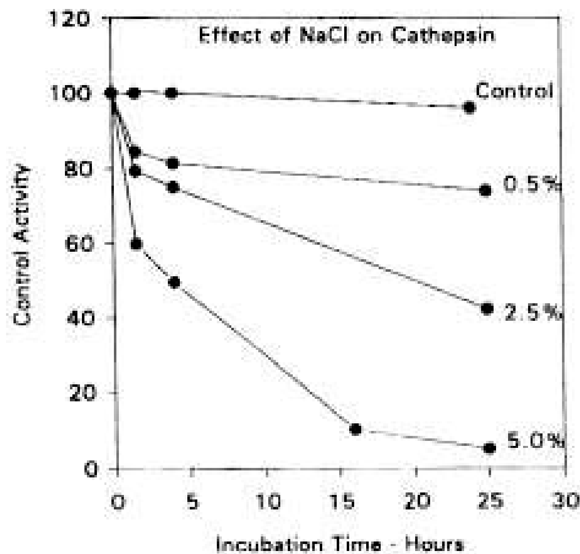


Figure 5.7 Effect of NaCl on the catheptic activity. Adapted from Reddi *et al.* (1972)

The tissues of such infected fish have little commercial value, but at present it is not known if it is the parasite or the host which secretes the proteolytic enzymes which autolyze the muscle.

In addition to their detrimental effect on texture, catheptic enzymes induce intentional autolytic changes in fermented fish products. For example, cathepsins are believed to be responsible for major textural changes during the fermentation of salted preserved Japanese squid and Crucian carp (Makinodan *et al.,* 1991, 1993).

Calpains

A second group of intracellular proteases called "calpains" or "calcium activated factor" (CAF) has recently been associated with fish muscle autolysis and is found in meats, finfish and crustaceans. Tenderness is probably the most important quality characteristic of red meat. It has been known for nearly a century that *post mortem* aging of red meat results in the tenderization process. Calpains have been found primarily responsible for the *post mortem* autolysis of meat through digestion of the z- line proteins of the myofibril. Although toughness is seldom a problem with unfrozen fish muscle, softening through autolysis is a serious problem limiting the commercial value. The calpains are intra-cellular endopeptidases requiring cysteine and calcium; μ-calpain requiring 5-50 μM Ca^{2+} , m-calpain requiring 150-1000 μM Ca^{2+} . Most calpains are active at physiological pH, making it reasonable to suspect their importance in fish-softening during chilled storage.

Studies have shown that in crustacean muscle, calpains are associated with moltinduced textural changes to the muscle and carry out non-specific generalized digestion of the myofibrillar proteins. However, vertebrate muscle calpains have been shown to be very specific, digesting primarily tropinin- T, desmin, titin and nebulin, attacking neither vertebrate actin or myosin (Koohmaraie, 1992). In contrast, fish calpains digest myosin (specifically the myosin heavy chain) to form an initial fragment with approximate molecular weight of 150 000 Da (Muramoto *et al.,* 1989). The same authors demonstrated that fish calpains were far more active at low temperatures than were mammalian calpains and that the rates of cleavage were species-specific, being most active against myosins with lowest heat stabilities. Thus, fish species adapted to colder environmental temperatures are more susceptible to calpain autolysis than those from tropical waters. Although calpain has been identified in several fish species including carp (Toyohara *et al.,* 1985), tilapia and shrimp (Wang *et al.,* 1993), as well as tuna, croaker, red seabream and trout (Muramoto *et al.,* 1989) to name a few, little work has to date demonstrated a "cause and effect" relationship between calpain activity and instrumental measurements of texture.

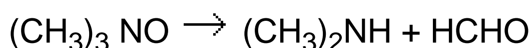
Collagenases

To this point, all of the *post mortem* autolytic changes described have involved changes within the muscle cell *per se.* However, the flesh of teleost fish is divided into blocks of muscle cells separated into "flakes" or myotomes by connective tissue called myocommata (Figure 3.3). Each muscle cell or fibre is surrounded with connective tissue which attaches to the myocommata at the ends of the cells by means of fine collagenous fibrils. During chilled storage, these fibrils deteriorate (Bremner and Hallett, 1985). More recently, it was shown that instrumental measurements of texture of chilled trout muscle decreased as the amount of type V collagen was solubilized, presumably due to the action of autolytic collagenase enzymes (Sato *et al.,* 1991). It is these enzymes which presumably cause "gaping" or breakdown of the myotome during long-term storage on ice or short term storage at high temperature. For Atlantic cod, it has been shown that upon reaching 17°C, gaping is inevitable presumably because of degradation of the connective tissue and rapid shortening of the muscle due to high temperature *rigor.*

The relatively short shelf life of chilled prawns due to softening of the tissue has also been shown to be due to the presence of collagenase enzymes (Nip *et al.,* 1985). The source of the collagenase enzymes in prawn is thought to be the hepatopancreas (digestive organ).

Autolytic changes during frozen storage

The reduction of trimethylamine oxide (TMAO), an osmoregulatory compound in many marine teleost fish, is usually due to bacterial action (section 5.3) but in some species an enzyme is present in the muscle tissue which is able to break down TMAO into dimethylamine (DMA) and formaldehyde (FA):



$$(CH_3)_3\,NO \rightarrow (CH_3)_2NH + HCHO$$

It is important to note that the amount of formaldehyde produced is equivalent to the dimethylamine formed but is of far greater commercial significance. Formaldehyde induces cross- linking of the muscle proteins making the muscle tough and readily lose its water holding capacity. The enzyme responsible for formal dehyde-induced toughening is called TMAO-ase or TMAO demethylase and is most commonly found in the gadoid fishes (cod family). Most of the TMAO demethylase enzymes reported to date weremembrane-bound and become most active when the tissue membranes are disrupted by freezing or artificially by detergent solubilization. Dark (red) muscle has a higher rate of activity than white muscle whereas other tissues such as kidney, spleen and gall bladder are extremely rich in the enzyme. Thus, it is important that minced fish is completely free of organ tissue such as kidney from gadoid species if toughening in frozen storage is to be avoided. It is often difficult to ensure that the kidney is removed prior to mechanical deboning since this particular organ runs the full length of the backbone and is adherent to it. The TMAO-ase enzyme has been isolated from the microsomal fraction in hake muscle (Parkin and Hultin, 1986) and the lysosomal membrane in kidney tissue (Gill *et al.,* 1992). It has been shown that the toughening of frozen hake muscle is correlated to the amount of formaldehyde produced, and that the rate of FA production is greatest at high frozen-storage temperatures (Gill *et al., 1979).* In addition, it has been shown that the amount of FA-induced toughening is enhanced by physical abuse to the catch prior to freezing and by temperature fluctuations during frozen storage. The most practical means of preventing the autolytic production of FA is to store fish at temperatures < -30°C to minimize temperature fluctuations in the cold store and to avoid rough handling or the application of physical pressure on the fish prior to freezing. The autolytic changes affecting the edibility of fresh and frozen fish are summarized in Table 5.3. Generally, the most important single factor affecting autolysis is physical disruption of the muscle cells. No attempt has been made here to deal with the alkaline proteases associated with the softening of cooked surimi products. An article by Kinoshita *et al.* (1990) deals with the heat-activated alkaline proteases associated with the softening in surimi-based products.

Table 5.3 Summary of Autolytic Changes in Chilled Fish

Enzyme(s)	Substrate	Changes Encountered	Prevention/Inhibition
glycolytic enzymes	glycogen	production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle high temperature rigor may result in gaping	fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible pre-rigor stress must be avoided

autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	loss of fresh fish flavour, gradual production of bitternes with Hx (later stages)	same as above rough handling or crushing accelerates breakdown
cathepsins	proteins, peptides	softening of tissue making processing difficult or impossible	rough handling during storage and discharge
chymotrypsin, trypsin, carboxy-peptidases	proteins, peptides	autolysis of visceral cavity in pelagics (belly-bursting)	problem increased with freezing/thawing or long- term chill storage
calpain	myofibrillar proteins	softening, molt-induced softening in crustaceans	removal of calcium thus preventing activation?
collagenases	connective tissue	gaping" of fillets softening	connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	formaldehyde-induced toughening of frozen gadoid fish	store fish at temperature <= -30°C physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

5.3 Bacteriological changes

The bacterial flora on live fish

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously and Liston (1980) states a normal range of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g (Shewan, 1962).

The bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1977). Fish caught in very cold, clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher counts. Very high numbers, i.e., 10^7 cfu/cm^2 are found on fish from polluted warm waters. Many different bacterial species can be found on the fish surfaces. The bacteria on temperate water fish are all classified according to their growth temperature range as either psychrotrophs or psychrophiles. Psychrotrophs (cold-tolerant) are bacteria capable of growth at 0°C but with optimum around 25°C. Psychrophiles (cold-loving) are

bacteria with maximum growth temperature around 20°C and optimum temperature at 15°C (Morita, 1975). In warmer waters, higher numbers of mesophiles can be isolated. The microflora on temperate water fish is dominated by psychrotrophic Gram-negative rodshaped bacteria belonging to the genera *Pseudomonas, Moraxella, Acinetobacter, Shewanella* and *Flavobacterium.* Members of the *Vibrionaceae (Vibrio* and *Photobacterium)* and the *Aeromonadaceae (Aeromonas* spp.) are also common aquatic bacteria and typical of the fish flora (Table 5.4). Gram-positive organisms as *Bacillus, Micrococcus, Clostridium, Lactobacillus* and coryneforms can also be found in varying proportions, but in general, Gram-negative bacteria dominate the microflora. Shewan (1977) concluded that Gram-positive *Bacillus* and Micrococcus dominate on fish from tropical waters. However, this conclusion has later been challenged by several studies which have found that the microflora on tropical fish species is very similar to the flora on temperate species (Acuff *et al.,*1984; Gram *et al.,* 1990; Lima dos Santos 1978; Surendran *et al.,* 1989). A microflora consisting of *Pseudomonas, Acinetobacter, Moraxella* and *Vibrio* has been found on newly-caught fish in several Indian studies (Surendran *et al.,* 1989). Several authors conclude, as Liston (1980), that the microflora on tropical fish often carry a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish.

Aeromonas spp. are typical of freshwater fish, whereas a number of bacteria require sodium for growth and are thus typical of marine waters. These include *Vibrio*, *Photobacterium* and *Shewanella*. However, although *Shewanella putrefaciens* is characterized as sodium-requiring, strains of *S. putrefaciens* can also be isolated from freshwater environments (DiChristina and DeLong, 1993; Gram et al., 1990; Spanggaard et al., 1993). Although *S. putrefaciens* has been isolated from tropical freshwaters, it is not important in the spoilage of freshwater fish (Lima dos Santos, 1978; Gram, 1990).

Table 5.4 Bacterial flora on fish caught in clean, unpolluted waters

Gram-negative	Gram-positive	Comments
Pseudomonas	*Bacillus*	
Moraxella	*Clostridium*	
Acinetobacter	*Micrococcus*	
Shewanella putrefaciens	*Lactobacillus*	
Flavobacterium	Coryneforms	
Cytophaga		
Vibrio *Photobacterium* *Aeromonas*		*Vibrio* and *Photobacterium* are typical of marine waters; *Aeromonas* is typical of freshwater

In polluted waters, high numbers of *Enterobacteriaceae* may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Fujioka *et al.,* 1988).

The taxonomy of S. *putrefaciens* has been rather confused. The organism was originally associated with the *Achromobacter* group but was later placed in the Shewan *Pseudomonas* group IV. Based on percentage of guanine+ cytosine (GC%) it was transferred to the genus *Alteromonas,* but on the basis of 5SRNA homology it was reclassified to a new genus, *Shewanella* (MacDonnell and Colwell, 1985). It has recently been suggested that the genus *Aeromonas* spp. which was a member of the *Vibrionaceae* family be transferred to its own family, the *Aeromonadaceae* (Colwell *et al.,* 1986).

Japanese studies have shown very high numbers of microorganisms in the gastrointestinal tract of fish, and as such numbers are much higher than in the surrounding water, this indicates the presence of a favourable ecological niche for the microorganisms. Similarly, Larsen *et al.* (1978) reported up to 10^7 cfu/g of vibrio-like organisms in the intestinal tract of cod and Westerdahl *et al.* (1991) also isolated high numbers of vibrio-like organisms from the intestines of turbot. *Photobacterium phosphoreum* which can be isolated from the surface can also be isolated in high numbers from the intestinal tract of some fish species (Dalgaard, 1993). On the contrary, some authors believe that the microflora of the gastrointestinal tract is merely a reflection of the environment and the food intake.

Microbial invasion

The flesh of healthy live or newly-caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh (Figure 5.8 a). When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibres. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during iced storage. Ruskol and Bendsen (1992) showed that bacteria can be detected by microscope in the flesh when the number of organisms on the skin surface increases above 10^6 cfu/cm^2 (Figure 5.6 b). This was seen at both iced and ambient temperatures. No difference was found in the invasive patterns of specific spoilage bacteria (e.g., S. *putrefaciens)* and non-spoilage bacteria.

Since only a limited number of organisms actually invade the flesh and microbial growth mainly takes place at the surface, spoilage is probably to a large extent a consequence of bacterial enzymes diffusing into the flesh and nutrients diffusing to the outside.

Fish spoil at very different rates (see also section 6.5), and differences in surface properties of fish have been proposed to explain this. Skins of fish have very different textures. Thus whiting *(Merlangius merlangus)* and cod *(Gadus morhua)* which have a very fragile integument spoil rapidly compared to several flatfish such as plaice that has a very robust dermis and epidermis. Furthermore, the latter group has a very thick slime layer, which includes several antibacterial components, such as antibodies, complement and bacteriolytic enzymes (Murray and Fletcher, 1976; Hjelmland *et al.,* 1983).

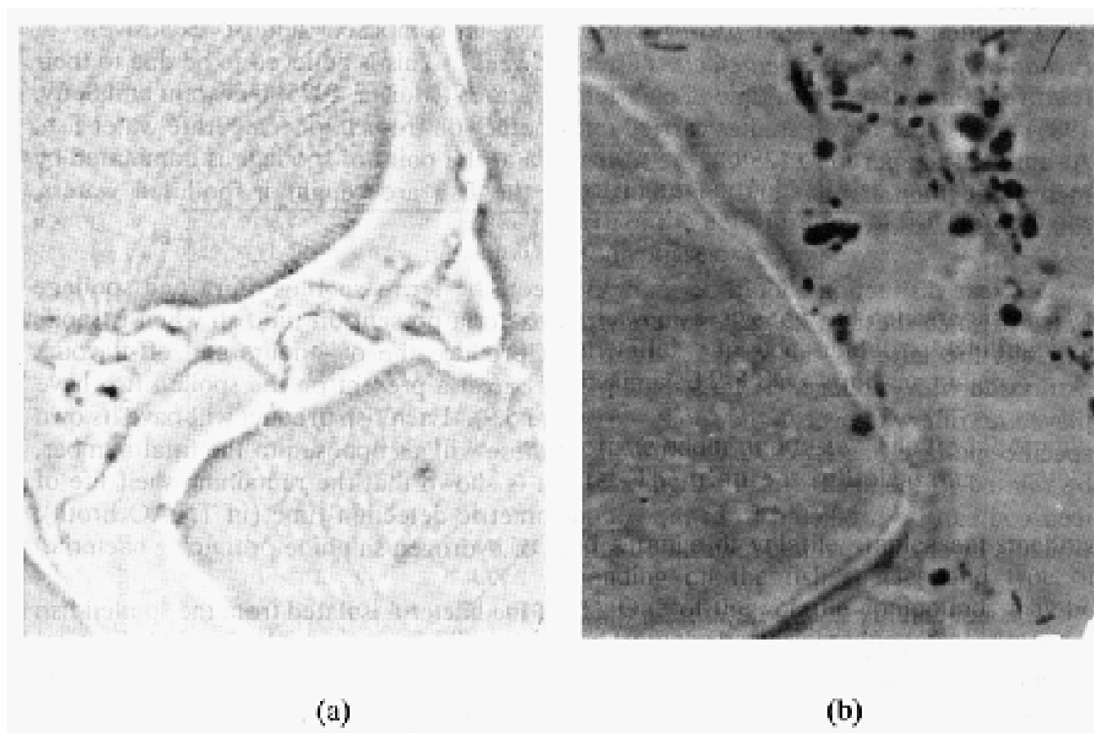


Figure 5.8 Histological section of (a) newly-caught cod and (b) cod fillets stored 12 days in ice. The section has been Giemsa-stained (Ruskol and Bendsen, 1992)

Changes in the microflora during storage and spoilage/Specific spoilage organisms

Bacteria on fish caught in temperate waters will enter the exponential growth phase almost immediately after the fish have died. This is also true when the fish are iced, probably because the microflora is already adapted to the chill temperatures. During ice storage, the bacteria will grow with a doubling time of approximately 1 day and will, after 2-3 weeks, reach numbers of 10^8-10^9 cfu/g flesh or cm^2 skin. During ambient storage, a slightly lower level of 10^7-10^8 cfu/g is reached in 24 hours. The bacteria on fish caught in tropical waters will often pass through a lag-phase of 1-2 weeks if the fish are stored in ice, whereafter exponential growth begins. At spoilage, the bacterial level on tropical fish is similar to the levels found on temperate fish species (Gram, 1990; Gram *et al.,* 1990).

If iced fish are stored under anaerobic conditions or if stored in CO_2 containing atmosphere, the number of the normal psychrotrophic bacteria such as S. *putrefaciens* and *Pseudomonas* is often much lower, i.e., 10^6-10^7 cfu/g than on the aerobically stored fish. However, the level of bacteria of psychrophilic character such as P. *phosphoreum* reaches a level of 10^7-10^8 cfu/g when the fish spoil (Dalgaard *et al.,* 1993).

The composition of the microflora also changes quite dramatically during storage. Thus, under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas* spp. and S. *putrefaciens* after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures (Morita, 1975; Devaraju and Setty, 1985) and is true for all studies carried out whether on tropical or temperate-water fish. At ambient temperature (25°C), the microflora at the point of spoilage is dominated by mesophilic

Vibrionaceae and, particularly if the fish are caught in polluted waters, *Enterobacteriaceae.*

A clear distinction should be made between the terms **spoilage flora** and **spoilage bacteria** since the first describes merely the bacteria present on the fish when it spoils whereas the latter is the specific group that produce the off-odours and off-flavours associated with spoilage. A large part of the bacteria present on the spoiled fish have played no role whatever in the spoilage (Figure 5.9). Each fish product will have its own specific spoilage bacteria and the number of these will, as opposed to the total number, be related to the shelf life. In Figure 5.10, it is shown that the remaining shelf life of iced cod can be predicted from the conductometric detection time (in TMAO broth), which is inversely correlated with the number of hydrogen sulphide-producing bacteria.

It is not an easy task to determine which of the bacteria isolated from the spoiled fish are those causing spoilage, and it requires extensive sensory, microbiological and chemical studies. First, the sensory, microbiological and chemical changes during storage must be studied and quantified, including a determination of the level of a given chemical compound that correlates with spoilage (the chemical spoilage indicator). Second, bacteria are isolated at the point of sensory rejection. Pure and mixed cultures of bacteria are screened in sterile fish substrates for their **spoilage potential**, i.e., their ability to produce sensory (off-odours) and chemical changes typical of the spoiling product. Finally, the selected strains are tested to evaluate their **spoilage activity**, i.e., if their growth rate and their qualitative and quantitative production of off-odours are similar to the measurements in the spoiled product (Dalgaard, 1993).

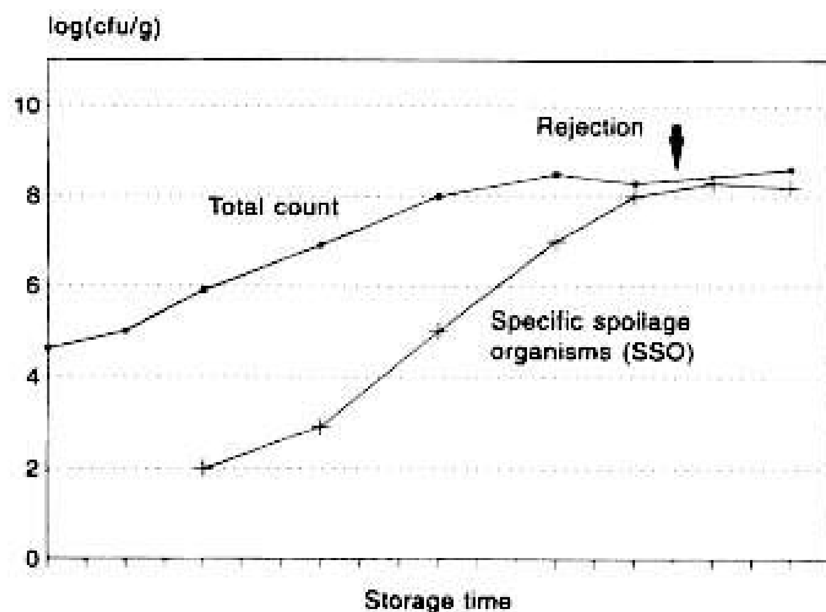


Figure 5.9 Changes in total counts and specific spoilage bacteria during storage (modified after Dalgaard (1993)

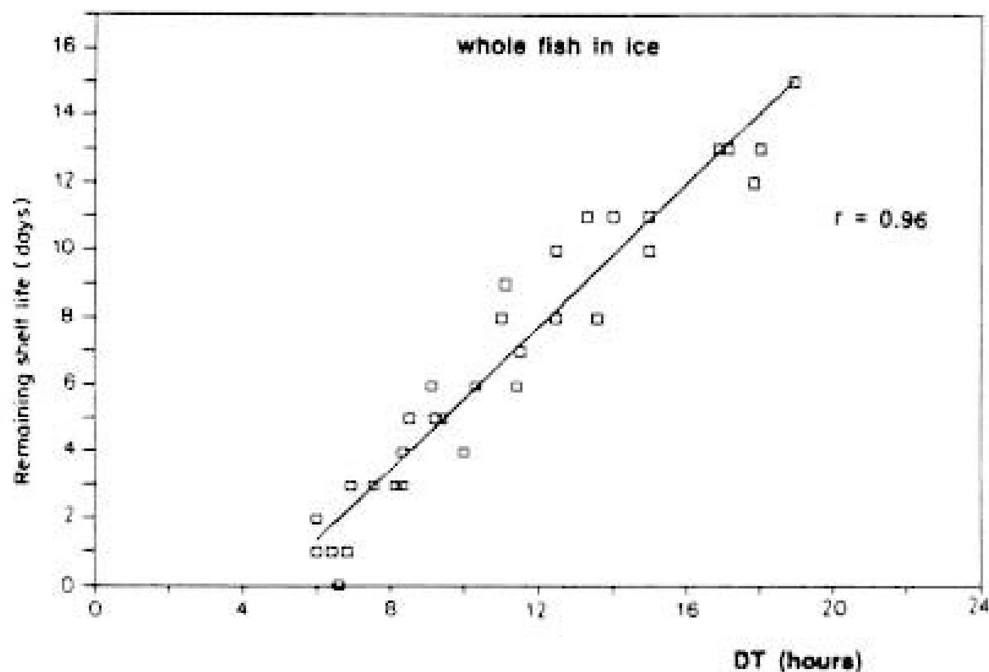


Figure 5.10 Comparison of remaining shelf life of iced cod and detection time in a TMAO broth (Jorgensen et al., 1988)

The latter step is particularly important, as some bacteria may produce the chemical compounds associated with spoilage but are unable to do so in significant amounts, and they are thus not the specific spoilage bacteria. When stored aerobically, levels of 10^8-10^9 cfu/g of specific spoilage bacteria are required to cause spoilage. The spoilage of packed fish is seen at a much lower level of 10^7 cfu P. *phosphoreum* per gramme. This relatively low level is probably due to the very large size (5 µm) of the bacterium resulting in a much higher yield of for example, TMA per cell (Dalgaard, 1993).

Spoilage potential and activity can be assessed in several fish substrates as sterile, raw fish juice (Lerke *et al.,* 1963), heat-sterilized fish juice (Castell and Greenough, 1957; Gram *et al. , 1987*; Dalgaard, 1993) or on sterile muscle blocks (Herbert *et al.,* 1971). The latter is the most complicated but is also that yielding results comparable to the product. If any of the fish juices are chosen, it is important that the growth rate of the spoilage bacteria in the model system is equal to the growth rate in the product.

A qualitative test for the ability of the bacteria to produce H_2S and/or reduce TMAO may also be used when the spoilage flora is screened for potential spoilage bacteria. A medium where the reduction of TMAO to TMA is seen as a redox indicator changes colour, and the formation of H_2S is evident from a black precipitation of FeS which has been developed for this purpose (Gram *et al.,*1987).

Shewanella putrefaciens has been identified as the specific spoilage bacteria of marine temperate- water fish stored aerobically in ice. If the product is vacuum-packed, P. phosphoreum participates in the spoilage and it becomes the specific spoilage bacteria of CO_2 packed fish (see section 6.3). The spoilage flora on iced tropical fish from marine

waters is composed almost exclusively of *Pseudomonas* spp. and *S. putrefaciens*. Some *Pseudomonas* spp. are the specific spoilers of iced stored tropical freshwater fish (Lima dos Santos, 1978; Gram et al., 1990) and are also, together with *S. putrefaciens*, spoilers of marine tropical fish stored in ice (Gillespie and MacRae, 1975; Gram, 1990).

At ambient temperature, motile aeromonads are the specific spoilers of aerobically stored freshwater fish (Gorzyka and Pek Poh Len, 1985; Gram *et al.,* 1990). Barile *et al.* (1985) showed that a large proportion of the flora on ambient-stored mackerel consisted of *S. putrefaciens,* indicating that this bacterium may also take part in the spoilage.

Table 5.5 gives an overview of the specific spoilage bacteria of fresh fish products stored in ice and at ambient temperature.

Table 5.5 Dominating microflora and specific spoilage bacteria at spoilage of fresh, white fish (cod) (from Huss, 1994)

Storage temperature	Packaging atmosphere	Dominating microflora	Specific spoilage organisms (SSO)	References
0°C	Aerobic	Gram-negative psychrotrophic, non-fermentative rods *(Pseudomonas spp., S. putrefaciens, Moraxella, Acinetobacter)*	S. *putrefaciens* *Pseudomonas* [3]	2,3,4,9
0°C	Vacuum	Gram-negative rods; psychrotrophic or with psychrophilic character (S. *putrefaciens, Photobacterium)*	S. *putrefaciens P. phosphoreum*	1,9
0°C	MAP[1]	Gram-negative fermentative rods with psychrophilic character *(Photobacterium)* Gram-negative non-fermentative psychrotrophic rods (1-10% of flora; *Pseudomonas, S. putrefaciens)* Gram-positive rods (LAB 2)	*P. phosphoreum*	1,7
5°C	Aerobic	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10

5°C	Vacuum	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*	10
5°C	MAP	Gram-negative psychrotrophic rods *(Vibrionaceae)*	*Aeromonas spp.*	6
20-30°C	Aerobic	Gram-negative mesophilic fermentative rods *(Vibrionaceae, Enterobacteriaceae)*	Motile *Aeromonas* spp. *(A. hydrophila)*	2,4,5,8

1) Modified Atmosphere Packaging (CO_2 containing)
2) LAB: Lactic Acid Bacteria
3) Fish caught in tropical waters or freshwaters tend to have a spoilage dominated by *Pseudomonas spp.*

References: 1) Battle *et al.* (1985); 2) Dalgaard *et al.* (1993); 3) Donald and Gibson (1992); 4) Gorczyca and Pek Poh Len (1985); 5) Gram *et al.* (1987); 6) Gram *et al.* (1990); 7) Gram and Dalgaard (pers. comm.); 8) Jorgensen and Huss (1989); 9) Lima dos Santos (1978); 10) van Spreekens (1977)

Biochemical changes induced by bacterial growth during storage and spoilage

Comparison of the chemical compounds developing in naturally spoiling fish and sterile fish has shown that most of the volatile compounds are produced by bacteria (Shewan, 1962) as shown in Figure 5.11. These include trimethylamine, volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecularweight compounds.

The substrates for the production of volatiles are the carbohydrates (e.g., lactate and ribose), nucleotides (e.g., inosine mono-phosphate and inosine) and other NPN molecules. The amino-acids are particularly important substrates for formation of sulphides and ammonia.

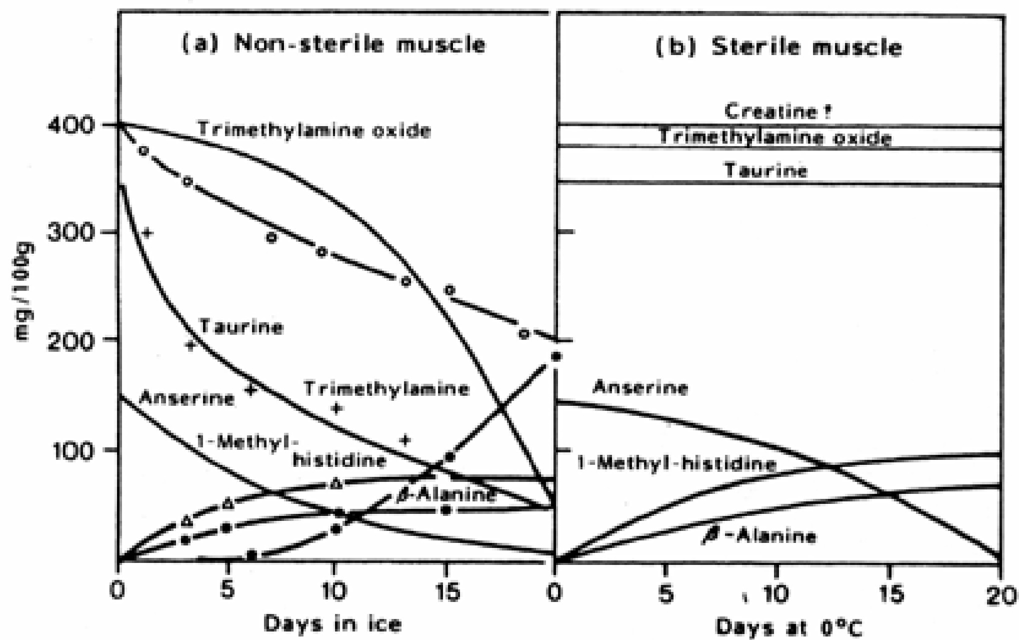


Figure 5.11 Changes in the nitrogenous extractives in a) spoiling and b) autolysing cod muscle (Shewan, 1962)

Microorganisms obtain far more energy from aerobic oxidation than from an anaerobic fermentation; thus the complete oxidation of 1 mole glucose (or other hexose) via Kreb's cycles yields 6 moles of CO_2 and 36 moles of ATP. On the contrary, the fermentation of 1 mole glucose gives only 2 moles of ATP and two moles of lactic acid. The initial aerobic growth on fish is dominated by bacteria using carbohydrates as substrate and oxygen as terminal electron-acceptor with the concurrent production of CO_2 and H_2O.

Reduction of Trimethylarnine Oxide (TMAO)

The growth of oxygen-consuming bacteria results in the formation of anaerobic or microaerophilic niches on the fish. This does, however, not necessarily favour the growth of anaerobic bacteria. Some of the bacteria present on fish are able to carry out a respiration (with the ATP advantage) by using other molecules as electron acceptor. It is typical of many of the specific spoilage bacteria on fish that they can use TMAO as electron acceptorin an anaerobic respiration. The reduced component, TMA, which is one of the dominant components of spoiling fish, has a typical fishy odour. The level of TMA found in fresh fish rejected by sensory panels varies between fish species, but is typically around 10-15 mg TMA-N/100 g in aerobically stored fish and at a level of 30 mg TMA-N/100 g in packed cod (Dalgaard *et al.,* 1993).

The TMAO reduction is mainly associated with the genera of bacteria typical of the marine environment *(Alteromonas, Photobactetium, Vibrio* and S. *putrefaciens),* but is also carried out by *Aeromonas* and intestinal bacteria of the *Enterobacteriaceae.* TMAO reduction has been studied in fermentative, facultative anaerobic bacteria like *E. coli* (Sakaguchi *et al.,* 1980) and *Proteus* spp. (Stenberg *et al.,* 1982) as well as in the non-

fermentative S. *putrefaciens* (Easter *et al,* 1983; Ringo *et al,* 1984). During aerobic growth, S. *putrefaciens* uses the Kreb's cycle to produce the electrons that are later channelled through the respiratory chain. Ringo *et al.* (1984) suggested that during anaerobic respiration S. *putrefaciens* also uses the complete Kreb's cycle (Figure 5.12), whereas it has recently been shown that in the anaerobic respiration in S. *putrefaciens,* only part of the Kreb's cycle is used (Figure 5.13) and electrons are also generated by another metabolic pathway, namely the serine pathway (Scott and Nealson, 1994). S. *putrefaciens* can use a variety of carbon sources as substrate in its TMAO-dependent anaerobic respiration, including formate and lactate. Compounds like acetate and succinate that are used in the oxygen respiration cannot be used when TMAO is terminal electron acceptor (DiChristina and DeLong, 1994) and on the contrary, acetate is a product of the anaerobic TMAO reduction (Ringo *et al.,* 1984; Scott and Nealson, 1994).

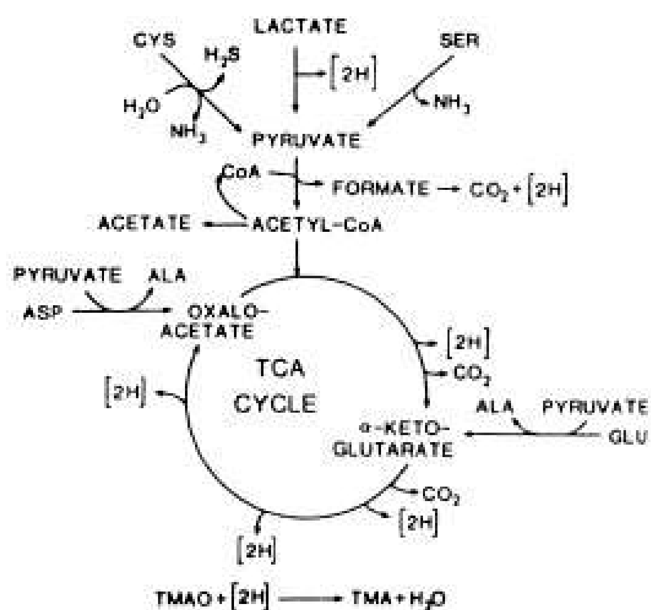


Figure 5.12 Anaerobic reduction of TMAO by *Shewanella putrefaciens* (formerly *Alteromonas)* as suggested by Ringo *et al.* (1984)

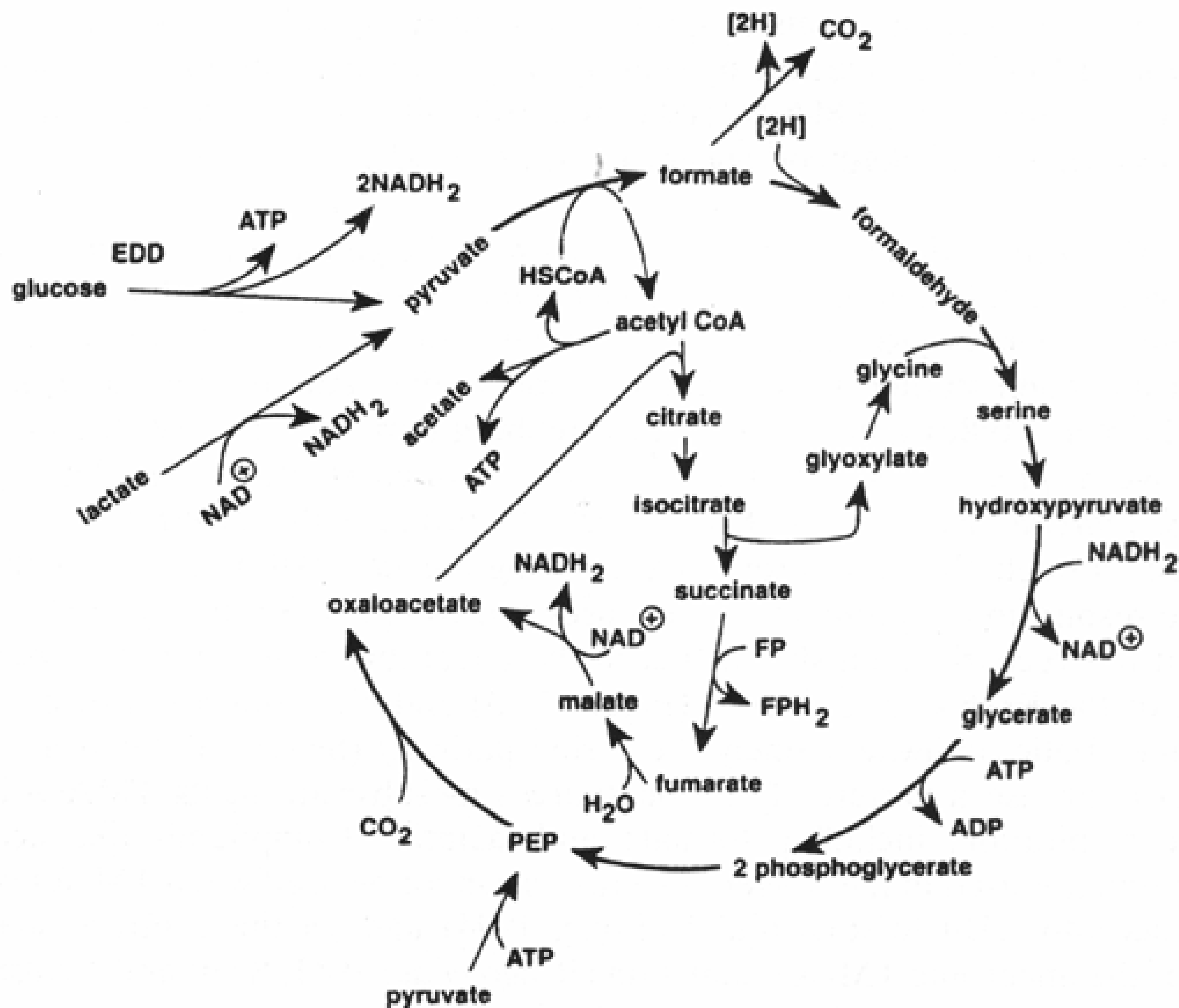


Figure 5.13 Proposed route of carbon during anaerobiosis for S. *putrefaciens* (Scott and Nealson, 1994)

Contrary to this, sugars and lactate are the main substrates generating electrons when *Proteus* spp. reduces TMAO. The reduction is accompanied by a production of acetate as the main product (Kjosbakken and Larsen, 1974).

TMAO is, as mentioned in section 4.4, a typical component of marine fish, and it has recently been reported that also some tropical freshwater fish contain high amounts of TMAO (Anthoni *et al.,* 1990). However, TMA is not necessarily a characteristic component during spoilage of such fish because spoilage is due to *Pseudomonas spp.* (Gram *et al.,* 1990).

The development of TMA is in many fish species paralleled by a production of hypoxanthine. Hypoxanthine can, as described in section 5.2. be formed by the autolytic decomposition of nucleotides, but it can also be formed by bacteria; and the rate of bacterial formation is higher than the autolytic. Both Jorgensen *et al.* (1988) and

Dalgaard (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod (Figure 5.14). Several of the spoilage bacteria produce hypoxanthine from inosine or inosine mono-phosphate, including *Pseudomonas* spp. (Surette *et al.,* 1988) S. *putrefaciens* (van Spreekens, 1977; Jorgensen and Huss, 1989; Gram, 1989) and P. *phosphoreum* (van Spreekens, 1977).

In cod and other gadoid fishes, TMA constitutes most of the so-called total volatile bases, TVB (also called total volatile nitrogen, TVN) until spoilage. However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB levels still rise due to formation of NH3 and other volatile amines. A little ammonia is also formed in the first weeks of iced storage due to autolysis. In some fish that do not contain TMAO or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVB is seen during storage, probably resulting from the deamination of amino-acids.

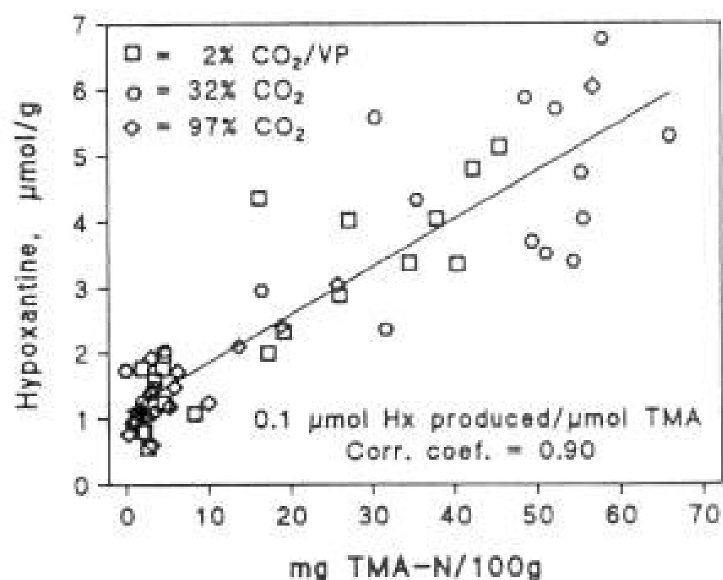


Figure 5.14 Relationship between contents of TMA and Hx during storage of packed cod in ice (Dalgaard *et al.,* 1993)

Volatile sulphur-compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. S. *putrefaciens* and some *Vibrionaceae* produce H_2S from the sulphur containing amino-acid 1-cysteine (Stenstroem and Molin, 1990; Gram *et al.,* 1987). On the contrary, neither *Pseudomonas* nor P. *phosphoreum* produce significant amounts of H_2S. Thus, hydrogen sulphide, which is typical of spoiling iced cod stored aerobically, is not produced in spoiling CO_2 packed fish (Dalgaard *et al.,* 1993). Methylmercaptan (CH_3SH) and dimethylsulphide ($(CH_3)_2S$) are both formed from the other sulphur-containing amino-acid, methionine. Taurine, which is also sulphur-containing, occurs as free amino-acid in very high concentrations in fish muscle. It disappears from the fish flesh during storage (Figure 5.11) but this is because of leakage rather than because of bacterial attack (Herbert and Shewan, 1975). The formation of compounds in naturally-spoiling cod as compared to sterile muscle is shown in Figure 5.15.

The volatile sulphur-compounds are very foul-smelling and can be detected even at ppb levels, so even minimal quantities have a considerable effect on quality. Ringo *et al. (1984)* have shown that cysteine is used as substrate in the Kreb's cycle when electrons are transferred to TMAO, and the formation of H_2S and TMA is thus to some extent a linked reaction (Figure 5.12).

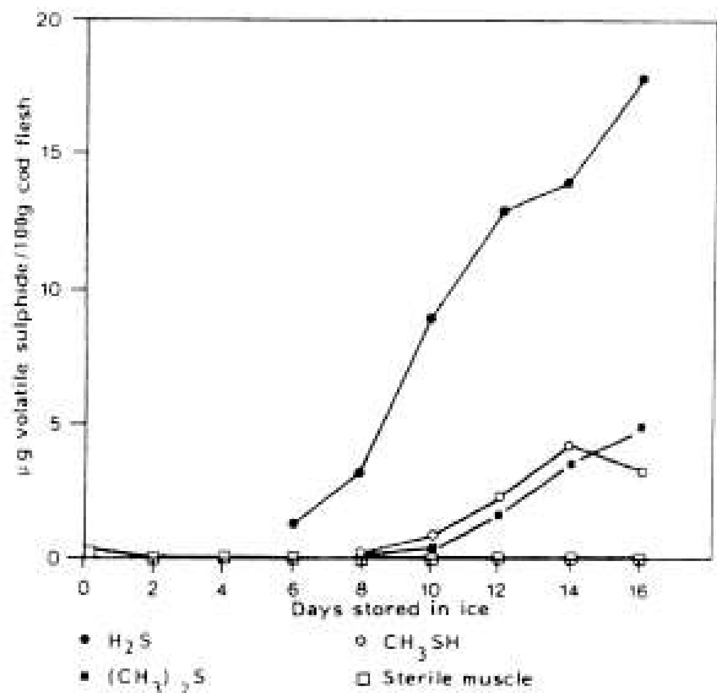


Figure 5.15 Production of HA CH_3SH and $(CH_3)_2S$ in naturally spoiling cod fillets and sterile muscle blocks (Herbert and Shewan, 1976)

Contrary to the iced spoilage by S. *putrefaciens* and the ambient spoilage by *Vibrionaceae* which is dominated by H_2S and TMA, the spoilage caused by *Pseudomonas* spp. is characterized by absence of these compounds (Gram *et al.,* 1989, Gram *et al.,* 1990). Fruity, rotten, sulphydryl odours and flavours are typical of the Pseudomonas spoilage of iced fish. *Pseudomonas* spp. produce a number of volatile aldehydes, ketones, esters and sulphides (Edwards *et al.,* 1987; Miller *et al.,* 1973 a, 1973 b). However, it is not known which specific compounds are responsible for the typical off-odours (Table 5.6). The fruity off-odours produced by *Pseudomonas fragi* originate from monoaminomonocarboxylic amino-acids.

Table 5.6 Typical spoilage compounds during spoilage of fresh fish stored aerobically or packed in ice or at ambient temperature

Specific spoilage organism	Typical spoilage compounds
Shewanella putrefaciens	*TMA, H_2S, CH_3SH, $(CH_3)_2S$, Hx*
Photobacterium phosphoreum	TMA, Hx
Pseudomonas spp.	ketones, aldehydes, esters, non-H_2S sulphides

Vibrionaceae	TMA, H_2S
anaerobic spoilers	NH_3, acetic, butyric and propionic acid

As mentioned above, TVB will continue to rise even after TMA has reached its maximum. This latter rise is due to proteolysis commencing when several of the free amino-acids have been used. Lerke *et al.* (1967) separated fish juice into a protein and a non-protein fraction and inoculated spoilage bacteria in each fraction and in the whole juice. The non-protein fraction of a fish juice spoiled as the whole juice whereas only faint off-odours were detected in the protein fraction of the juice. Although some authors have used the number of proteolytic bacteria as indicators of spoilage, it must be concluded that the turnover of the protein fraction is not of major importance in spoilage of fresh fish.

Some of the compounds typically formed by bacteria during spoilage of fish are shown in Table 5.7 together with the substrate used for the formation.

Table 5.7 Substrate and off-odour/off-flavour compounds produced by bacteria during spoilage of fish

Substrate	**Compounds produced by bacterial action**
TMAO	TMA
cysteine	H_2S
methionine	CH_3SH, $(CH_3)_2S$
carbohydrates and lactate	acetate, CO_2, H_2O
inosine, IMP	hypoxanthine
amino-acid s (glycine, serine, leucine)	esters, ketones, aldehydes
amino-acids, urea	NH_3

The formation of TMA is accompanied by a formation of ammonia during anaerobic storage of herring and mackerel (Haaland and Njaa, 1988). Prolonged anaerobic storage of fish results in vigorous production of NH_3 owing to further degradation of the amino-acids, and in the accumulation of lower fatty acids as acetic, butyric and propionic acid. The very strong NH_3-producers were found to be obligate anaerobes belonging to the family Bacteroidaceae genus *Fusobacterium* (Kjosbakken and Larsen, 1974; Storroe *et al.,* 1975, 1977). These organisms grow only in the spoiled fish extract and have little or no proteolytic activity relying on already hydrolysed proteins.

During iced storage of fresh fatty fish, changes in the lipid fraction is caused almost exclusively by chemical action, e.g., oxidation, whereas bacterial attack on the lipid fraction contributes little to the spoilage profile. During storage of lightly preserved fish, lipid hydrolysis caused by bacteria may be part of the spoilage profile.

5.4 Lipid oxidation and hydrolysis

The two distinct reactions in fish lipids of importance for quality deterioration are:

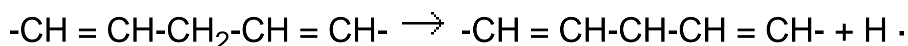
- oxidation
- hydrolysis

They result in production of a range of substances among which some have unpleasant (rancid) taste and smell. Some may also contribute to texture changes by binding covalently to fish muscle proteins. The various reactions are either *nonenzymatic* or catalyzed by *microbial* enzymes or by *intracellular* or *digestive* enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature.

Fatty fish are, of course, particularly susceptible to lipid degradation which can create severe quality problems even on storage at subzero temperatures.

Oxidation

The large amount of polyunsaturated fatty acid moieties found in fish lipids (see section 4.2) makes them highly susceptible to oxidation by an autocatalytic mechanism (Figure 5.16). The process is initiated as described below by abstraction of a hydrogen atom from the central carbon of the *pentadiene structure* found in most fatty acid acyl chains containing more than one double bond:



$$-CH = CH-CH_2-CH = CH- \rightarrow -CH = CH-CH-CH = CH- + H \cdot$$

Contrary to the native molecule, the lipid radical (L) reacts very quickly with atmospheric oxygen making a peroxy-radical (LOO) which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical L. This propagation continues until one of the radicals is removed by reaction with another radical or with an *antioxidant* (AH) whose resulting radical (A) is much less reactive. The hydroperoxides produced in relatively large amounts during propagation are tasteless, and it is therefore perhaps not surprising that the widely used "peroxide value" (section 8.2) usually correlates rather poorly to sensorial properties.

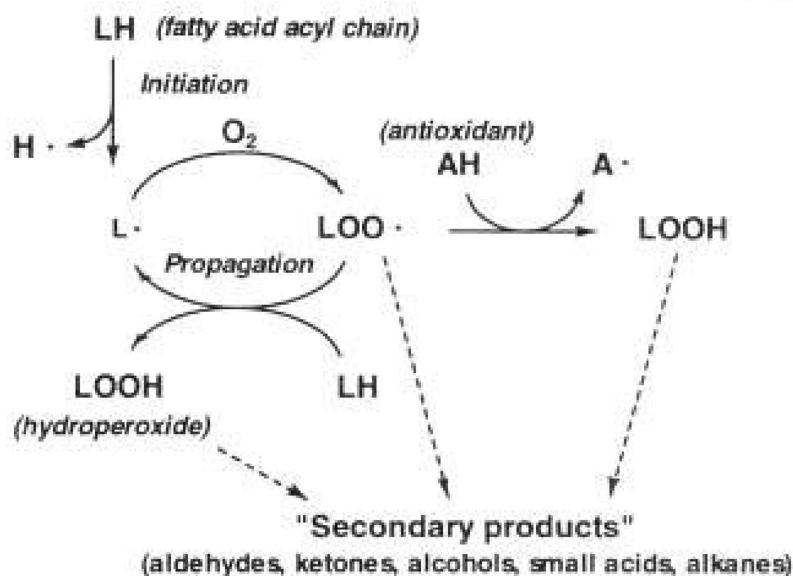


Figure 5.16 Autoxidation of polyunsaturated lipid

The hydroperoxides are readily broken down, catalyzed by heavy metal ions, to secondary autoxidation products of shorter carbon chain-length. These secondary products - mostly aldehydes, ketones, alcohols, small carboxylic acids and alkanes - give rise to a very broad odour spectrum and in some cases to a yellowish discoloration. Several of the aldehydes can be determined as "thiobarbituric acid-reactive substances" (section 8.2).

Metal ions are very important in the first step of lipid autoxidation - the initiation process - in catalyzing the formation of reactive oxygen species as for example the hydroxyl radical (OH). This radical immediately reacts with lipids or other molecules at the site where it is generated. The high reactivity may explain that free fatty acids have been found to be more susceptible to oxidation than the corresponding bound ones, because the amount of iron in the aqueous phase is probably greater than the amount bound to the surface of cellular membranes and lipid droplets.

Fatty acid hydroperoxides may also be formed enzymatically, catalyzed by *lipoxygenase* which is present in variable amounts in different fish tissues. A relatively high activity has been found in the gills and under the skin of many species. The enzyme is unstable and is probably important for lipid oxidation only in fresh fish. Cooking or freezing/thawing rather effectively destroys the enzyme activity.

The living cells possess several protection mechanisms directed against lipid oxidation products. An enzyme, glutathione peroxidase, exists which reduces hydroperoxides in the cellular membranes to the corresponding hydroxy-compounds. This reaction demands supply of reduced glutathione and will therefore cease post mortem when the cell is depleted of that substance. The membranes also contain the phenolic compound a-tocopherol (Vitamin E) which is considered the most important natural antioxidant. Tocopherol can donate a hydrogen atom to the radicals L- or LOO- functioning as the molecule AH in Figure 5.16. It is generally assumed, that the resulting tocopheryl radical

reacts with ascorbic acid (Vitamin C) at the lipid/water interface regenerating the tocopherol molecule. Other compounds, for example the carotenoids, may also function as antioxidants. Wood smoke contains phenols which may penetrate the fish surface during smoking and thereby provide some protection against lipid oxidation.

Hydrolysis

During storage, a considerable amount of free fatty acids (FFA) appears (Figure 5.17). The phenomenon is more profound in ungutted than in gutted fish probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase (TL in Figure 5.18) originating from the digestive tract or excreted by certain microorganisms. Cellular lipases may also play a minor role.

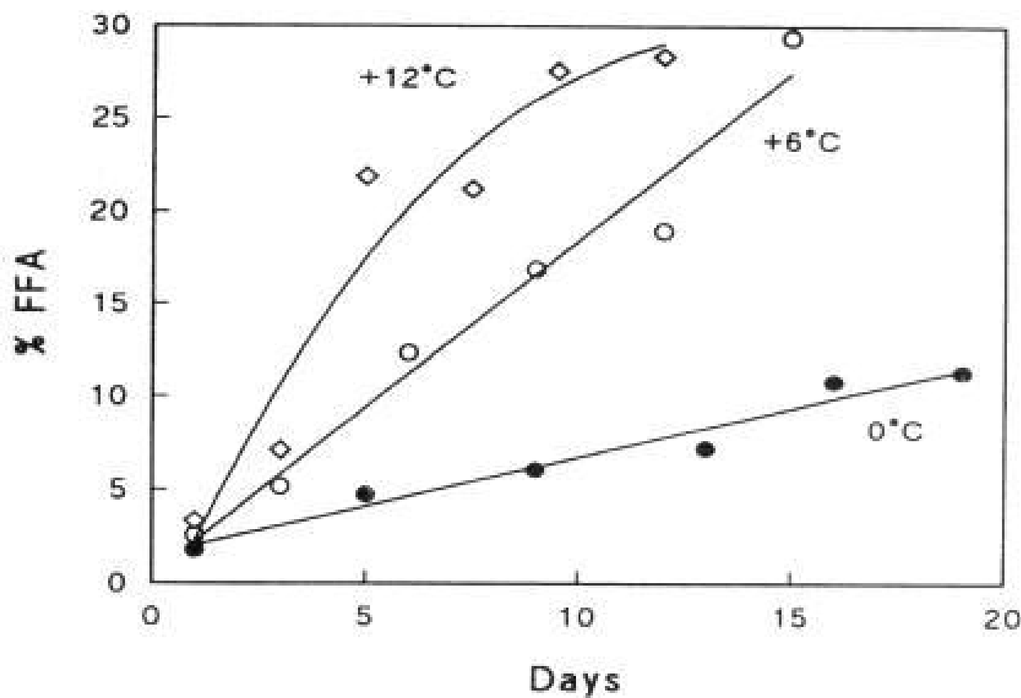
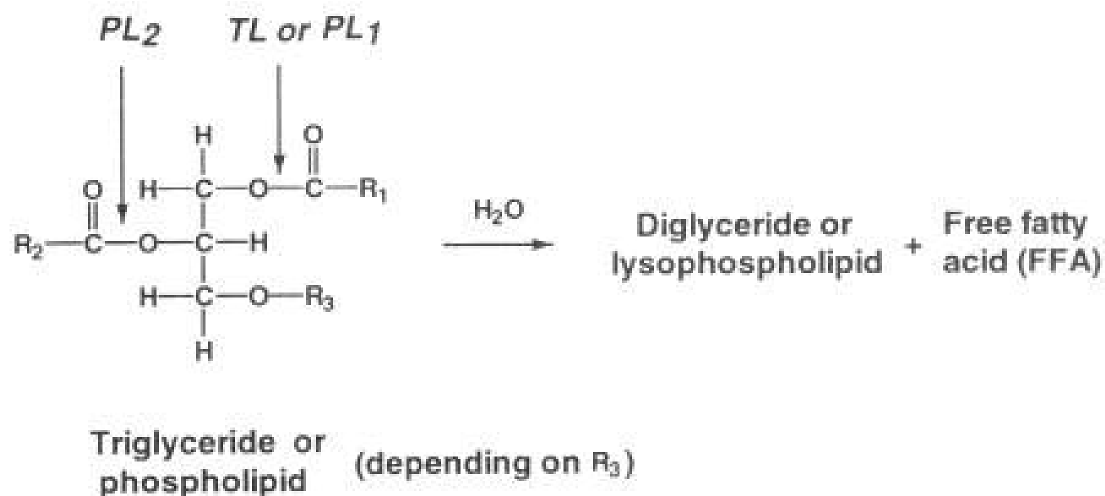


Figure 5.17 The development of free fatty acids in herring stored at different temperatures (Technological Laboratory, Danish Ministry of Fisheries, Annual Report, 1971)



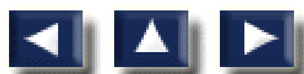


Figure 5.18 Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: PL_1 & PL_2 phospholipases;TL, triglyceride lipase

In lean fish, for example Atlantic cod, production of free fatty acids also occurs, even at low temperatures. The enzymes responsible are believed to be cellular phospholipases - in particular phospholipase A_2 (PL2 in Figure 5.18) - although a correlation between activity of these enzymes and the rate of appearance of FFA has as yet not been firmly established. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely of the polyunsaturated type, and hydrolysis therefore often leads to increased oxidation as well. Furthermore, the fatty acids themselves may cause a "soapy" off-flavour.

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6. QUALITY CHANGES AND SHELF LIFE OF CHILLED FISH

6.1. The effect of storage temperature
6.2. The effect of hygiene during handling
6.3. The effect of anaerobic conditions and carbon dioxide
6.4. The effect of gutting
6.5. The effect of fish species, fishing ground and season

6.1 The effect of storage temperature

Chill storage (0-25°C)

It is well known that both enzymatic and microbiological activity are greatly influenced by temperature. However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity (Figure 6.1).

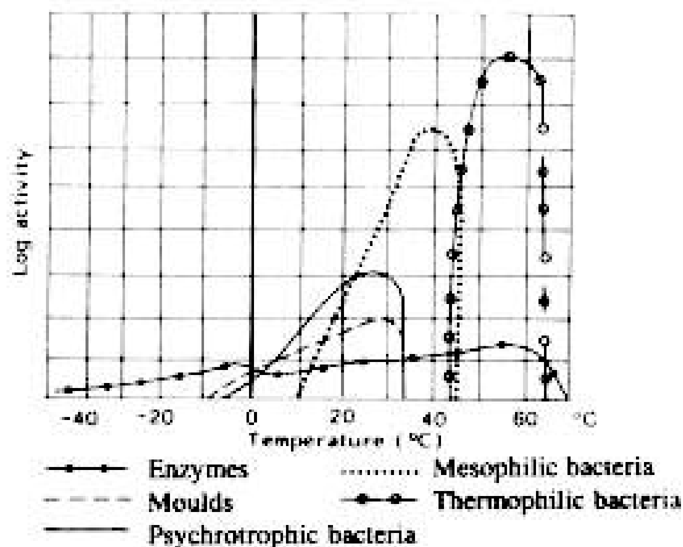

Figure 6.1 Relative enzyme activity and growth rate of bacteria in relation to temperature (Andersen et *al.,* 1965)

Many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0°C Figure 6.2 shows the effect of temperature on the growth rate of the fish spoilage bacterium *Shewanella Putrefaciens.* At 0°C the growth rate is less than one-tenth of the rate at the optimum growth temperature.

Microbial activity is responsible for spoilage of most fresh fish products. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures. In industrialized countries it is common practice to store fresh fish in ice (at 0°C) and the shelf life at different storage temperatures (at t°C) has been expressed by the relative rate of spoilage (RRS), defined as shown in Equation 6.a (Nixon, 1971).

$$\text{Relative rate of spoilage at } t\,^{\circ}C = \frac{\text{keeping time at } 0^{\circ}C}{\text{keeping time at } t\,^{\circ}C} \qquad 6.a$$

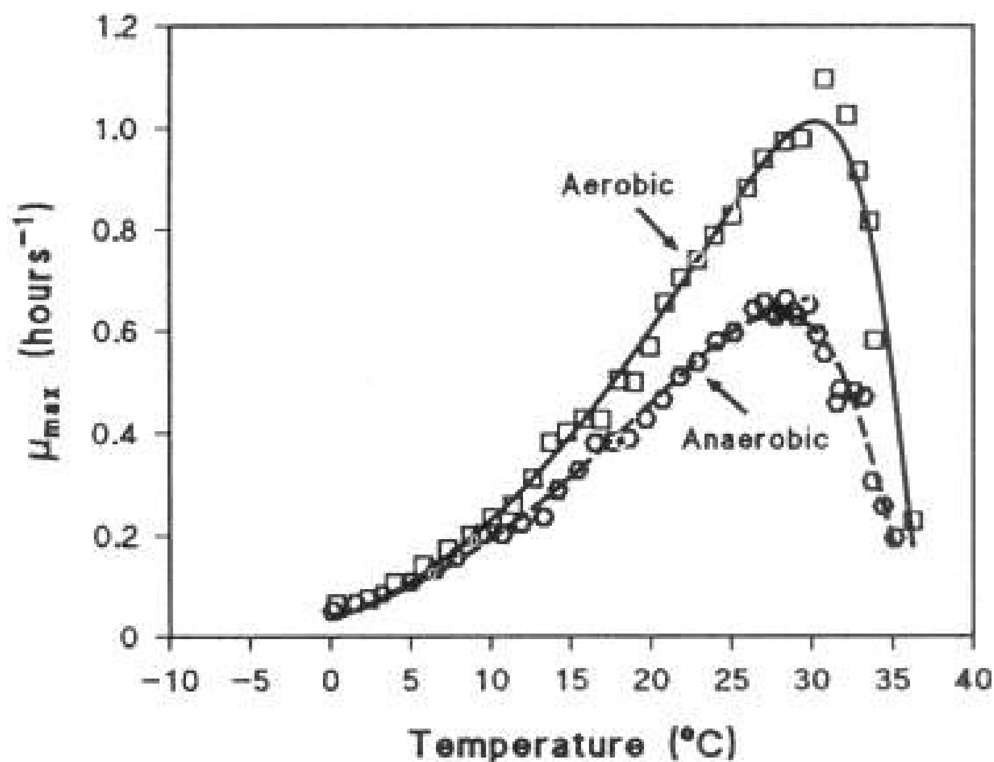

Figure 6.2 Effect of temperature on the maximum specific growth rate (μmax) of *Shewanella putrefaciens* grown in a complex medium containing TMAO (Dalgaard, 1993)

While broad differences are observed in shelf lives of the various seafood products, the effect of temperature on RRS is similar for fresh fish in general. Table 6.1 shows an example with different seafood products.

Table 6.1 Shelf lives in days and relative rates of spoilage (RRS) of seafood products stored at different temperatures

	0°C		**5°C**		**10°C**	
	shelf life	**RRS**	**shelf life**	**RRS**	**shelf life**	**RRS**
Crab claw[a]	10.1	1	5.5	1.8	2.6	3.9
Salmon[b]	11.8	1	8.0	1.5	3.0	3.9
Sea bream[c]	32.0	1	-	-	8.0	4.0

Packed cod[d)]	14	1	6.0	2.3	3.0	4.7

a) Cann et al. (1985); b) Cann et al. (1984); c) Olley and Quarmby (1981); d) Cann et al. (1983)

The relationship between shelf life and temperature has been thoroughly studied by Australian researchers (Olley and Ratkowsky, 1973 a, 1973 b). Based on data from the literature they found that the relationship between temperature and RRS could be expressed as an S-shaped general spoilage curve (Figure 6.3). Particularly at low temperatures (e.g., < 10°C this curve is similar to, and confirms the results of Spencer and Baines (1964). These authors, 10 years earlier, found a straight line relationship between RRS and the storage temperatures of cod from the North Sea (Figure 6.3).

The effect of temperature on the rate of chemical reactions is often described by the Arrhenius Equation. This Equation, however, has been shown not to be accurate when used for the effect of a wide range of temperatures, on growth of microorganisms and on spoilage of foods (Olley and Ratkowsky, 1973 b; Ratkowsky *et* al., 1982). Ratkowsky *et al.* (1982) suggested the 2-parameter square root model (Equation 6.b) for the effect of sub-optimal temperature on growth of microorganisms

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad 6b$$

T is the absolute temperature (Kelvin) and T_{min} in a parameter expressing the theoretical minimum temperature of growth. The square root of the microbial growth rates plotted against the temperature form a straight line from which T_{min} is determined. Several psychrotrophic bacteria isolated from fish products have T_{min} values of about 263 Kelvin (-10°C) (Ratkowsky *et al.,* 1982; Ratkowsky *et al.,* 1983). Based on this T_{min} value, a spoilage model has been developed. It has been assumed that the relative microbial growth rate would be similar to the relative rate of spoilage. The relative rate concept (Equation 6.a) was then combined with the simple square root model (Equation 6.b) to give a temperature spoilage model (Equation 6.c). As just described, this model was derived from growth of psychrotrophic model has been shown to give good estimates of the effect of temperature on bacteria (T_{min} = -10°C) but the RRS of chilled fresh fish as shown in Figure studies (Storey, 1985; Gibson, 1985). 6.1 and also confirmed in other

$$\sqrt{Relative\ rate\ of\ spoilage} = \frac{b(t°C - (-10°C))}{b(0°C - (-10°C))} = 0.1 * t°C + 1 \qquad 6.c$$

If the shelf life of a fish product is known at a given temperature, the shelf life at other storage temperatures can be calculated from the spoilage models. The effect of temperature, shown in Table 6.2, as calculated from Equation 6.c for products with different shelf lives when stored at 0°C.

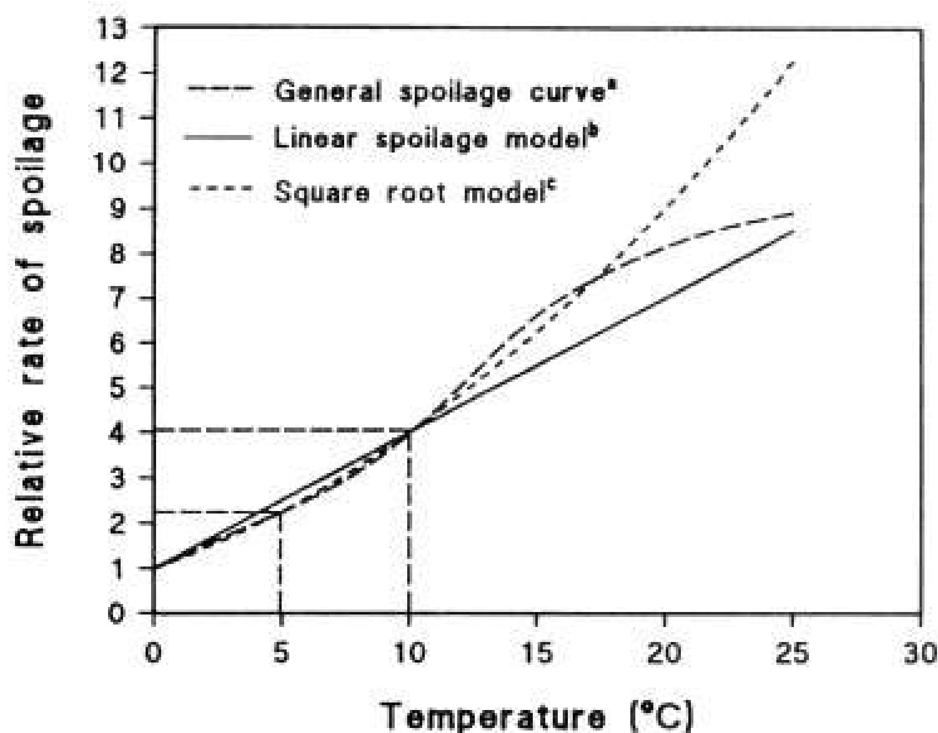

Figure 6.3 Effect of temperature on the relative rate of spoilage of fresh fish products. a) the general spoilage curve (Olley and Ratkowsky, 1973 a); b) the linear spoilage model suggested by Spencer and Baines (1964); c) the square root spoilage model derived from growth for psychrotrophic bacteria (Equation 6. c)

The effect of time/temperature storage conditions on product shelf life has been shown to be cumulative (Charm et al, 1972). This allows spoilage models to be used for prediction of the effect of variable temperatures on product keepability. An electronic time/temperature function integrator for shelf life prediction was developed, based on Equation 6.c. The instrument predicts RRS accurately, but a high price has limited its practical application (Owen and Nesbitt, 1984; Storey, 1985).

Table 6.2 Predicted shelf lives of fish products stored at different temperatures

Shelf life in days of product stored in ice (0° C)	**Shelf life at chill temperatures (days)**		
	5° C	**10°C**	**15°C**
6	2.7	1.5	1
10	4.4	2.5	1.6
14	6.2	3.5	2.2
18	8	4.5	2.9

The temperature history of a product, e.g., through a distribution system, can be determined by a temperature logger. Using a spoilage model and simple PC software, the effect of a given storage temperature profile can then be predicted. McMeekin *et al.* (1993) reviewed the literature on application of temperature loggers and on predictive temperature models. A product temperature profile also allows growth of pathogenic microorganisms to be estimated from safety models. Computers and temperature loggers are today available at reasonable prices and it is most likely that spoilage and safety models will be used frequently in the future.

The microflora responsible for spoilage of fresh fish changes with changes in storage temperature. At low temperatures (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas spp.* and *Pseudomonas s*pp. cause spoilage (Table 5.5). However, at high storage temperatures (15-30°C) different species of Vibrionaceae, Enterobacteriaceae and Gram-positive organisms are responsible for spoilage (Gram *et al.,* 1987; Gram *et al.,* 1990; Liston, 1992). Equation 6.c does not take into account the change in spoilage microflora. Nevertheless, reasonable estimates of RRS are obtained for whole fresh fish, for packed fresh fish and for superchilled fresh fish products (Figure 6.3; Gibson and Ogden, 1987; Dalgaard and Huss, 1994). For tropical fish, however, the average relative rate of spoilage of a large number of species stored at 20°-30°C was approximately 25 times higher than at 0°C The RRS of tropical fish is thus more than twice as high as estimated from the temperature models shown in Figure 6.3. Tropical fish are likely to be exposed to high temperatures and a new tropical spoilage model, covering the range of temperatures from 0°-30°C, was

recently developed (Equation 6.d; Dalgaard and Huss, 1994). Figure 6.4 shows that the natural logarithm of RRS of tropical fish is linearly related to the storage temperature. This figure also shows the differences between the new tropical model and previous spoilage models developed for fish from temperate waters.

*Ln (relative rate of spoilage for tropical fish) = 0. 12 * t °C*
6.d

Temperature models based on the relative rate concept do not take into account the initial product quality. Inaccurate shelf life predictions, therefore, may be obtained for products with variable initial quality. Spencer and Baines (1964), however, suggested that both the effect of the initial product quality and the effect of storage temperature could be predicted. At a constant storage temperature measurements of quality will change linearily from an initial to a final level reached when the product is no longer acceptable (Equation 6.e). Shelf life at a given temperature and a given initial quality is determined (Equation 6.e) and then the shelf life at other temperatures can be determined from a temperature spoilage model.

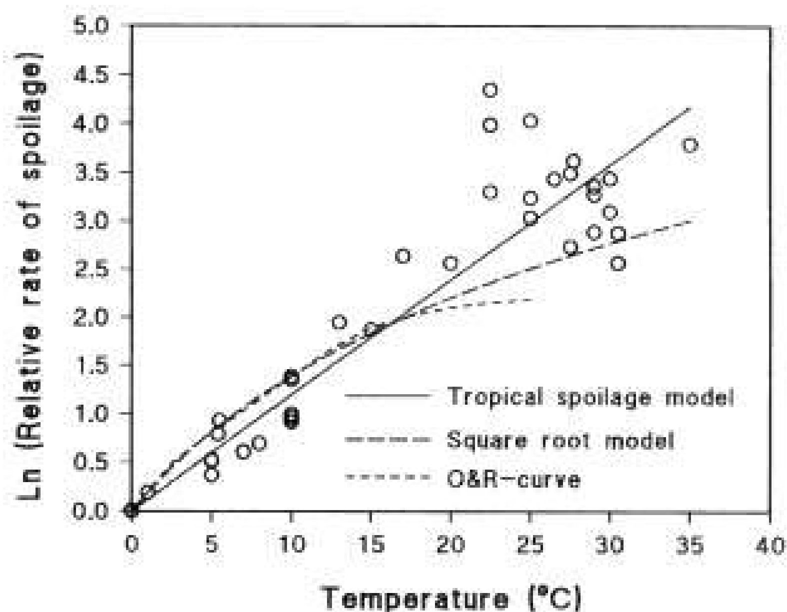

Figure 6.4 Natural logarithm of the relative rateof spoilage of tropical fish species plotted against storage temperatures (Dalgaard and Huss, 1994)

$$\textit{Shelf life} = \frac{\textit{final} - \textit{initial level of a quality indicator}}{\textit{rate of spoilage at the actual storage conditions}} \qquad 6.e$$

Much later, the demerit point system, also known as the quality index method, was developed and has proved most useful for obtaining a straight line

relationship between quality scores and storage time (see section 8.1). Bremner et al. (1987) suggested that the rate of change in quality scores, determined by the demerit point system, couldbe quantitatively described at different temperatures by Equation 6.c. Gibson (1985) related microbiological conductance detection times (DT), determined with the Malthus Growth Analyzer, to shelf life of cod. At storage temperatures from 0° to 10°C the daily rate of change in DT values was well predicted by Equation 6.c, and shelf lives were predicted at different temperatures from initial and final DT values and from the temperature spoilage model.

Many aspects of fresh fish spoilage remain to be studied; e.g., the activity of the microorganisms responsible for spoilage at different storage temperatures. Despite this lack of understanding, the relative rate concept has made it possible to quantify and mathematically describe the effect of temperature on the rate of spoilage of various types of fish products. These temperature spoilage models allow time/temperature function integration to be used for evaluation of production, distribution and storage conditions, and when combined with methods for determination of initial product quality, shelf life of various fish products can be predicted.

Apart from the actual storage temperature, the delay before chilling is of great importance. Thus, it can be observed that if white-fleshed, lean fish enter rigor mortis at temperatures above + 17°C, the muscle tissue may be ruptured through severe muscle contractions and weakening of the connective tissue (Love, 1973). The flakes in the fillets separate from each other and this "gaping" ruins the appearance. The fish also become difficult to fillet (Table 6.3) and the water- binding capacity decreases.

Table 6.3 Fillet yield of gutted cod (Hansen, 1981)

	Percentage fillet yield	
	Iced 1 hour after catch	**Iced 6 ½ hour after catch**
Yield of fillets	48.4	46.5
Yield after trimming	43.3	40.4

Rapid chilling is also crucial for the quality of fatty fish. Several experiments have shown that herring and garfish *(Belone belone)* have a significantly reduced

storage life if they are exposed to sun and wind for 4-6 hours before chilling. The reason for the observed rapid quality loss is oxidation of the lipids, resulting in rancid off-flavours. It should be noted, however, that high temperatures are only partly responsible for the speed of the oxidation processes. Direct sunlight combined with wind may have been more important in this experiment as it is difficult to stop autocatalytic oxidation processes once they have been initiated (see section 5.1).

Superchilling (0°C to -4°C)

Storage of fish at temperatures between 0°C and -4°C is called superchilling or partial freezing. The shelf life of various fish and shellfish can be extended by storage at subzero temperatures. The square root spoilage model (Equation 6.c) gives a reasonable description of RRS of superchilled products (Figure 6.5). The shelf life predicted by the square root model at -1°C, -2°C and -3°C for a product that keeps 14 days in ice is 17, 22 and 29 days, respectively.

Superchilling extends the shelf life of fish products. The technique can be used, for example where productive fishing grounds are so far from ports and consumers that normal icing is insufficient for good quality products to be landed and sold. The application of superchilling to replace transport of live fish has also been studied in Japan (Aleman *et al.,* 1982).

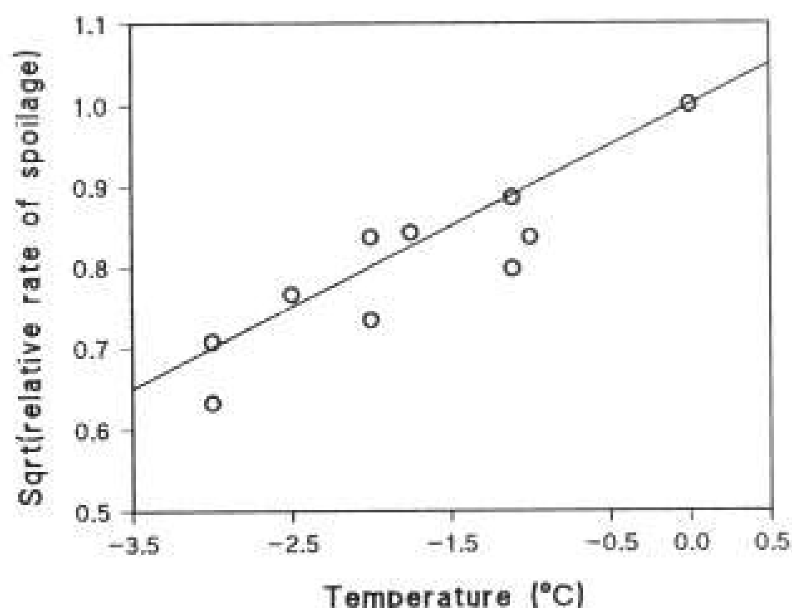

Figure 6.5 Square root plot of the relative rate of spoilage of superchilled cod, shrimp and mullet. The solid line shows relative rates of spoilage predicted by Equation 6.c (Dalgaard and Huss, 1994)

The technology needed to use superchilling at sea as well as for storage on-land

is available today. The "Frigido-system", developed in Portugal in the 1960s, uses heat exchanges in the fish holds. Sub- zero temperatures were kept constant (±0.5°C) and the fish:ice ratio was reduced from the normal 1:1 to 3:1. Sub-zero storage temperatures in fishing vessels can also be obtained in refrigerated sea water (RSW) where the freezing point of water is reduced by NaCl or other freezing point depressors. Compared to ice storage, the RSW systems chill fish more rapidly, reduce the exposure to oxygen, reduce the pressures that often occur when fish are iced and also give significant labour-saving (Nelson and Barnett, 1973). Promising results have been obtained with superchilling, but both technical problems and problems in relation to product quality have been observed. Unloading of fish is difficult when heat exchanges are used in fishing vessels and RSW increases the corrosion of the vessels (Partmann, 1965; Barnett *et* al., 1971). Also, superchilling extends product shelf life, but a negative effect on freshness/prime quality has been observed for some fish species. Merritt (1965) found that cod stored at -2°C for 10 days had an appearance and texture inferior to fish stored at 0°C in ice. The drip of the superchilled fish was increased and at -3°C the texture of whole cod made them unsuitable for filleting. RSW storage gives several fish species a salty taste due to the take-up of sea water (Barnett *et al.,* 1971; Shaw and Botta, 1975; Reppond and Collins, 1983; Reppond *et al.,* 1985). This negative effect of RSW, however, has not been found in all studies (Lemon and Regier, 1977; Olsen *et al.,* 1993). As opposed to cod and several other fish species, the prime quality of superchilled shrimp from Pakistan was increased from 8 days in ice to 16 days in NaCl-ice at -3°C (Fatima *et al.,* 1988). Also, both freshness (measured by a K-value of 20%) and shelf life of cultured carp *(Cyrinus carpio),* cultured rainbow trout *(Salmo gairdnerii)* and mackerel *(Scomber japonicus)* have been improved by superchilling at -3°C as compared to storage at 0°C (Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

The percentage of frozen water in superchilled fish is highly temperature-dependent (-1°C = 19%; -2°C = 55%; -3°C = 70%; -4°C = 76%) (Ronsivalli and Baker, 1981). It has been suggested that negative effects of superchilling on drip loss, appearance, and texture of cod and haddock are due to formation of large ice crystals, protein denaturation and increased enzymatic activity in the partially frozen fish (Love and Elerian, 1964). Simpson and Haard (1987), however, found only very little difference in biochemical and chemical deterioration of cod *(Gadus morhua)* stored at 0°C and at - 3°C In Japanese studies with seabass, carp, rainbow trout and mackerel, it has been shown that the drip loss as well as several biochemical and chemical deteriorative reactions were reduced in superchilled fish, compared to ice storage (Uchiyama and Kato, 1974; Kato *et al.,* 1974; Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

Superchilling has been used industrially with a few fish species such as tuna and salmon. The negative effects on sensory quality found for some other species

may have limited the practical application of the technique. Nevertheless, it seems that shelf life of at least some seafood products is improved considerably by superchilling. Consequently, for selected products, superchilling may well be more suitable than other technologies.

6.2 The effect of hygiene during handling

Onboard handling

Much emphasis has been placed on hygienic handling of the fish from the moment of catching in order to ensure good quality and long storage life. The importance of hygiene during handling onboard has been tested in a series of experiments where various hygienic measures were employed (Huss *et al.,* 1974). The quality and storage life of completely aseptically treated fish (aseptic handling) were compared with fish iced in clean plastic boxes with clean ice (clean handling) and with fish treated badly, i.e., iced in old, dirty wooden boxes (normal handling). As expected, a considerable difference is found in the bacterial contamination of the three batches (Figure 6.6). However, a similar difference in the organoleptic quality is not detected. During the first week of storage no difference whatsoever is found. Only during the second week does the initial contamination level become important and the heavily contaminated fish have a reduction in storage life of a few days compared with the other samples. These results are not surprising if it is kept in mind that bacterial activity is normally only important in the later stages of the storage period as illustrated in Figure 5.1.

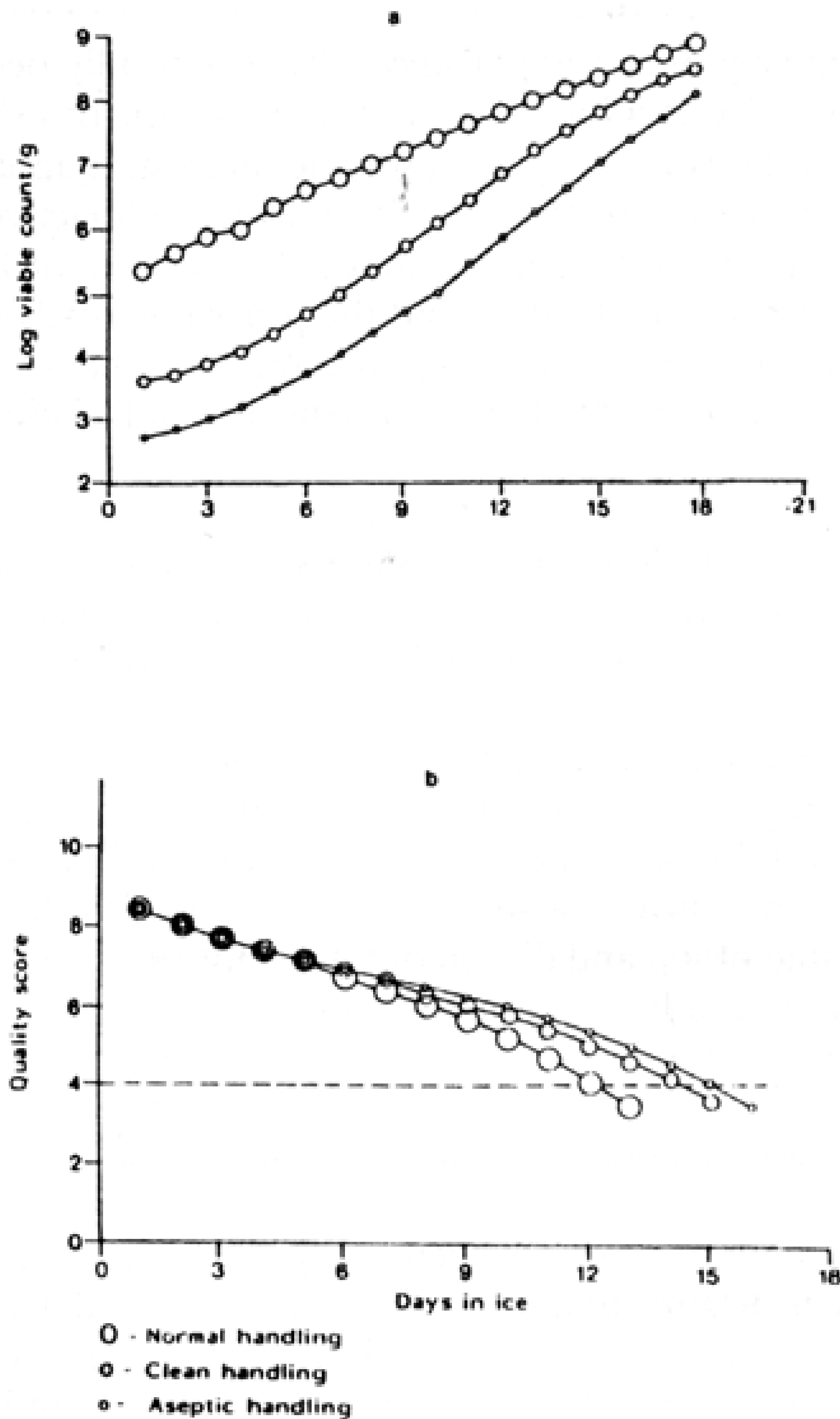

Figure 6.6 Bacterial growth (a) and organoleptic quality (b) of plaice stored at 0° C with initial high, medium and low bacterial counts (after Huss *et al.,* 1974)

On the basis of these data it seems sensible to advocate reasonably hygienic handling procedures including use of clean fish boxes. Very strict hygienic measures do not seem to have great importance. In comparison with the impact of quick and effective chilling, the importance of hygiene is minor.

The above-named observations have influenced the discussion about the design of fish boxes. Normally, fish are iced in boxes stacked on top of each other. In this connection it has been argued that fish boxes should have a construction that prevents the ice melt-water from one box draining into the box underneath it. In a system like this, some bacterial contamination of fish in the bottom boxes would be avoided, as melt-water usually contains a large number of bacteria. However, practical experience as well as experiments (Peters *et al.,* 1974) have shown that this type of contamination is unimportant, and it may be concluded that fish boxes allowing the drainage of melt-water from upper into lower boxes are advantageous because the chilling becomes more effective.

Inhibition or reduction of the naturally occurring microflora

In spite of the relatively minor importance of the naturally occurring microflora in the quality of the fish, much effort has been put into reduction or inhibition of this microflora. Many of these methods are only of academic interest. Among these are (at least until now) attempts to prolong the storage life by using radioactive irradiation. Doses of 100 000 - 200 000 rad are sufficient to reduce the number of bacteria and prolong storage life (Hansen, 1968; Connell, 1975), but the process is costly and, to many people, unacceptable in connection with human food. Another method which has been rejected because of concern about public health is treatment with antibiotics incorporated in the ice.

A method that has been used with some success over recent years is treatment with CO_2, which can be applied either in containers with chilled seawater or as part of a modified atmosphere during distribution or in retail packages (see section 6.3).

It should also be mentioned that washing with chlorinated water has been tried as a means of decontaminating fish. However, the amount of chlorine necessary to prolong the storage life creates off- flavours in the fish meat (Huss, 1971). The newly-caught fish should be washed in clean seawater without any additives. The purpose of the washing is mainly to remove visible blood and dirt, and it does not cause any significant reduction in the number of bacteria and has no effect on storage life.

6.3 The effect of anaerobic conditions and carbon dioxide

High CO_2 concentrations can reduce microbial growth and may therefore extend the shelf life of food products, where spoilage is caused by microbial activity (Killeffer, 1930; Coyne, 1933). Technological aspects of modified atmosphere packaging (MAP) have since been studied. Today, materials and techniques for

storage of bulk or retail packed foods are available.

This section discusses the effect of anaerobic conditions and modified atmospheres on the shelf life of fish products. The safety aspects are reviewed in Farber (1991) and Reddy et al. (1992).

Effect on microbial spoilage

Vacuum packaging (VP) and MAP, with high CO_2 levels (25% - 100%), extends the shelf life of meat products by several weeks or months (Table 6.4). In contrast, the shelf life of fresh fish is not affected by VP and only a small increase in shelf life can be obtained by MAP (Table 6.4).

Table 6.4 Effect of packaging on the shelf life of chilled fish and meat products

Type of product	Storage temp.	Shelf life (weeks)		
		Air	VP[a]	MAP[b]
Meat (beef, pork, poultry)	1.0 - 4.4°C	1 - 3	1 - 12	3 - 21
Lean fish (cod, pollock, rockfish, trevally)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Fatty fish (herring, salmon, trout)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Shellfish (crabs, scampi, scallops)	0.0 - 4.0°C	½ - 2	-	½ - 3
Warmwater fish (sheepshead, swordfish, tilapia)	2.0 - 4.0°C	½ - 2	-	2 - 4

a) VP: Vacuum packed
b) MAP: Modified atmosphere packed (High CO_2 concentrations (25 - 100%)

Differences in spoilage microflora. and in pH are mainly responsible for the observed differences in the shelf life of fish and meat products. Spoilage of meat under aerobic conditions is caused by strict aerobic Gram-negative organisms, primarily *Pseudomonas spp.* These organisms are strongly inhibited by

anaerobic conditions and by CO_2. Consequently, they do not play any role in the spoilage of packed meat. Instead the microflora, of VP and MAP meat products changes to be dominated by Gram-positive organisms (Lactic Acid Bacteria), which are much more resistant to CO_2 (Molin, 1983; Dainty and Mackey, 1992). Fish stored under aerobic conditions are also spoiled by Gram negative-organisms, primarily *Shewanella putrefaciens* (see section 5.3).

The spoilage flora on some packed fish products was found to be dominated by Grampositive microorganisms and in this way the microflora, was similar to the flora on packed meats; see Stammen *et al.* (1990) for a review. For packed cod, however, the Gram-negative organism *Photobacterium phosphoreum* has been identified as the organism responsible for spoilage. The growth rate of this organism is increased under anaerobic conditions (Figure 6.7) and this may explain the importance of the organism in VP cod.

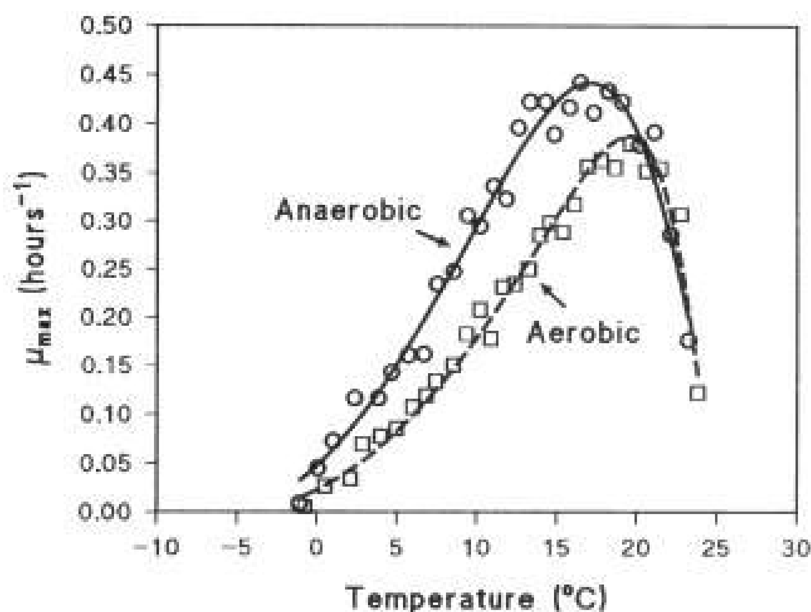

Figure 6.7 Effect of oxygen and temperature on the maximum specific growth rate (max of *Photobacterium phosphoreum* grown in a complex medium containing TMAO (Dalgaard, 1993)

In CO_2-packed fish, the growth of *Shewanella putrefaciens* and of many other microorganisms found on live fish is strongly inhibited. In contrast P. *phosphoreum* was shown to be highly resistent to CO_2 (Figure 6.8). It was also shown that the limited effect of CO_2 on growth of this bacteria correspond very well with the limited effect of CO_2 on the shelf life of packed fresh cod. P. *phosphoreum* reduces TMAO to TMA while very little H_2S is produced during growth in fish substrates. Spoiled VP and MAP cod is characterized by high levels of TMA, but little or no development of the putrid or H_2S odours typical for

some aerobically stored spoiled fish. The growth characteristics of P. *phosphoreum* and the metabolic activity of the organism thus explain both the short shelf life and the spoilage pattern of packed cod (Dalgaard, 1994 a).

The shelf life of VP and MAP cod is similar to various other sea food products (Table 6.4). P. *phosphoreum* is widespread in the marine environment and it seems likely that this organism or other highly CO_2 resistent microorganisms are responsible for spoilage of packed sea food products (Baumann and Baumann, 1981; van Spreekens, 1974; Dalgaard *et al.,* 1993).

The best effect of MAP storage on shelf life has been obtained with fish from warm waters. The shelf life of these products, however, is still relatively short compared to meat products (Table 6.4).

Very low bacterial level (10^5-10^6 cfu/g) has been found at the time of sensory rejection of some packed fish products. In these cases non-microbial reactions may have been responsible for spoilage.

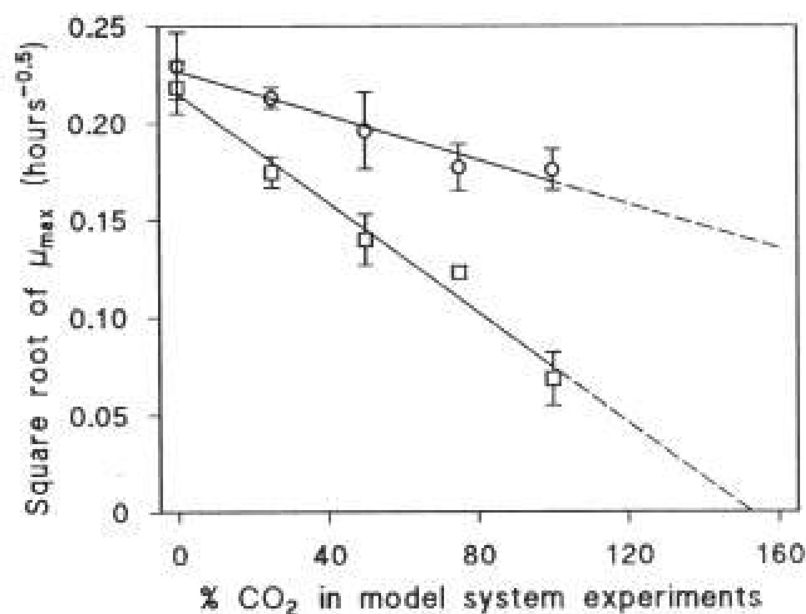

Figure 6.8 Effect of CO_2 on the maximum specific growth rate (μmax) of *Photobacterium phosphoreum* (circles) and of *Shewanella putrefaciens* (squares). Experiments were carried out at 0°C (Dalgaard, 1994 b)

Effect of non-microbial spoilage reactions

CO_2 is dissolved in the water phase of the flesh of MAP fish and a decrease in pH of about 0.2- 0.3 units is observed, depending on the CO_2 concentration in the surrounding gaseous atmosphere. The water-holding capacity of muscle

proteins is decreased by decreased pH and an increased drip loss is expected for fish stored in high CO_2 concentrations. Increased drip has been found for cod fillets, red hake, salmon, and shrimps (Fey and Regenstein, 1982; Layrisse and Matches, 1984; Dalgaard *et al.,* 1993) but not for herring, red snapper, trevally, Dungeness crab, and rockfish (Cann *et al.,* 1983; Gerdes *et al.* 1991; Parking and Brown, 1983 and Parkin *et al.,* 1981).

Coyne (1933) and many later studies have found the textural quality of fish stored in 100% CO_2 to be reduced. However, up to 60% CO_2 has no negative effect on the texture of cod. The colour of the belly flaps, of cornea, and of the skin may be altered for whole fish stored in high CO_2 concentrations (Haard, 1992). Packaging may also stimulate the formation of metmyoglobulin in red-fleshed fish and thereby result in a darkening of fish muscles. Although oxygen-containing modified atmospheres have been used, the development of rancid off-odours in fatty fish species has not been registered as a problem (Haard, 1992).

Carbon dioxide used in combination with refrigerated seawater systems

Storage of fish in refrigerated seawater (RSW) was discussed in section 6. 1. Only the effect of addition of CO_2 to RSW will be considered in this section. Table 6.5 shows the effect of RSW and RSW + CO_2 on the shelf life of various fish products, as compared to storage in ice.

Table 6.5 Shelf life of various fish products stored in Refrigerated Seawater (RSW) and in RSW with added CO_2

Type of product	Storage temp. in RSW	Shelf life (days)			References
		Ice (0° C)	RSW	RSW +CO_2	
Pacific cod	-1.1°C	6-9	-	9-12	Reppond and Collins (1983)
Pink shrimp	-1.1°C	-	4-5	6	Barnett et al. (1978)
Herring	-1.0°C	-	8-8.5	10	Hansen et al. (1970)
Walleye Pollock	-1.0°C	6-8	4-6	6-8	Reppond et al. (1979, 1985)

Rockfish	-0.6°C	-	7-10	17	Barnett et al. (1971)
Chum Salmon	-0.6°C	-	7-11	18	Barnett et al. (1971)
Silver Hake	0-1°C	4-5	4-5	5	Hiltz et al. (1976)
Capelin	+0.2 - -1.5°C	6	2	2	Shaw and Botta (1975)

An evident shelf life-extending effect of CO_2 is only seen with some species. Several negative effects of adding CO_2 to RSW-systems have been observed. The fish colour and texture were negatively influenced, and CO_2 dissolved in the flesh made mackerel unsuitable for canning (Longard and Regier, 1974; Lemon and Regier, 1977).

CO_2 acidifies the seawater, and a lowered pH inhibits the enzymatic reactions that otherwise lead to black spots in shrimps and prawns. The shelf life of pink shrimps can be more than doubled by storage in RSW + CO_2, where, compared to ice storage, colour, texture, flavour, and odour were improved (Nelson and Barnett, 1973). RSW+CO_2 stored prawns, however, may be unacceptably tough and have a "soft shell" appearance (Ruello, 1974).

Sea water acidified by CO_2 is highly corrosive. Therefore, inert materials are needed in RSW+CO_2 systems, e.g., for heat exchange. These materials are available, but their cost must be taken into account when the application of RSW + CO_2 systems is evaluated (Nelson and Barnett, 1973).

Future application of carbon dioxide for shelf life extension

For most MAP seafoods, the production of TMA is delayed by only a few days compared to aerobic or anaerobic storage. This indicates that fish products in general are contaminated with a highly CO_2 resistent microflora of TMAO reducing organisms. Very high CO_2 concentrations can inhibit microbial growth but high levels of CO_2 have a negative effects on other aspects of the fish quality. MAP has found little practical application with fish products as compared to meat products. The main reasons for this are probably that:

- MAP used with retail packs is an expensive technique
- the prime fish quality is not improved
- only small shelf life extensions are obtained

- MAP cannot replace good chilling or good hygienic production conditions
- toxin production of *Clostridium botulinum* is increased for bacteria growing under anaerobic conditions, and this may be of importance for the safety of packed fish (Huss *et al.,* 1980; Reddy *et al.,* 1992).

Packaging, however, can be used simply because packed products are more convenient to handle, e.g., in supermarkets. According to the EEC Council Directive of *22 July* 1991 (91/493/EEC), VP and MAP fish products are considered as fresh products. Consequently, CO_2 can be used for preservation of fresh fish products, when a shelf life extension of only a few days is found to be sufficient.

The negative effect Of CO_2 on fish colour is primarily a problem for whole fish and the negative effect of CO_2 on texture and drip loss is only observed with high CO_2 concentrations. A pronounced effect on growth of *S. putrefaciens* and on many other bacteria is obtained with even moderate CO_2 concentrations (40-80%). It is therefore likely that, in the future, MAP will be used in combination with preservation techniques that has been developed specifically to inhibit growth of CO_2 resistent TMAO reducing marine spoilage bacteria such as P. *phosphoreum.*

The effect of MAP also seems to depend on fish species and further studies are needed to determine if MAP can give interesting shelf life extensions for other fish species, e.g., those from warm waters. Finally, high CO_2 concentrations could be used for fish intended for fishmeal as the negative effects of CO_2 on colour and texture in this case are less important.

6.4 The effect of gutting

It is a common experience that the quality and storage life of many fish decrease if they have not been gutted. During feeding periods the fish contain many bacteria in the digestive system and strong digestive enzymes are produced. The latter will be able to cause a violent autolysis *post mortem,* which may give rise to strong off-flavour especially in the belly area, or even cause belly-burst. On the other hand, gutting means exposing the belly area and cut surfaces to the air thereby rendering them more susceptible to oxidation and discoloration. Thus, many factors such as the age of the fish, the species, amount of lipid, catching ground and method, etc., should be taken into consideration before deciding whether or not gutting is advantageous.

Fatty species

In most cases,small- and medium-sized fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is partly that a large number of small fish are caught at the same time and partly because of problems with discoloration and the acceleration of rancidity.

However, problems may arise with ungutted fish during periods of heavy feeding due to belly- burst. The reactions leading to belly-burst are complex and not fully understood. It is known that the strength of the connective tissue is decreased during these periods and that post mortem pH is normally lower in well-fed fish, this also weakens the connective tissue (Figure 6.9). Furthermore, it seems that the type of feed ingested may play an important role in the belly-burst phenomenon.

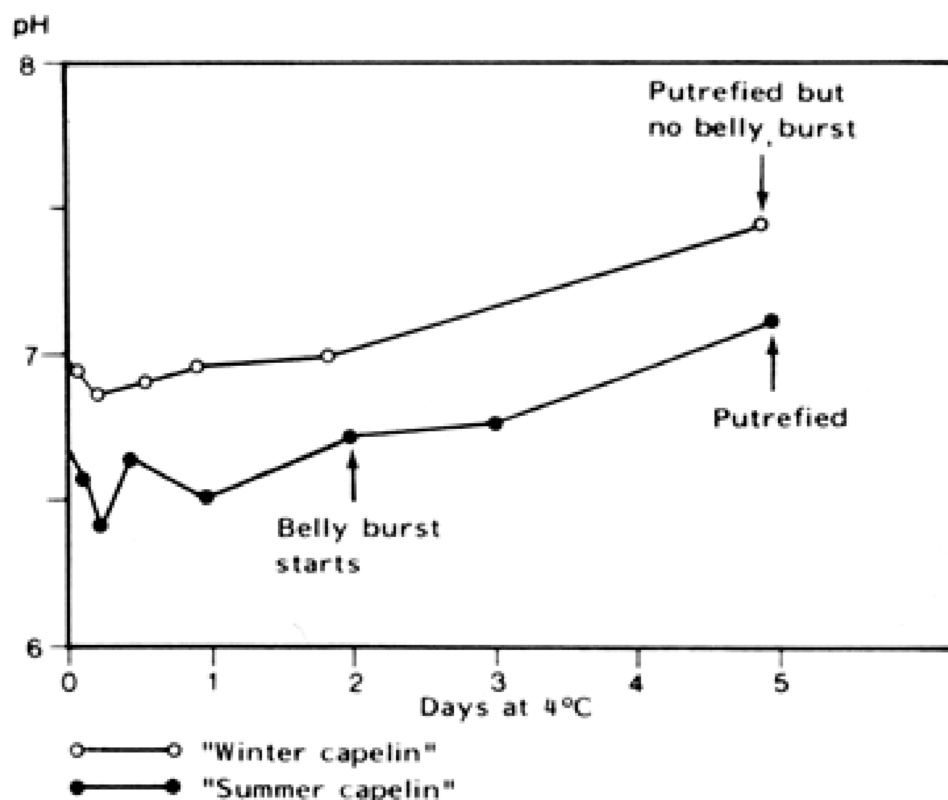

Figure 6.9 pH in winter capelin (o) and summer capelin (·) during storage at +4° C (Gildberg, 1978)

Lean species

In most North European countries, the gutting of lean species is compulsory. It is based on the assumption that the quality of these species suffers if they are not gutted. In the case of cod, it has been shown that omission causes a considerable quality loss and a reduction in the storage life of five or six days. After only two days from catch, discoloration of the belly area is visible and the

raw fillet acquires an offensive cabbagey odour. As seen in Figure 6.10, these odours are removed to some extent by boiling.

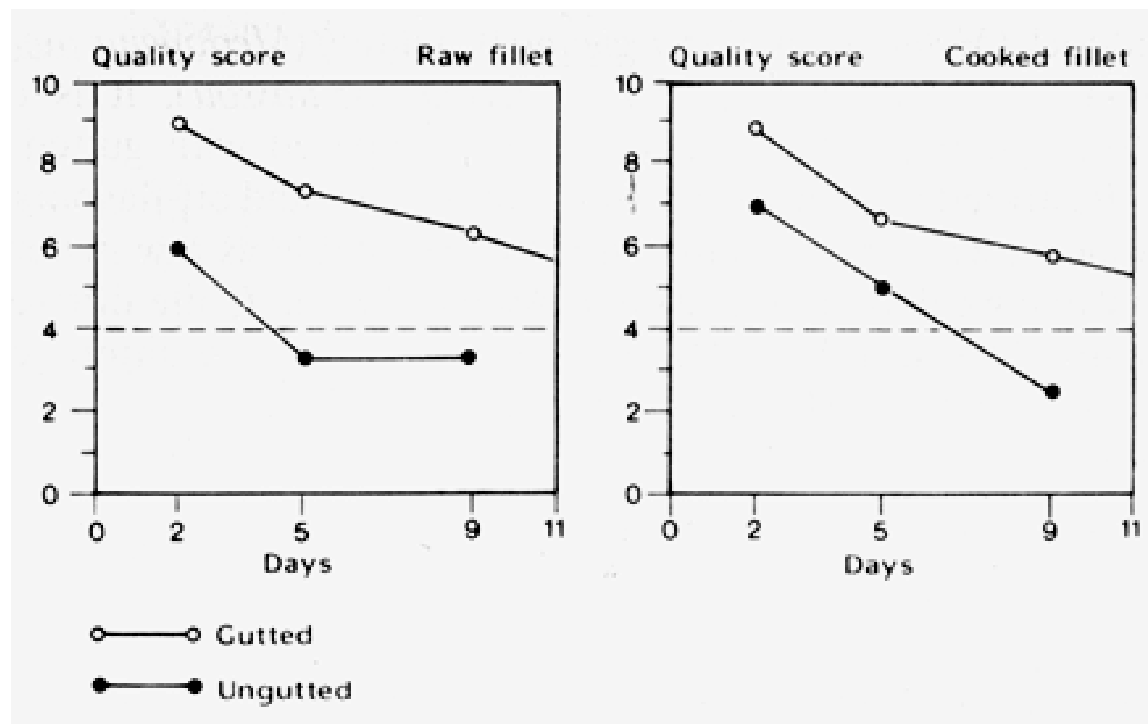

Figure 6.10 Organoleptic quality of raw and boiled fillet, respectively from gutted (o) and ungutted (·) iced cod (Huss, 1976)

These volatile, foul-smelling compounds are mostly found in the gut and surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself (Figure 6.11). These chemical parameters are, therefore, not useful for distinguishing between gutted and ungutted fish (Huss and Asenjo, 1976).

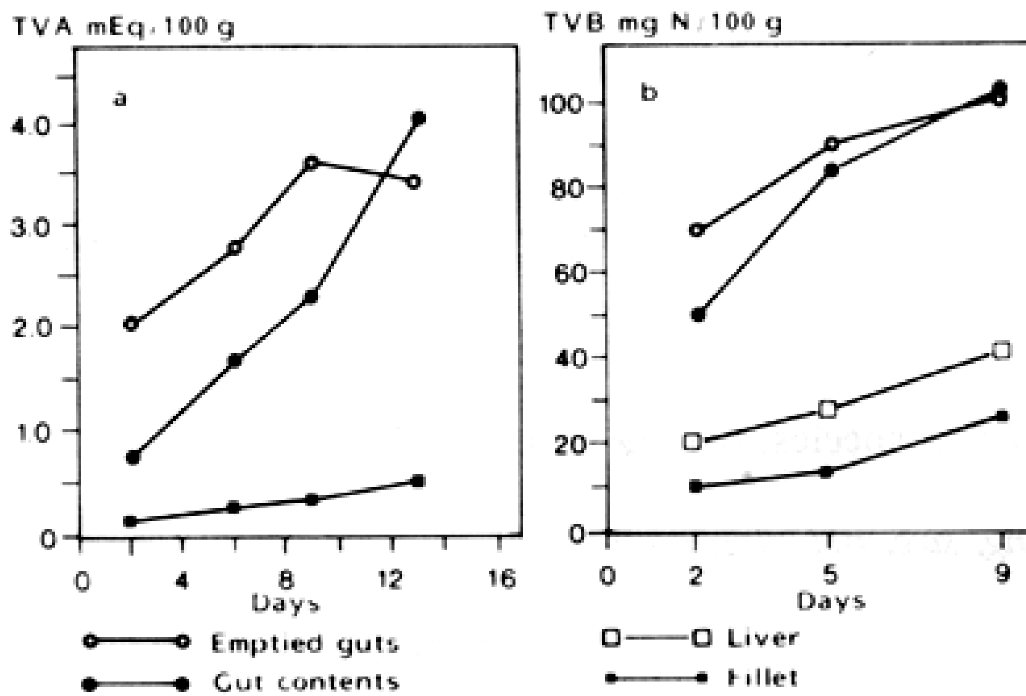

Figure 6.11 Development of (a) volatile acids in iced, ungutted saithe *(Polacchius* virens) and (b) volatile bases in iced, ungutted cod *(Gadus morhua)* *(Huss* and Asenjo, 1976)

Similar experiments with other cod-like species show a more differentiated picture. In the case of haddock *(Melanogrammus aeglefinus),* whiting *(Merlangius merlangus,* saithe *(Pollachius virens)* and blue whiting *(Micromesistius poutassou),* it is observed that ungutted fish stored at 0°C suffer a quality loss compared with gutted fish, but the degree varies as illustrated in Figure 6.12. Some off-odours and off-flavours are detected, but ungutted haddock, whiting and saithe are still acceptable as raw material for frozen fillets after nearly one week on ice (Huss and Asenjo, 1976). Quite different results are obtained with South American hake *(Merluccius gayi),* where no difference is observed between gutted and ungutted fish (Huss and Asenjo, 1977 b).

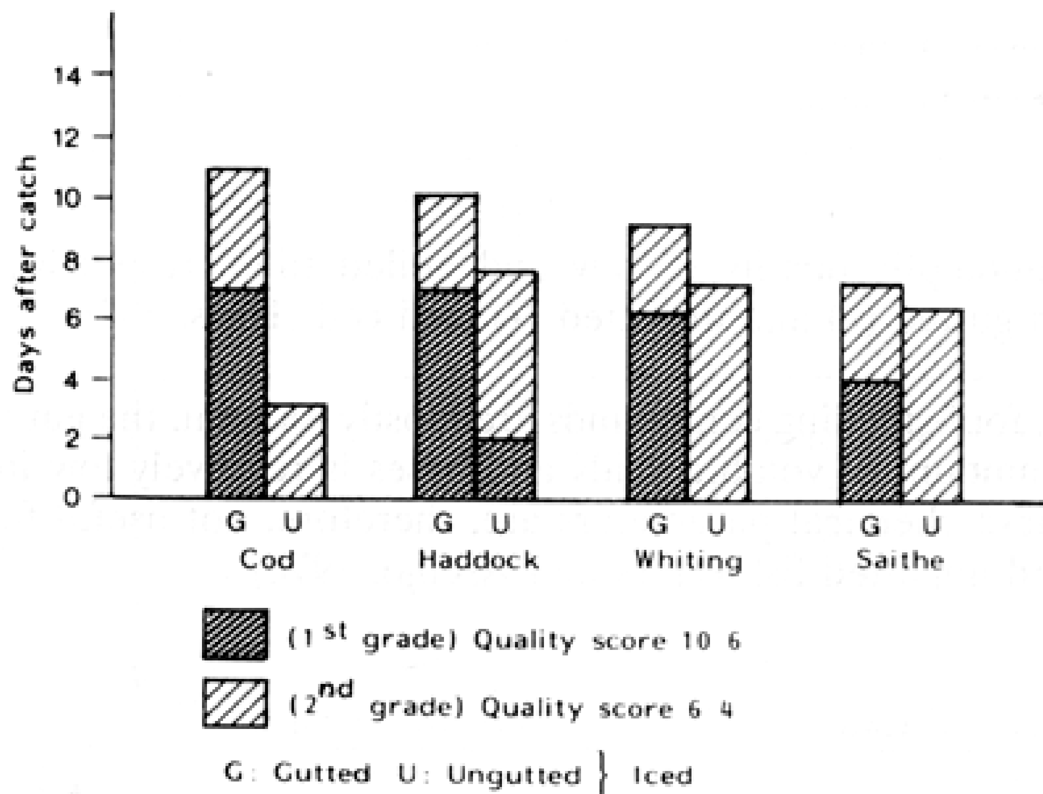

Figure 6.12 Quality and storage life of gutted and ungutted lean fish stored in ice (Huss and Asenjo, 1976)

6.5 The effect of fish species, fishing ground and season

Influence of handling, size, pH, skin properties

The spoilage rate and shelf life of fish is affected by many parameters and, as stated in section 5, fish spoil at different rates. In general it can be stated that larger fish spoil more slowly than small fish, flat fish keep better than round fish, lean fish keep longer than fatty fish under aerobic storage and bony fish are edible longer than cartilaginous fish (Table 6.6). Several factors probably contribute to these differences and whereas some are clear, many are still on the level of hypotheses.

Table 6.6 Intrinsic factors affecting spoilage rate of fish species stored in ice

Factors affecting spoilage rate	Relative spoilage rate	
	fast	slow

size	small fish	larger fish
post mortem pH	high pH	low pH
fat content	fatty species	lean species
skin properties	thin skin	thick skin

Rough handling will, as outlined in section 5.2, result in a faster spoilage rate. This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. The surface/volume ratio of larger fish is lower than that of smaller fish, and, as bacteria are found on the outside, this is probably the reason for the longer shelf life of the former. This is true within a species but may not be universally so.

Post mortem pH varies between species but is, as described in section 5.2, higher than in warm- blooded animals. The long rigor period and the corresponding low pH (5.4-5.6) of the very large flatfish, halibut (*Hippoglossus hipoglossus*), has been offered as an explanation for its relatively long iced storage life (Table 6.7). However, mackerel will often also experience a low pH and this seems to have little effect on shelf life. As can be seen from Table 6.7, fatty fish are in general rejected sensorically long before lean fish. This is mainly due to the appearance of oxidative rancidity.

The skin of the fatty pelagic fish is often very thin, and this may contribute to the faster spoilage rate. This allows enzymes and bacteria to penetrate more quickly. On the contrary, the thick skin of flatfish and the antibacterial compounds found in the slime of these fish may also contribute to the keepability of flatfish. As described earlier, the slime of flat fish contains bacteriolytic enzymes, antibodies and various other antibacterial substances (Hjelmland et al., 1983; Murray and Fletcher, 1976). Although large differences exist in the content of TMAO, this does not seem to affect the shelf life of aerobically-stored fish but rather the chemical spoilage profile of the species.

Table 6.7 Shelf life of various fish species from temperate and tropical waters. Prepared from data published by Lima dos Santos (1981); Poulter *et al.*(1981); and Gram (1989)

Species	Fish type	Shelf life (days in ice)	
		temperate	tropical
Marine species		2-24	6-35
cod, haddock	lean	9-15	
whiting	lean	7-9	
hake	lean	7-15	
bream	lean/low fat		10-31
croaker	lean		8-22
snapper	lean		10-28
grouper	lean		6-28
catfish	lean		16-19
pandora	lean		8-21
jobfish	lean		16-35
spadefish	lean/low fat		21-26
batfish	lean		21-24
sole, plaice,	flat	7-21	21
flounder	flat	7-18	
halibut	flat	21-24	

mackerel[1)]	high/ low fat	4-19	14-18
summer herring	high fat	2-6	
winter herring	low fat	7-12	
sardine	high fat	3-8	9-16
Freshwater species		9-17	6-40
catfish	lean	12-13	15-27
trout	low fat	9-11	16-24
perch	lean/ low fat	8-17	13-32
tilapia	lean		10-27
mullet	lean		12-26
carp	lean/ low fat		16-21
lungfish	lean/ low fat		11-25
Haplochromis	lean		6
shad	medium fat		25
corvina	medium fat		30
bagré	medium fat		25
chincuna	fatty		40

pacu	fatty		40

1) fat content and shelf life subject to seasonal variation.

In general, the slower spoilage of some fish species has been attributed to a slower bacterial growth, and Liston (1980) stated that "different spoilage rates seem to be related at least partly to the rate of increase of bacteria on them".

Influence of water temperature on iced shelf life

Of all the factors affecting shelf life, most interest has focused on the possible difference in iced shelf life between fish caught in warm, tropical waters and fish caught in cold, temperate waters. In the mid- and late sixties it was reported that some tropical fish kept 20-30 days when stored in ice (Disney *et al.,* 1969). This is far longer than for most temperate species and several studies have been conducted assessing the shelf life of tropical species. Comparison of the data is, as pointed out by Lima dos Santos (1981), difficult as no clear definition has been given on a "tropical" fish species and as experiments have been carried out using different sensory and bacteriological analyses.

Several authors have concluded that fish taken from warm waters keep better than fish from temperate waters (Curran and Disney, *1979;* Shewan, 1977) whereas Lima dos Santos (1981) concluded that also some temperate water fish species keep extremely well and that the longer shelf lives in general are found in fresh water fish species compared to marine species. However, he also noted that shelf life of more than 3 weeks, which is often observed for fish caught in tropical waters (Table 6.7),never occurs when fish from temperate waters are stored in ice. The iced shelf life of marine fish from temperate waters varies from 2 to 21 days which does not differ significantly from the shelf life of temperate freshwater fish ranging from *9* to 20 days. Contrary to this, fish caught in tropical marine waters keep for 12-35 days when stored in ice and tropical freshwater fish from *6* to 40 days. Although very wide variations occur, tropical fish species often have prolonged shelf lives when stored in ice as shown in Table 6.6. When comparisons are made, data on fatty fish like herring and mackerel should probably be omitted as spoilage is mainly due to oxidation.

Several hypotheses have been launched trying to explain the often prolonged iced spoilage of tropical fish. Some authors have noted an absence in development of TMA and TVN during storage and suggested that the spoilage of tropical fish is not caused by bacteria (Nair *et al.,* 1971). The lack of development of TMA and TVN may be explained by a spoilage dominated by *Pseudomonas* spp.; however, qualitative bacteriological analyses must be carried out to confirm

or reject this suggestion. Low bacterial counts have been claimed in some studies, but often inappropriate media have been used for the examination and too high incubation temperatures (30°C) have not allowed the psychrotrophic spoilage bacteria to grow on the agar plates.

Reviewing the existing literature on storage trials of tropical fish species leads to the conclusion that the overall sensory, chemical and bacteriological changes occurring during spoilage of tropical fish species are similar to those described for temperate species.

Psychrotrophic bacteria belonging to *Pseudomonas spp*. and *Shewanella putrefaciens* dominate the spoilage flora of iced stored fish. Differences exist, as described in section 5.3, in the spoilage profile depending on the dominating bacterial species. *Shewanella* spoilage is characterized by TMA and sulphides (H_2S) whereas the *Pseudomonas* spoilage is characterized by absence of these compounds and occurrence of sweet, rotten sulphydryl odours. As this is not typical of temperate, marine fish species which have been widely studied, this may explain the hypothesis that bacteria are not involved in the spoilage process of tropical fish.

Despite the different odour profiles, the level at which the offensive off-odours are detected sensorially is more or less the same. In model systems (sterile fish juice) 10^8-10^9 cfu/ml of both types of bacteria is the level at which spoilage is evident.

As outlined in section 5.3, the relatively high post*mortem* pH is one of the reasons for the relatively short shelf life of fresh fish as compared to, for instance, chill stored beef. It has been suggested that tropical fish species, such as the halibut from temperate waters, reach a very low pH, and that this explains the longer shelf life. However, pH values of 6-7 have been found in the studies of tropical fish species where pH has been measured (Gram, 1989). As the differences in skin properties are believed to contribute to the longer shelf life of flatfish, it has been suggested that this factor explained the extended shelf lives. It is indeed true that fish from warm waters often have very thick skin, but no systematic investigation has been carried out on the skin properties.

As spoilage of fish is caused by bacterial action, most hypotheses dealing with the long iced shelf life of tropical fish species have centred around differences in bacterial flora. Shewan (1977) attributed the long iced shelf lives to the lower number of psychrotrophs on tropical fish. However, in 1977 only a very limited number of studies of the bacterial flora on tropical fish were published. During the last 10- 15 years several investigations have concluded that Gram-negative rod-shaped bacteria (e.g., *Pseudomonas, Moraxella* and *Acinetobacter)* dominate on many fish caught in tropical waters (Gram, 1989; Surendram *et al.,* 1989; Acuff *et*

al., 1984). Similarly, Sieburth (1967) concluded that the composition of the bacterial flora in Narragansett Bay did not change during a 2-year survey even though the water temperature fluctuated with 23°C on a year-round basis. Gram (1989) showed that 40-90% of the bacteria found on Nile perch were able to grow at 7°C. The number of psychrotrophic bacteria is within one log unit of the total count, and the level of psychrotrophic organisms is not *per se low* enough to account for the extended iced storage lives of tropical fish; Jorgensen *et al.* (1989) showed that a two log difference in number of spoilage bacteria only resulted in a difference of 3 days in the shelf life of iced cod.

As described in section 5, the bacterial flora on temperate water fish species resume growth immediately after the fish have been caught and rarely is a lag phase seen. Contrary to this, Gram (1989) concluded that a bacterial lag phase of 1-2 weeks is seen when tropical fish are stored in ice. Also, the subsequent growth of psychrotrophic bacteria is often slower on iced tropical than on iced temperate water fish. This is in agreement with Liston (1980) who attributed differences in shelf life to differences in bacterial growth rates. Although a large part of the bacteria on tropical fish are capable of growth at chill temperatures, they will (as this has never been necessary) require a period of adaptation (i.e., the lag phase and slow growth phase). Gram (1989) illustrated this by investigating the growth rate at 0°C of fish spoilage bacteria that had either been pre-cultured at 20°C or at 5°C. For some strains, the same bacterial strain would grow more quickly at 0°C if pre-cultured at 5°C than if pre-cultured at 20°C (Table 6.8). Preculturing was done with several sub-culture steps at each temperature. Similarly, Sieburth (1967) showed that although the taxonomic composition of the bacterial flora in Narrangansett Bay did not change with fluctuating temperature, the growth profile of the bacteria fluctuated following the water temperature. However, the adaptation hypothesis does not explain why some tropical fish spoil at rates comparable to temperate water fish.

Table 6.8 Generation times at 0°C for fish spoilage bacteria pre-cultured at high (20°C) or low (5°C) temperatures

Species	**Origin**	**Pre-culture temperature (°C)**	**Subsequent generation time (hours) at 0°C**
Aeromonas spp.	spoiled chilled trout	5 20	11 20
Pseudomonas spp.	iced cod (Denmark)	5 20	9 14

	spoiled iced sardine (Senegal)	5 20	12 14
Shewanella spp.	iced cod (Denmark)	5 20	8 17
	iced sole (Senegal)	5 20	9 17

It can be concluded that many factors affect shelf life of fish and that differences in the physiology of the bacterial flora are likely to be of major importance.

Off flavours related to fishing ground

Occasionally fish with off-flavours are caught, and in certain localities this is a fairly common phenomenon. Several of these off-flavours can be attributed to their feeding on different compounds or organisms. The planktonic mollusc, *Spiratella helicina,* gives rise to an off-flavour described as "mineral oil" or "petrol". It is caused by dimthyl-B-propiothetin which is converted to dimethylsulphide in the fish (Connell, 1975). The larvae of *Mytilus* spp. cause a bitter taste in herring. A very well known off-flavour is the muddy-earthy taint in many freshwater fish. The flavour is mainly caused by two compounds: geosmin (1a, 10ß-dimethyl-9a-decalol) and 2-methylisoborneol, which also are part of the chemical profile of wine with cork flavour. Geosmin, the odour of which is detectable in concentrations of 0.01-0.1 µg/l, is produced by several bacterial taxa, notably the actinomycetes *Streptomyces* and *Actinomyces.*

An iodine-like flavour is found in some fish and shrimp species in the marine environment. This is caused by volatile bromophenolic compounds; and it has been suggested that the compounds are formed by marine algae, sponges and Bryozoa and become distributed through the food chain (Anthoni *et al.,* 1990).

Oil taint may be found in the fish flesh in areas of the world where off-shore exploitation of oil is intensive or in areas where large oil spills occur. The fraction of the crude oil that is soluble in water is responsible for the off-flavours. This is caused by the accumulation of various hydrocarbon compounds, where particularly the aromatic compounds are strong flavourants (Martinsen *et al.,* 1992).

Figure 6.13 The situation on a South American hake trawler. The fishermen have spent considerable time and effort gutting the fish, where rapid chilling of whole, ungutted fish would have been more beneficial to quality

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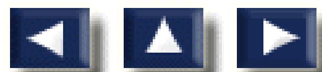

6. QUALITY CHANGES AND SHELF LIFE OF CHILLED FISH

6.1. The effect of storage temperature
6.2. The effect of hygiene during handling
6.3. The effect of anaerobic conditions and carbon dioxide
6.4. The effect of gutting
6.5. The effect of fish species, fishing ground and season

6.1 The effect of storage temperature

Chill storage (0-25°C)

It is well known that both enzymatic and microbiological activity are greatly influenced by temperature. However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity (Figure 6.1).

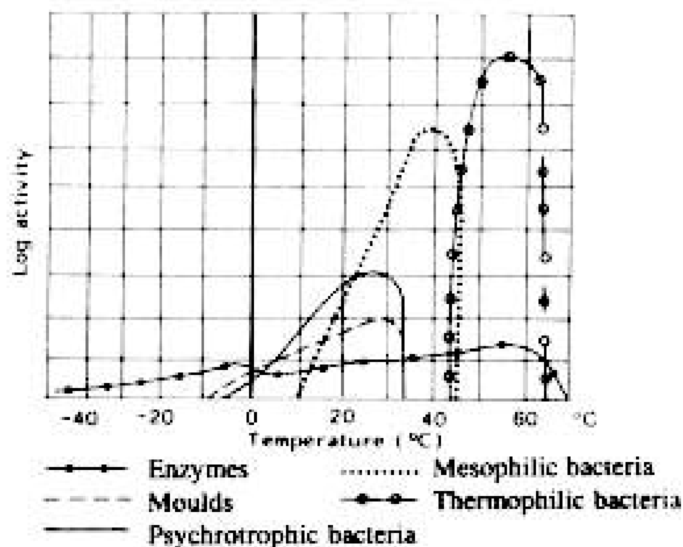

Figure 6.1 Relative enzyme activity and growth rate of bacteria in relation to temperature (Andersen et *al.,* 1965)

Many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0°C Figure 6.2 shows the effect of temperature on the growth rate of the fish spoilage bacterium *Shewanella Putrefaciens.* At 0°C the growth rate is less than one-tenth of the rate at the optimum growth temperature.

Microbial activity is responsible for spoilage of most fresh fish products. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures. In industrialized countries it is common practice to store fresh fish in ice (at 0°C) and the shelf life at different storage temperatures (at t°C) has been expressed by the relative rate of spoilage (RRS), defined as shown in Equation 6.a (Nixon, 1971).

$$\text{Relative rate of spoilage at } t\,^{\circ}C = \frac{\text{keeping time at } 0\,^{\circ}C}{\text{keeping time at } t\,^{\circ}C} \qquad 6.a$$

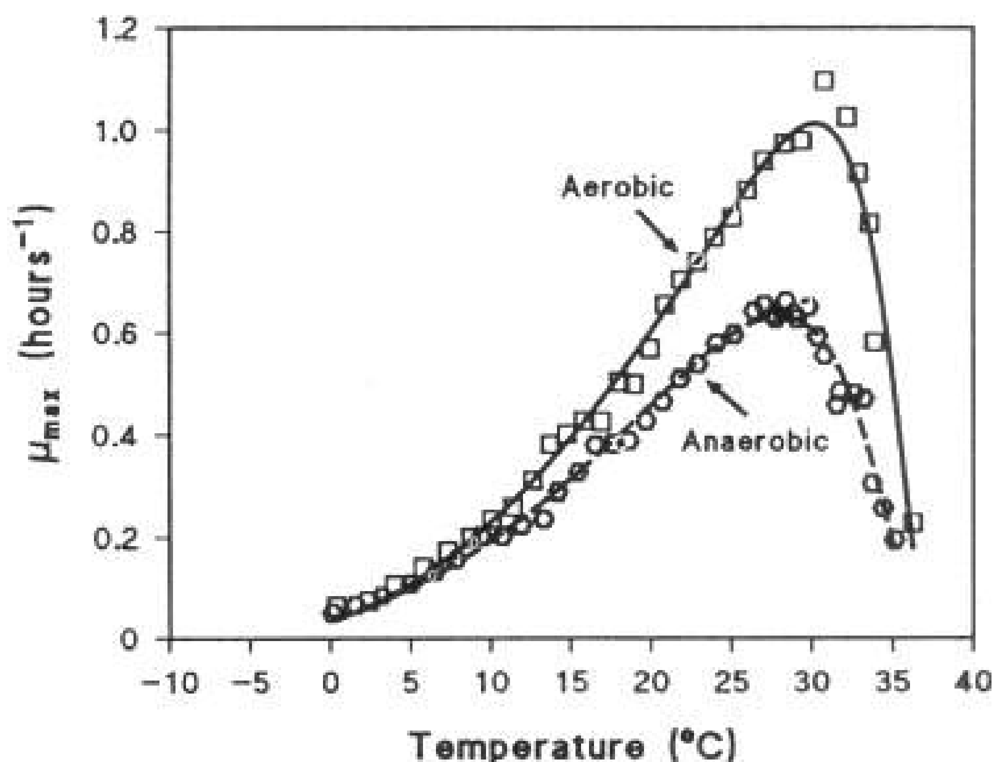

Figure 6.2 Effect of temperature on the maximum specific growth rate (μmax) of *Shewanella putrefaciens* grown in a complex medium containing TMAO (Dalgaard, 1993)

While broad differences are observed in shelf lives of the various seafood products, the effect of temperature on RRS is similar for fresh fish in general. Table 6.1 shows an example with different seafood products.

Table 6.1 Shelf lives in days and relative rates of spoilage (RRS) of seafood products stored at different temperatures

	0°C		**5°C**		**10°C**	
	shelf life	**RRS**	**shelf life**	**RRS**	**shelf life**	**RRS**
Crab claw[a]	10.1	1	5.5	1.8	2.6	3.9
Salmon[b]	11.8	1	8.0	1.5	3.0	3.9
Sea bream[c]	32.0	1	-	-	8.0	4.0

Packed cod[d)]	14	1	6.0	2.3	3.0	4.7

a) Cann et al. (1985); b) Cann et al. (1984); c) Olley and Quarmby (1981); d) Cann et al. (1983)

The relationship between shelf life and temperature has been thoroughly studied by Australian researchers (Olley and Ratkowsky, 1973 a, 1973 b). Based on data from the literature they found that the relationship between temperature and RRS could be expressed as an S-shaped general spoilage curve (Figure 6.3). Particularly at low temperatures (e.g., < 10°C this curve is similar to, and confirms the results of Spencer and Baines (1964). These authors, 10 years earlier, found a straight line relationship between RRS and the storage temperatures of cod from the North Sea (Figure 6.3).

The effect of temperature on the rate of chemical reactions is often described by the Arrhenius Equation. This Equation, however, has been shown not to be accurate when used for the effect of a wide range of temperatures, on growth of microorganisms and on spoilage of foods (Olley and Ratkowsky, 1973 b; Ratkowsky *et* al., 1982). Ratkowsky *et al.* (1982) suggested the 2-parameter square root model (Equation 6.b) for the effect of sub-optimal temperature on growth of microorganisms

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad \text{6b}$$

T is the absolute temperature (Kelvin) and T_{min} in a parameter expressing the theoretical minimum temperature of growth. The square root of the microbial growth rates plotted against the temperature form a straight line from which T_{min} is determined. Several psychrotrophic bacteria isolated from fish products have T_{min} values of about 263 Kelvin (-10°C) (Ratkowsky *et al.,* 1982; Ratkowsky *et al.,* 1983). Based on this T_{min} value, a spoilage model has been developed. It has been assumed that the relative microbial growth rate would be similar to the relative rate of spoilage. The relative rate concept (Equation 6.a) was then combined with the simple square root model (Equation 6.b) to give a temperature spoilage model (Equation 6.c). As just described, this model was derived from growth of psychrotrophic model has been shown to give good estimates of the effect of temperature on bacteria (T_{min} = -10°C) but the RRS of chilled fresh fish as shown in Figure studies (Storey, 1985; Gibson, 1985). 6.1 and also confirmed in other

$$\sqrt{\text{Relative rate of spoilage}} = \frac{b(t\,°C - (-10°C))}{b(0°C - (-10°C))} = 0.1 * t\,°C + 1 \qquad 6.c$$

If the shelf life of a fish product is known at a given temperature, the shelf life at other storage temperatures can be calculated from the spoilage models. The effect of temperature, shown in Table 6.2, as calculated from Equation 6.c for products with different shelf lives when stored at 0°C.

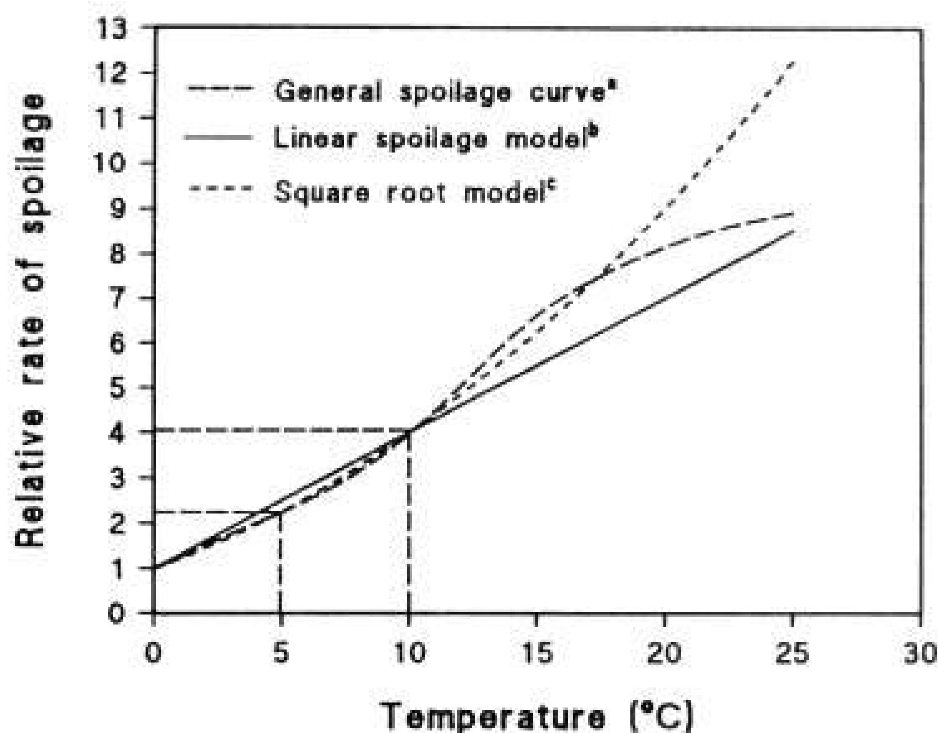

Figure 6.3 Effect of temperature on the relative rate of spoilage of fresh fish products. a) the general spoilage curve (Olley and Ratkowsky, 1973 a); b) the linear spoilage model suggested by Spencer and Baines (1964); c) the square root spoilage model derived from growth for psychrotrophic bacteria (Equation 6. c)

The effect of time/temperature storage conditions on product shelf life has been shown to be cumulative (Charm et al, 1972). This allows spoilage models to be used for prediction of the effect of variable temperatures on product keepability. An electronic time/temperature function integrator for shelf life prediction was developed, based on Equation 6.c. The instrument predicts RRS accurately, but a high price has limited its practical application (Owen and Nesbitt, 1984; Storey, 1985).

Table 6.2 Predicted shelf lives of fish products stored at different temperatures

Shelf life in days of product stored in ice (0°C)	**Shelf life at chill temperatures (days)**		
	5°C	**10°C**	**15°C**
6	2.7	1.5	1
10	4.4	2.5	1.6
14	6.2	3.5	2.2
18	8	4.5	2.9

The temperature history of a product, e.g., through a distribution system, can be determined by a temperature logger. Using a spoilage model and simple PC software, the effect of a given storage temperature profile can then be predicted. McMeekin *et al.* (1993) reviewed the literature on application of temperature loggers and on predictive temperature models. A product temperature profile also allows growth of pathogenic microorganisms to be estimated from safety models. Computers and temperature loggers are today available at reasonable prices and it is most likely that spoilage and safety models will be used frequently in the future.

The microflora responsible for spoilage of fresh fish changes with changes in storage temperature. At low temperatures (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas spp.* and *Pseudomonas s*pp. cause spoilage (Table 5.5). However, at high storage temperatures (15-30°C) different species of Vibrionaceae, Enterobacteriaceae and Gram-positive organisms are responsible for spoilage (Gram *et al.,* 1987; Gram *et al.,* 1990; Liston, 1992). Equation 6.c does not take into account the change in spoilage microflora. Nevertheless, reasonable estimates of RRS are obtained for whole fresh fish, for packed fresh fish and for superchilled fresh fish products (Figure 6.3; Gibson and Ogden, 1987; Dalgaard and Huss, 1994). For tropical fish, however, the average relative rate of spoilage of a large number of species stored at 20°-30°C was approximately 25 times higher than at 0°C The RRS of tropical fish is thus more than twice as high as estimated from the temperature models shown in Figure 6.3. Tropical fish are likely to be exposed to high temperatures and a new tropical spoilage model, covering the range of temperatures from 0°-30°C, was

recently developed (Equation 6.d; Dalgaard and Huss, 1994). Figure 6.4 shows that the natural logarithm of RRS of tropical fish is linearly related to the storage temperature. This figure also shows the differences between the new tropical model and previous spoilage models developed for fish from temperate waters.

*Ln (relative rate of spoilage for tropical fish) = 0. 12 * t °C*
6.d

Temperature models based on the relative rate concept do not take into account the initial product quality. Inaccurate shelf life predictions, therefore, may be obtained for products with variable initial quality. Spencer and Baines (1964), however, suggested that both the effect of the initial product quality and the effect of storage temperature could be predicted. At a constant storage temperature measurements of quality will change linearily from an initial to a final level reached when the product is no longer acceptable (Equation 6.e). Shelf life at a given temperature and a given initial quality is determined (Equation 6.e) and then the shelf life at other temperatures can be determined from a temperature spoilage model.

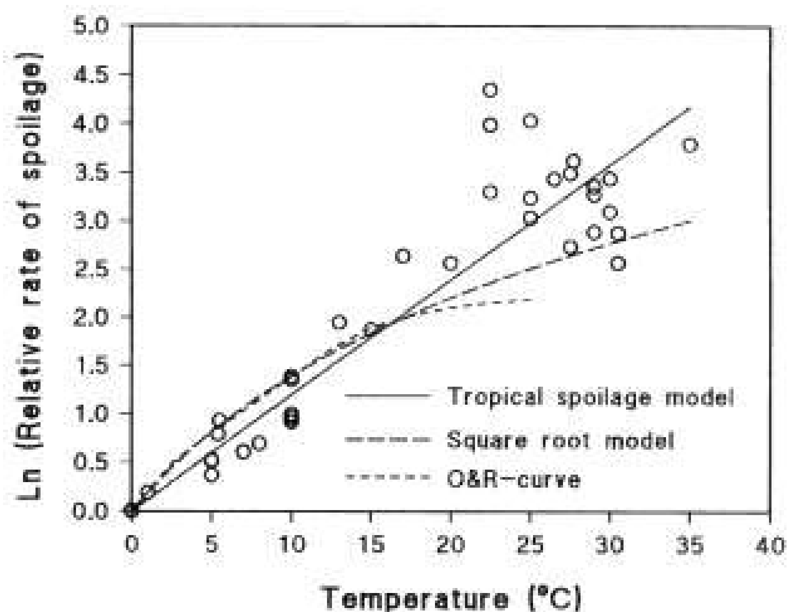

Figure 6.4 Natural logarithm of the relative rateof spoilage of tropical fish species plotted against storage temperatures (Dalgaard and Huss, 1994)

$$\text{Shelf life} = \frac{\text{final} - \text{initial level of a quality indicator}}{\text{rate of spoilage at the actual storage conditions}} \qquad 6.e$$

Much later, the demerit point system, also known as the quality index method, was developed and has proved most useful for obtaining a straight line

relationship between quality scores and storage time (see section 8.1). Bremner et al. (1987) suggested that the rate of change in quality scores, determined by the demerit point system, couldbe quantitatively described at different temperatures by Equation 6.c. Gibson (1985) related microbiological conductance detection times (DT), determined with the Malthus Growth Analyzer, to shelf life of cod. At storage temperatures from 0° to 10°C the daily rate of change in DT values was well predicted by Equation 6.c, and shelf lives were predicted at different temperatures from initial and final DT values and from the temperature spoilage model.

Many aspects of fresh fish spoilage remain to be studied; e.g., the activity of the microorganisms responsible for spoilage at different storage temperatures. Despite this lack of understanding, the relative rate concept has made it possible to quantify and mathematically describe the effect of temperature on the rate of spoilage of various types of fish products. These temperature spoilage models allow time/temperature function integration to be used for evaluation of production, distribution and storage conditions, and when combined with methods for determination of initial product quality, shelf life of various fish products can be predicted.

Apart from the actual storage temperature, the delay before chilling is of great importance. Thus, it can be observed that if white-fleshed, lean fish enter rigor mortis at temperatures above + 17°C, the muscle tissue may be ruptured through severe muscle contractions and weakening of the connective tissue (Love, 1973). The flakes in the fillets separate from each other and this "gaping" ruins the appearance. The fish also become difficult to fillet (Table 6.3) and the water- binding capacity decreases.

Table 6.3 Fillet yield of gutted cod (Hansen, 1981)

	Percentage fillet yield	
	Iced 1 hour after catch	**Iced 6 ½ hour after catch**
Yield of fillets	48.4	46.5
Yield after trimming	43.3	40.4

Rapid chilling is also crucial for the quality of fatty fish. Several experiments have shown that herring and garfish *(Belone belone)* have a significantly reduced

storage life if they are exposed to sun and wind for 4-6 hours before chilling. The reason for the observed rapid quality loss is oxidation of the lipids, resulting in rancid off-flavours. It should be noted, however, that high temperatures are only partly responsible for the speed of the oxidation processes. Direct sunlight combined with wind may have been more important in this experiment as it is difficult to stop autocatalytic oxidation processes once they have been initiated (see section 5.1).

Superchilling (0°C to -4°C)

Storage of fish at temperatures between 0°C and -4°C is called superchilling or partial freezing. The shelf life of various fish and shellfish can be extended by storage at subzero temperatures. The square root spoilage model (Equation 6.c) gives a reasonable description of RRS of superchilled products (Figure 6.5). The shelf life predicted by the square root model at -1°C, -2°C and -3°C for a product that keeps 14 days in ice is 17, 22 and 29 days, respectively.

Superchilling extends the shelf life of fish products. The technique can be used, for example where productive fishing grounds are so far from ports and consumers that normal icing is insufficient for good quality products to be landed and sold. The application of superchilling to replace transport of live fish has also been studied in Japan (Aleman *et al.,* 1982).

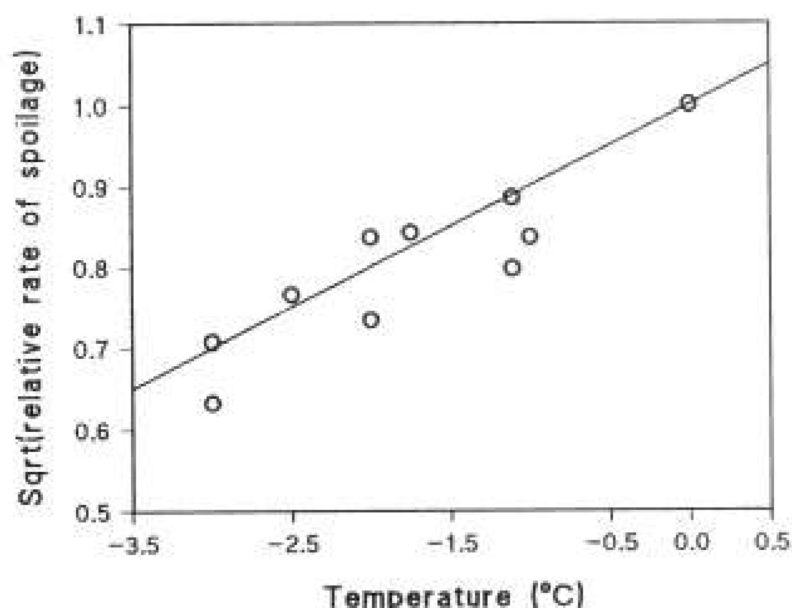

Figure 6.5 Square root plot of the relative rate of spoilage of superchilled cod, shrimp and mullet. The solid line shows relative rates of spoilage predicted by Equation 6.c (Dalgaard and Huss, 1994)

The technology needed to use superchilling at sea as well as for storage on-land

is available today. The "Frigido-system", developed in Portugal in the 1960s, uses heat exchanges in the fish holds. Sub- zero temperatures were kept constant (±0.5°C) and the fish:ice ratio was reduced from the normal 1:1 to 3:1. Sub-zero storage temperatures in fishing vessels can also be obtained in refrigerated sea water (RSW) where the freezing point of water is reduced by NaCl or other freezing point depressors. Compared to ice storage, the RSW systems chill fish more rapidly, reduce the exposure to oxygen, reduce the pressures that often occur when fish are iced and also give significant labour-saving (Nelson and Barnett, 1973). Promising results have been obtained with superchilling, but both technical problems and problems in relation to product quality have been observed. Unloading of fish is difficult when heat exchanges are used in fishing vessels and RSW increases the corrosion of the vessels (Partmann, 1965; Barnett *et* al., 1971). Also, superchilling extends product shelf life, but a negative effect on freshness/prime quality has been observed for some fish species. Merritt (1965) found that cod stored at -2°C for 10 days had an appearance and texture inferior to fish stored at 0°C in ice. The drip of the superchilled fish was increased and at -3°C the texture of whole cod made them unsuitable for filleting. RSW storage gives several fish species a salty taste due to the take-up of sea water (Barnett *et al.,* 1971; Shaw and Botta, 1975; Reppond and Collins, 1983; Reppond *et al.,* 1985). This negative effect of RSW, however, has not been found in all studies (Lemon and Regier, 1977; Olsen *et al.,* 1993). As opposed to cod and several other fish species, the prime quality of superchilled shrimp from Pakistan was increased from 8 days in ice to 16 days in NaCl-ice at -3°C (Fatima *et al.,* 1988). Also, both freshness (measured by a K-value of 20%) and shelf life of cultured carp *(Cyrinus carpio),* cultured rainbow trout *(Salmo gairdnerii)* and mackerel *(Scomber japonicus)* have been improved by superchilling at -3°C as compared to storage at 0°C (Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

The percentage of frozen water in superchilled fish is highly temperature-dependent (-1°C = 19%; -2°C = 55%; -3°C = 70%; -4°C = 76%) (Ronsivalli and Baker, 1981). It has been suggested that negative effects of superchilling on drip loss, appearance, and texture of cod and haddock are due to formation of large ice crystals, protein denaturation and increased enzymatic activity in the partially frozen fish (Love and Elerian, 1964). Simpson and Haard (1987), however, found only very little difference in biochemical and chemical deterioration of cod *(Gadus morhua)* stored at 0°C and at - 3°C In Japanese studies with seabass, carp, rainbow trout and mackerel, it has been shown that the drip loss as well as several biochemical and chemical deteriorative reactions were reduced in superchilled fish, compared to ice storage (Uchiyama and Kato, 1974; Kato *et al.,* 1974; Uchiyama *et al.,* 1978 a, 1978 b; Aleman *et al.,* 1982).

Superchilling has been used industrially with a few fish species such as tuna and salmon. The negative effects on sensory quality found for some other species

may have limited the practical application of the technique. Nevertheless, it seems that shelf life of at least some seafood products is improved considerably by superchilling. Consequently, for selected products, superchilling may well be more suitable than other technologies.

6.2 The effect of hygiene during handling

Onboard handling

Much emphasis has been placed on hygienic handling of the fish from the moment of catching in order to ensure good quality and long storage life. The importance of hygiene during handling onboard has been tested in a series of experiments where various hygienic measures were employed (Huss *et al.,* 1974). The quality and storage life of completely aseptically treated fish (aseptic handling) were compared with fish iced in clean plastic boxes with clean ice (clean handling) and with fish treated badly, i.e., iced in old, dirty wooden boxes (normal handling). As expected, a considerable difference is found in the bacterial contamination of the three batches (Figure 6.6). However, a similar difference in the organoleptic quality is not detected. During the first week of storage no difference whatsoever is found. Only during the second week does the initial contamination level become important and the heavily contaminated fish have a reduction in storage life of a few days compared with the other samples. These results are not surprising if it is kept in mind that bacterial activity is normally only important in the later stages of the storage period as illustrated in Figure 5.1.

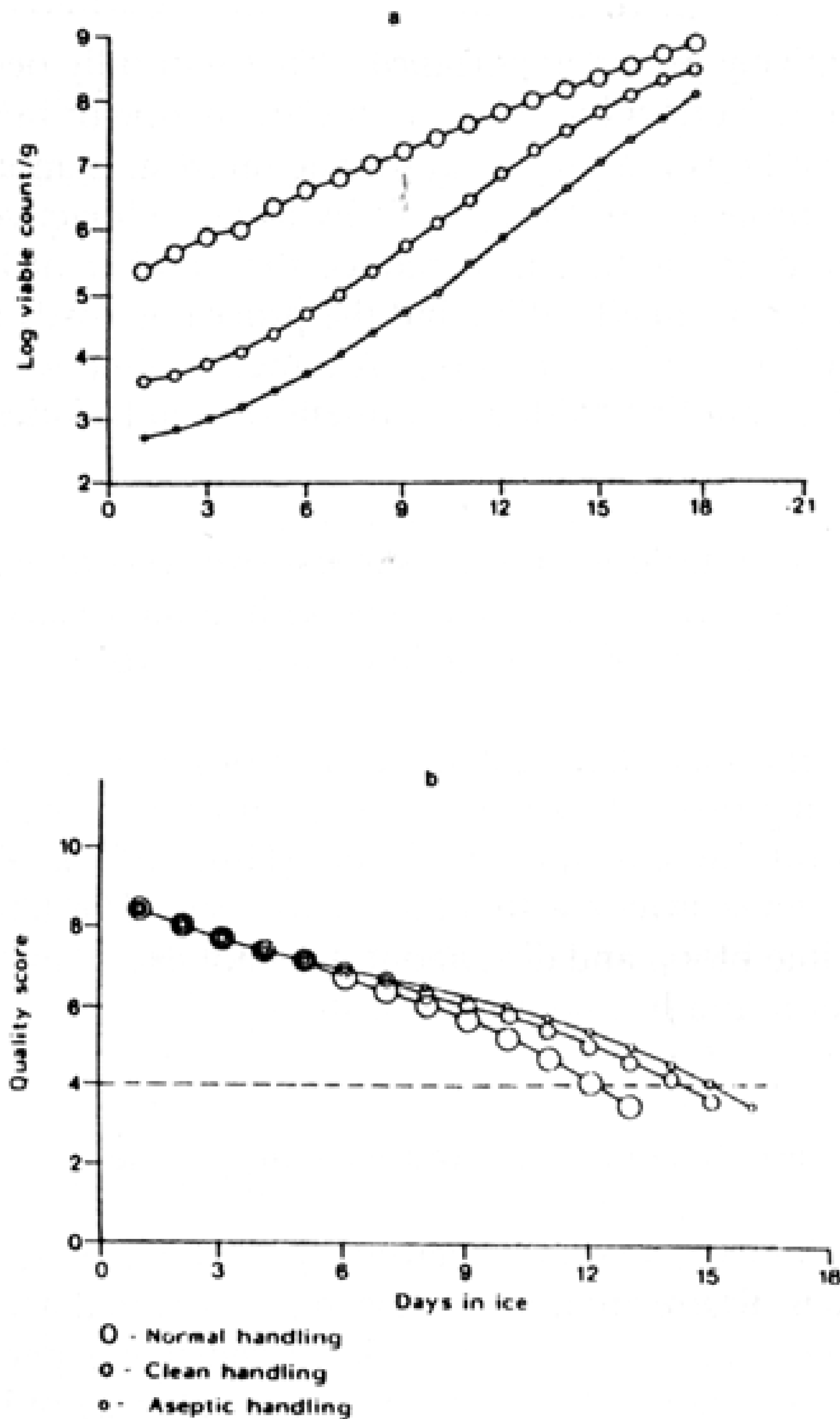

Figure 6.6 Bacterial growth (a) and organoleptic quality (b) of plaice stored at 0° C with initial high, medium and low bacterial counts (after Huss *et al.,* 1974)

On the basis of these data it seems sensible to advocate reasonably hygienic handling procedures including use of clean fish boxes. Very strict hygienic measures do not seem to have great importance. In comparison with the impact of quick and effective chilling, the importance of hygiene is minor.

The above-named observations have influenced the discussion about the design of fish boxes. Normally, fish are iced in boxes stacked on top of each other. In this connection it has been argued that fish boxes should have a construction that prevents the ice melt-water from one box draining into the box underneath it. In a system like this, some bacterial contamination of fish in the bottom boxes would be avoided, as melt-water usually contains a large number of bacteria. However, practical experience as well as experiments (Peters *et al.,* 1974) have shown that this type of contamination is unimportant, and it may be concluded that fish boxes allowing the drainage of melt-water from upper into lower boxes are advantageous because the chilling becomes more effective.

Inhibition or reduction of the naturally occurring microflora

In spite of the relatively minor importance of the naturally occurring microflora in the quality of the fish, much effort has been put into reduction or inhibition of this microflora. Many of these methods are only of academic interest. Among these are (at least until now) attempts to prolong the storage life by using radioactive irradiation. Doses of 100 000 - 200 000 rad are sufficient to reduce the number of bacteria and prolong storage life (Hansen, 1968; Connell, 1975), but the process is costly and, to many people, unacceptable in connection with human food. Another method which has been rejected because of concern about public health is treatment with antibiotics incorporated in the ice.

A method that has been used with some success over recent years is treatment with CO_2, which can be applied either in containers with chilled seawater or as part of a modified atmosphere during distribution or in retail packages (see section 6.3).

It should also be mentioned that washing with chlorinated water has been tried as a means of decontaminating fish. However, the amount of chlorine necessary to prolong the storage life creates off- flavours in the fish meat (Huss, 1971). The newly-caught fish should be washed in clean seawater without any additives. The purpose of the washing is mainly to remove visible blood and dirt, and it does not cause any significant reduction in the number of bacteria and has no effect on storage life.

6.3 The effect of anaerobic conditions and carbon dioxide

High CO_2 concentrations can reduce microbial growth and may therefore extend the shelf life of food products, where spoilage is caused by microbial activity (Killeffer, 1930; Coyne, 1933). Technological aspects of modified atmosphere packaging (MAP) have since been studied. Today, materials and techniques for

storage of bulk or retail packed foods are available.

This section discusses the effect of anaerobic conditions and modified atmospheres on the shelf life of fish products. The safety aspects are reviewed in Farber (1991) and Reddy et al. (1992).

Effect on microbial spoilage

Vacuum packaging (VP) and MAP, with high CO_2 levels (25% - 100%), extends the shelf life of meat products by several weeks or months (Table 6.4). In contrast, the shelf life of fresh fish is not affected by VP and only a small increase in shelf life can be obtained by MAP (Table 6.4).

Table 6.4 Effect of packaging on the shelf life of chilled fish and meat products

Type of product	Storage temp.	Shelf life (weeks)		
		Air	VP[a]	MAP[b]
Meat (beef, pork, poultry)	1.0 - 4.4°C	1 - 3	1 - 12	3 - 21
Lean fish (cod, pollock, rockfish, trevally)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Fatty fish (herring, salmon, trout)	0.0 - 4.0°C	1 - 2	1 - 2	1 - 3
Shellfish (crabs, scampi, scallops)	0.0 - 4.0°C	½ - 2	-	½ - 3
Warmwater fish (sheepshead, swordfish, tilapia)	2.0 - 4.0°C	½ - 2	-	2 - 4

a) VP: Vacuum packed
b) MAP: Modified atmosphere packed (High CO_2 concentrations (25 - 100%)

Differences in spoilage microflora. and in pH are mainly responsible for the observed differences in the shelf life of fish and meat products. Spoilage of meat under aerobic conditions is caused by strict aerobic Gram-negative organisms, primarily *Pseudomonas spp.* These organisms are strongly inhibited by

anaerobic conditions and by CO_2. Consequently, they do not play any role in the spoilage of packed meat. Instead the microflora, of VP and MAP meat products changes to be dominated by Gram-positive organisms (Lactic Acid Bacteria), which are much more resistant to CO_2 (Molin, 1983; Dainty and Mackey, 1992). Fish stored under aerobic conditions are also spoiled by Gram negative-organisms, primarily *Shewanella putrefaciens* (see section 5.3).

The spoilage flora on some packed fish products was found to be dominated by Grampositive microorganisms and in this way the microflora, was similar to the flora on packed meats; see Stammen *et al.* (1990) for a review. For packed cod, however, the Gram-negative organism *Photobacterium phosphoreum* has been identified as the organism responsible for spoilage. The growth rate of this organism is increased under anaerobic conditions (Figure 6.7) and this may explain the importance of the organism in VP cod.

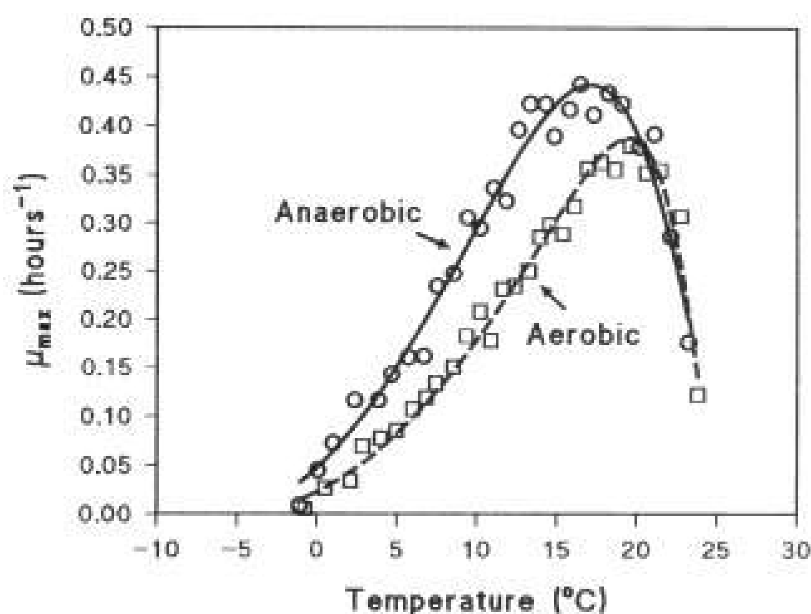

Figure 6.7 Effect of oxygen and temperature on the maximum specific growth rate (max of *Photobacterium phosphoreum* grown in a complex medium containing TMAO (Dalgaard, 1993)

In CO_2-packed fish, the growth of *Shewanella putrefaciens* and of many other microorganisms found on live fish is strongly inhibited. In contrast P. *phosphoreum* was shown to be highly resistent to CO_2 (Figure 6.8). It was also shown that the limited effect of CO_2 on growth of this bacteria correspond very well with the limited effect of CO_2 on the shelf life of packed fresh cod. P. *phosphoreum* reduces TMAO to TMA while very little H_2S is produced during growth in fish substrates. Spoiled VP and MAP cod is characterized by high levels of TMA, but little or no development of the putrid or H_2S odours typical for

some aerobically stored spoiled fish. The growth characteristics of P. *phosphoreum* and the metabolic activity of the organism thus explain both the short shelf life and the spoilage pattern of packed cod (Dalgaard, 1994 a).

The shelf life of VP and MAP cod is similar to various other sea food products (Table 6.4). P. *phosphoreum* is widespread in the marine environment and it seems likely that this organism or other highly CO_2 resistent microorganisms are responsible for spoilage of packed sea food products (Baumann and Baumann, 1981; van Spreekens, 1974; Dalgaard *et al.,* 1993).

The best effect of MAP storage on shelf life has been obtained with fish from warm waters. The shelf life of these products, however, is still relatively short compared to meat products (Table 6.4).

Very low bacterial level (10^5-10^6 cfu/g) has been found at the time of sensory rejection of some packed fish products. In these cases non-microbial reactions may have been responsible for spoilage.

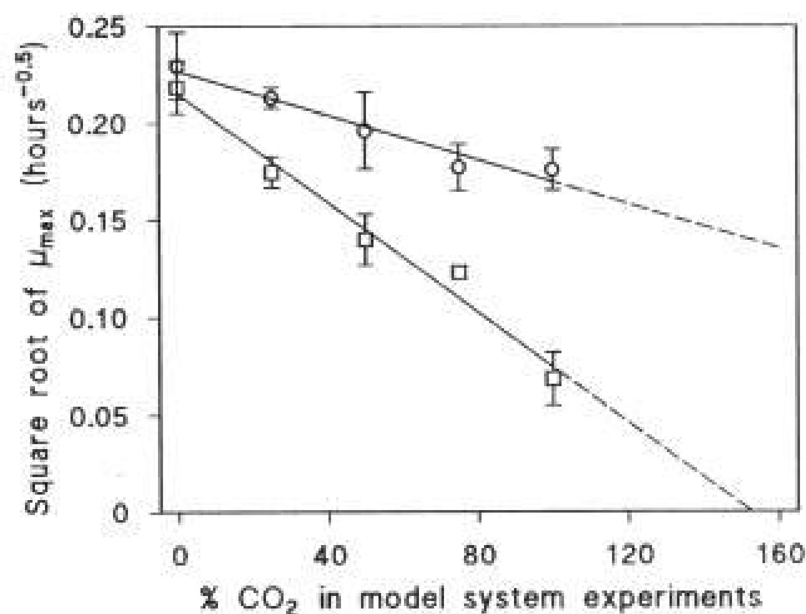

Figure 6.8 Effect of CO_2 on the maximum specific growth rate (μmax) of *Photobacterium phosphoreum* (circles) and of *Shewanella putrefaciens* (squares). Experiments were carried out at 0°C (Dalgaard, 1994 b)

Effect of non-microbial spoilage reactions

CO_2 is dissolved in the water phase of the flesh of MAP fish and a decrease in pH of about 0.2- 0.3 units is observed, depending on the CO_2 concentration in the surrounding gaseous atmosphere. The water-holding capacity of muscle

proteins is decreased by decreased pH and an increased drip loss is expected for fish stored in high CO_2 concentrations. Increased drip has been found for cod fillets, red hake, salmon, and shrimps (Fey and Regenstein, 1982; Layrisse and Matches, 1984; Dalgaard *et al.,* 1993) but not for herring, red snapper, trevally, Dungeness crab, and rockfish (Cann *et al.,* 1983; Gerdes *et al.* 1991; Parking and Brown, 1983 and Parkin *et al.,* 1981).

Coyne (1933) and many later studies have found the textural quality of fish stored in 100% CO_2 to be reduced. However, up to 60% CO_2 has no negative effect on the texture of cod. The colour of the belly flaps, of cornea, and of the skin may be altered for whole fish stored in high CO_2 concentrations (Haard, 1992). Packaging may also stimulate the formation of metmyoglobulin in red-fleshed fish and thereby result in a darkening of fish muscles. Although oxygen-containing modified atmospheres have been used, the development of rancid off-odours in fatty fish species has not been registered as a problem (Haard, 1992).

Carbon dioxide used in combination with refrigerated seawater systems

Storage of fish in refrigerated seawater (RSW) was discussed in section 6. 1. Only the effect of addition of CO_2 to RSW will be considered in this section. Table 6.5 shows the effect of RSW and RSW + CO_2 on the shelf life of various fish products, as compared to storage in ice.

Table 6.5 Shelf life of various fish products stored in Refrigerated Seawater (RSW) and in RSW with added CO_2

Type of product	Storage temp. in RSW	Shelf life (days)			References
		Ice (0° C)	RSW	RSW +CO_2	
Pacific cod	-1.1°C	6-9	-	9-12	Reppond and Collins (1983)
Pink shrimp	-1.1°C	-	4-5	6	Barnett et al. (1978)
Herring	-1.0°C	-	8-8.5	10	Hansen et al. (1970)
Walleye Pollock	-1.0°C	6-8	4-6	6-8	Reppond et al. (1979, 1985)

Rockfish	-0.6°C	-	7-10	17	Barnett et al. (1971)
Chum Salmon	-0.6°C	-	7-11	18	Barnett et al. (1971)
Silver Hake	0-1°C	4-5	4-5	5	Hiltz et al. (1976)
Capelin	+0.2 - -1.5°C	6	2	2	Shaw and Botta (1975)

An evident shelf life-extending effect of CO_2 is only seen with some species. Several negative effects of adding CO_2 to RSW-systems have been observed. The fish colour and texture were negatively influenced, and CO_2 dissolved in the flesh made mackerel unsuitable for canning (Longard and Regier, 1974; Lemon and Regier, 1977).

CO_2 acidifies the seawater, and a lowered pH inhibits the enzymatic reactions that otherwise lead to black spots in shrimps and prawns. The shelf life of pink shrimps can be more than doubled by storage in RSW + CO_2, where, compared to ice storage, colour, texture, flavour, and odour were improved (Nelson and Barnett, 1973). RSW+CO_2 stored prawns, however, may be unacceptably tough and have a "soft shell" appearance (Ruello, 1974).

Sea water acidified by CO_2 is highly corrosive. Therefore, inert materials are needed in RSW+CO_2 systems, e.g., for heat exchange. These materials are available, but their cost must be taken into account when the application of RSW + CO_2 systems is evaluated (Nelson and Barnett, 1973).

Future application of carbon dioxide for shelf life extension

For most MAP seafoods, the production of TMA is delayed by only a few days compared to aerobic or anaerobic storage. This indicates that fish products in general are contaminated with a highly CO_2 resistent microflora of TMAO reducing organisms. Very high CO_2 concentrations can inhibit microbial growth but high levels of CO_2 have a negative effects on other aspects of the fish quality. MAP has found little practical application with fish products as compared to meat products. The main reasons for this are probably that:

- MAP used with retail packs is an expensive technique
- the prime fish quality is not improved
- only small shelf life extensions are obtained

- MAP cannot replace good chilling or good hygienic production conditions
- toxin production of *Clostridium botulinum* is increased for bacteria growing under anaerobic conditions, and this may be of importance for the safety of packed fish (Huss *et al.,* 1980; Reddy *et al.,* 1992).

Packaging, however, can be used simply because packed products are more convenient to handle, e.g., in supermarkets. According to the EEC Council Directive of *22 July* 1991 (91/493/EEC), VP and MAP fish products are considered as fresh products. Consequently, CO_2 can be used for preservation of fresh fish products, when a shelf life extension of only a few days is found to be sufficient.

The negative effect Of CO_2 on fish colour is primarily a problem for whole fish and the negative effect of CO_2 on texture and drip loss is only observed with high CO_2 concentrations. A pronounced effect on growth of *S. putrefaciens* and on many other bacteria is obtained with even moderate CO_2 concentrations (40-80%). It is therefore likely that, in the future, MAP will be used in combination with preservation techniques that has been developed specifically to inhibit growth of CO_2 resistent TMAO reducing marine spoilage bacteria such as P. *phosphoreum.*

The effect of MAP also seems to depend on fish species and further studies are needed to determine if MAP can give interesting shelf life extensions for other fish species, e.g., those from warm waters. Finally, high CO_2 concentrations could be used for fish intended for fishmeal as the negative effects of CO_2 on colour and texture in this case are less important.

6.4 The effect of gutting

It is a common experience that the quality and storage life of many fish decrease if they have not been gutted. During feeding periods the fish contain many bacteria in the digestive system and strong digestive enzymes are produced. The latter will be able to cause a violent autolysis *post mortem,* which may give rise to strong off-flavour especially in the belly area, or even cause belly-burst. On the other hand, gutting means exposing the belly area and cut surfaces to the air thereby rendering them more susceptible to oxidation and discoloration. Thus, many factors such as the age of the fish, the species, amount of lipid, catching ground and method, etc., should be taken into consideration before deciding whether or not gutting is advantageous.

Fatty species

In most cases,small- and medium-sized fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is partly that a large number of small fish are caught at the same time and partly because of problems with discoloration and the acceleration of rancidity.

However, problems may arise with ungutted fish during periods of heavy feeding due to belly- burst. The reactions leading to belly-burst are complex and not fully understood. It is known that the strength of the connective tissue is decreased during these periods and that post mortem pH is normally lower in well-fed fish, this also weakens the connective tissue (Figure 6.9). Furthermore, it seems that the type of feed ingested may play an important role in the belly-burst phenomenon.

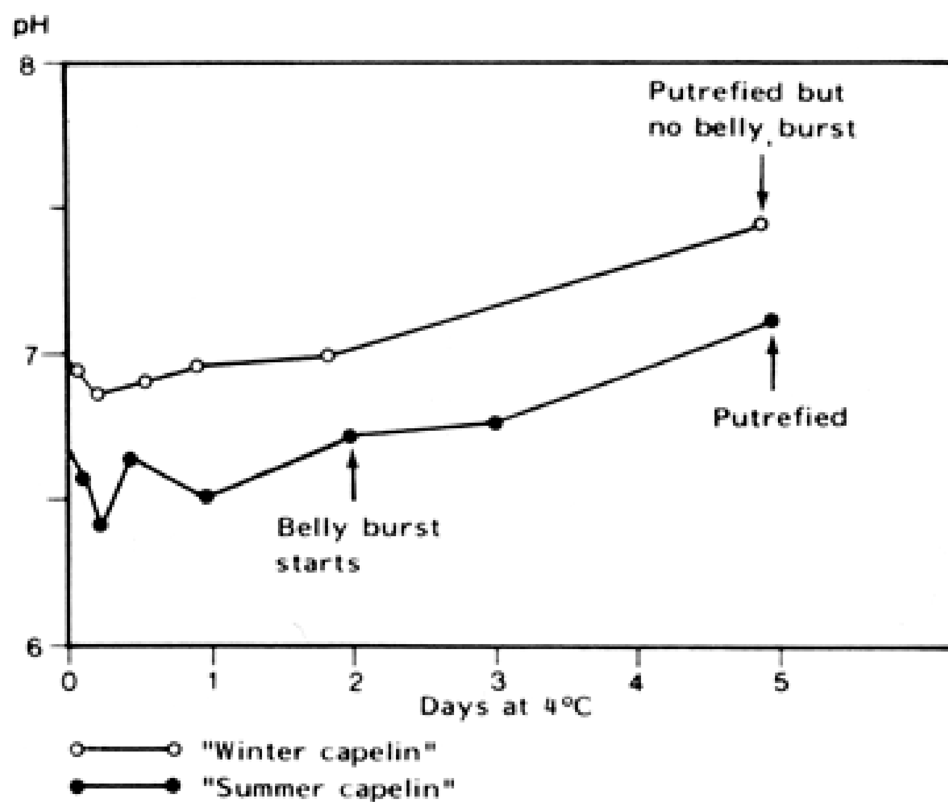

Figure 6.9 pH in winter capelin (o) and summer capelin (·) during storage at +4° C (Gildberg, 1978)

Lean species

In most North European countries, the gutting of lean species is compulsory. It is based on the assumption that the quality of these species suffers if they are not gutted. In the case of cod, it has been shown that omission causes a considerable quality loss and a reduction in the storage life of five or six days. After only two days from catch, discoloration of the belly area is visible and the

raw fillet acquires an offensive cabbagey odour. As seen in Figure 6.10, these odours are removed to some extent by boiling.

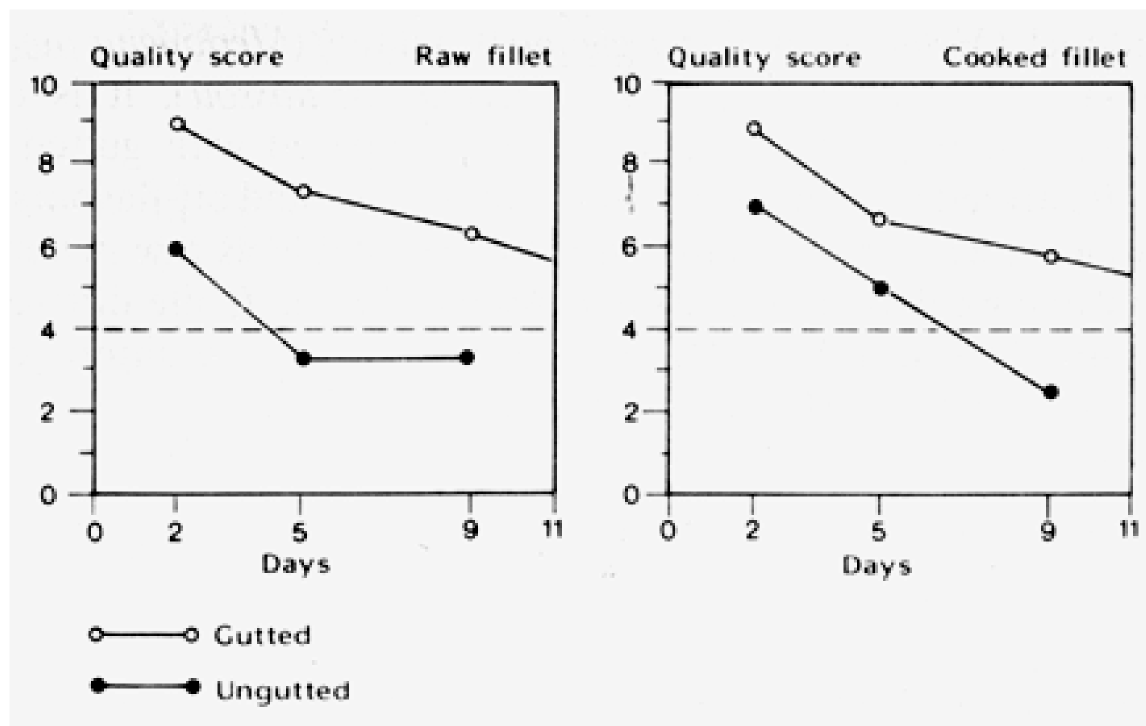

Figure 6.10 Organoleptic quality of raw and boiled fillet, respectively from gutted (o) and ungutted (·) iced cod (Huss, 1976)

These volatile, foul-smelling compounds are mostly found in the gut and surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself (Figure 6.11). These chemical parameters are, therefore, not useful for distinguishing between gutted and ungutted fish (Huss and Asenjo, 1976).

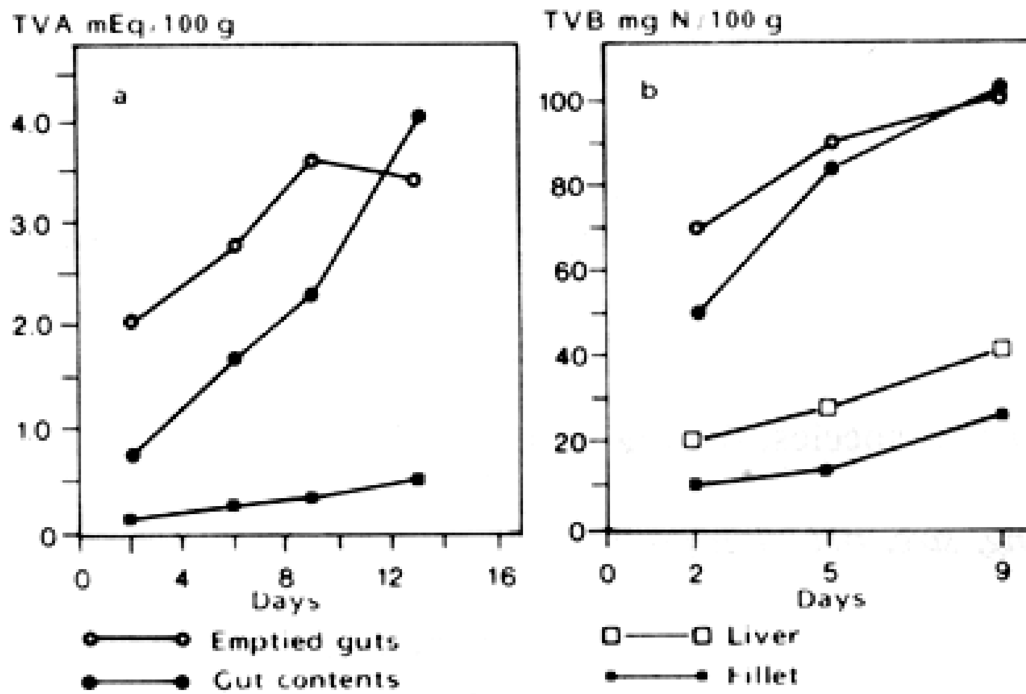

Figure 6.11 Development of (a) volatile acids in iced, ungutted saithe *(Polacchius* virens) and (b) volatile bases in iced, ungutted cod *(Gadus morhua)* *(Huss* and Asenjo, 1976)

Similar experiments with other cod-like species show a more differentiated picture. In the case of haddock *(Melanogrammus aeglefinus),* whiting *(Merlangius merlangus,* saithe *(Pollachius virens)* and blue whiting *(Micromesistius poutassou),* it is observed that ungutted fish stored at 0°C suffer a quality loss compared with gutted fish, but the degree varies as illustrated in Figure 6.12. Some off-odours and off-flavours are detected, but ungutted haddock, whiting and saithe are still acceptable as raw material for frozen fillets after nearly one week on ice (Huss and Asenjo, 1976). Quite different results are obtained with South American hake *(Merluccius gayi),* where no difference is observed between gutted and ungutted fish (Huss and Asenjo, 1977 b).

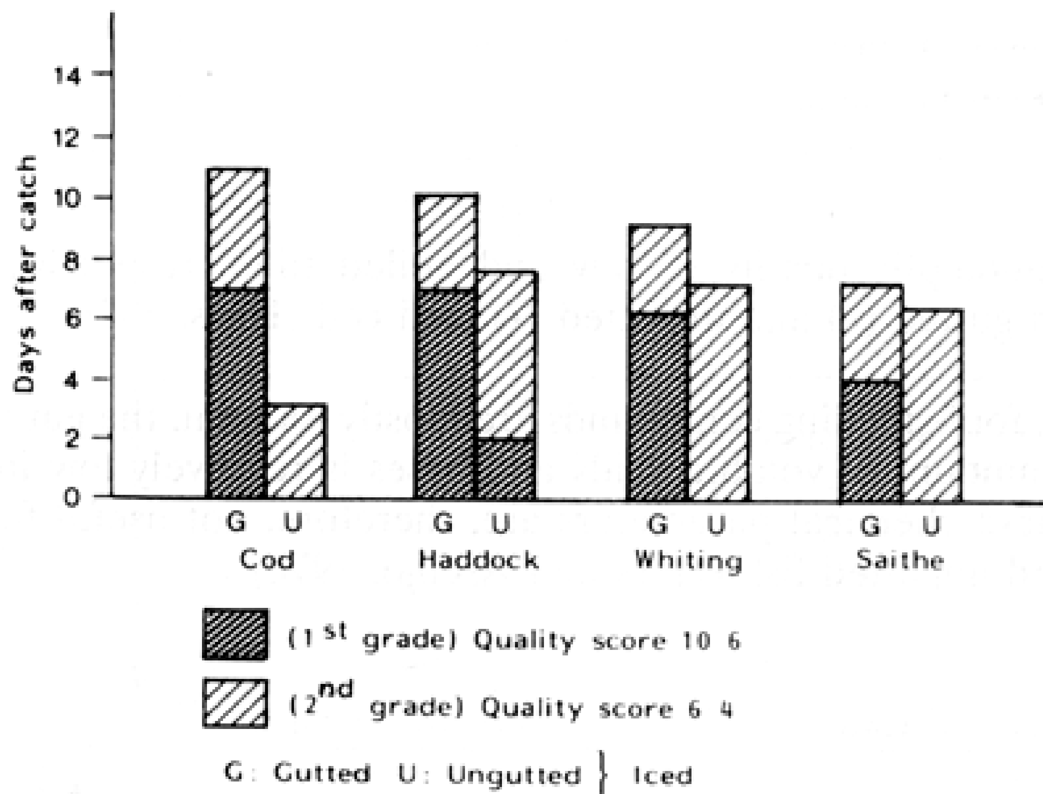

Figure 6.12 Quality and storage life of gutted and ungutted lean fish stored in ice (Huss and Asenjo, 1976)

6.5 The effect of fish species, fishing ground and season

Influence of handling, size, pH, skin properties

The spoilage rate and shelf life of fish is affected by many parameters and, as stated in section 5, fish spoil at different rates. In general it can be stated that larger fish spoil more slowly than small fish, flat fish keep better than round fish, lean fish keep longer than fatty fish under aerobic storage and bony fish are edible longer than cartilaginous fish (Table 6.6). Several factors probably contribute to these differences and whereas some are clear, many are still on the level of hypotheses.

Table 6.6 Intrinsic factors affecting spoilage rate of fish species stored in ice

Factors affecting spoilage rate	Relative spoilage rate	
	fast	slow

size	small fish	larger fish
post mortem pH	high pH	low pH
fat content	fatty species	lean species
skin properties	thin skin	thick skin

Rough handling will, as outlined in section 5.2, result in a faster spoilage rate. This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. The surface/volume ratio of larger fish is lower than that of smaller fish, and, as bacteria are found on the outside, this is probably the reason for the longer shelf life of the former. This is true within a species but may not be universally so.

Post mortem pH varies between species but is, as described in section 5.2, higher than in warm- blooded animals. The long rigor period and the corresponding low pH (5.4-5.6) of the very large flatfish, halibut (*Hippoglossus hipoglossus*), has been offered as an explanation for its relatively long iced storage life (Table 6.7). However, mackerel will often also experience a low pH and this seems to have little effect on shelf life. As can be seen from Table 6.7, fatty fish are in general rejected sensorically long before lean fish. This is mainly due to the appearance of oxidative rancidity.

The skin of the fatty pelagic fish is often very thin, and this may contribute to the faster spoilage rate. This allows enzymes and bacteria to penetrate more quickly. On the contrary, the thick skin of flatfish and the antibacterial compounds found in the slime of these fish may also contribute to the keepability of flatfish. As described earlier, the slime of flat fish contains bacteriolytic enzymes, antibodies and various other antibacterial substances (Hjelmland et al., 1983; Murray and Fletcher, 1976). Although large differences exist in the content of TMAO, this does not seem to affect the shelf life of aerobically-stored fish but rather the chemical spoilage profile of the species.

Table 6.7 Shelf life of various fish species from temperate and tropical waters. Prepared from data published by Lima dos Santos (1981); Poulter *et al.*(1981); and Gram (1989)

Species	Fish type	Shelf life (days in ice)	
		temperate	tropical
Marine species		2-24	6-35
cod, haddock	lean	9-15	
whiting	lean	7-9	
hake	lean	7-15	
bream	lean/low fat		10-31
croaker	lean		8-22
snapper	lean		10-28
grouper	lean		6-28
catfish	lean		16-19
pandora	lean		8-21
jobfish	lean		16-35
spadefish	lean/low fat		21-26
batfish	lean		21-24
sole, plaice,	flat	7-21	21
flounder	flat	7-18	
halibut	flat	21-24	

mackerel[1)]	high/ low fat	4-19	14-18
summer herring	high fat	2-6	
winter herring	low fat	7-12	
sardine	high fat	3-8	9-16
Freshwater species		9-17	6-40
catfish	lean	12-13	15-27
trout	low fat	9-11	16-24
perch	lean/ low fat	8-17	13-32
tilapia	lean		10-27
mullet	lean		12-26
carp	lean/ low fat		16-21
lungfish	lean/ low fat		11-25
Haplochromis	lean		6
shad	medium fat		25
corvina	medium fat		30
bagré	medium fat		25
chincuna	fatty		40

pacu	fatty		40

1) fat content and shelf life subject to seasonal variation.

In general, the slower spoilage of some fish species has been attributed to a slower bacterial growth, and Liston (1980) stated that "different spoilage rates seem to be related at least partly to the rate of increase of bacteria on them".

Influence of water temperature on iced shelf life

Of all the factors affecting shelf life, most interest has focused on the possible difference in iced shelf life between fish caught in warm, tropical waters and fish caught in cold, temperate waters. In the mid- and late sixties it was reported that some tropical fish kept 20-30 days when stored in ice (Disney *et al.,* 1969). This is far longer than for most temperate species and several studies have been conducted assessing the shelf life of tropical species. Comparison of the data is, as pointed out by Lima dos Santos (1981), difficult as no clear definition has been given on a "tropical" fish species and as experiments have been carried out using different sensory and bacteriological analyses.

Several authors have concluded that fish taken from warm waters keep better than fish from temperate waters (Curran and Disney, *1979;* Shewan, 1977) whereas Lima dos Santos (1981) concluded that also some temperate water fish species keep extremely well and that the longer shelf lives in general are found in fresh water fish species compared to marine species. However, he also noted that shelf life of more than 3 weeks, which is often observed for fish caught in tropical waters (Table 6.7),never occurs when fish from temperate waters are stored in ice. The iced shelf life of marine fish from temperate waters varies from 2 to 21 days which does not differ significantly from the shelf life of temperate freshwater fish ranging from *9* to 20 days. Contrary to this, fish caught in tropical marine waters keep for 12-35 days when stored in ice and tropical freshwater fish from *6* to 40 days. Although very wide variations occur, tropical fish species often have prolonged shelf lives when stored in ice as shown in Table 6.6. When comparisons are made, data on fatty fish like herring and mackerel should probably be omitted as spoilage is mainly due to oxidation.

Several hypotheses have been launched trying to explain the often prolonged iced spoilage of tropical fish. Some authors have noted an absence in development of TMA and TVN during storage and suggested that the spoilage of tropical fish is not caused by bacteria (Nair *et al.,* 1971)*.* The lack of development of TMA and TVN may be explained by a spoilage dominated by *Pseudomonas* spp.; however, qualitative bacteriological analyses must be carried out to confirm

or reject this suggestion. Low bacterial counts have been claimed in some studies, but often inappropriate media have been used for the examination and too high incubation temperatures (30°C) have not allowed the psychrotrophic spoilage bacteria to grow on the agar plates.

Reviewing the existing literature on storage trials of tropical fish species leads to the conclusion that the overall sensory, chemical and bacteriological changes occurring during spoilage of tropical fish species are similar to those described for temperate species.

Psychrotrophic bacteria belonging to *Pseudomonas spp*. and *Shewanella putrefaciens* dominate the spoilage flora of iced stored fish. Differences exist, as described in section 5.3, in the spoilage profile depending on the dominating bacterial species. *Shewanella* spoilage is characterized by TMA and sulphides (H_2S) whereas the *Pseudomonas* spoilage is characterized by absence of these compounds and occurrence of sweet, rotten sulphydryl odours. As this is not typical of temperate, marine fish species which have been widely studied, this may explain the hypothesis that bacteria are not involved in the spoilage process of tropical fish.

Despite the different odour profiles, the level at which the offensive off-odours are detected sensorially is more or less the same. In model systems (sterile fish juice) 10^8-10^9 cfu/ml of both types of bacteria is the level at which spoilage is evident.

As outlined in section 5.3, the relatively high post*mortem* pH is one of the reasons for the relatively short shelf life of fresh fish as compared to, for instance, chill stored beef. It has been suggested that tropical fish species, such as the halibut from temperate waters, reach a very low pH, and that this explains the longer shelf life. However, pH values of 6-7 have been found in the studies of tropical fish species where pH has been measured (Gram, 1989). As the differences in skin properties are believed to contribute to the longer shelf life of flatfish, it has been suggested that this factor explained the extended shelf lives. It is indeed true that fish from warm waters often have very thick skin, but no systematic investigation has been carried out on the skin properties.

As spoilage of fish is caused by bacterial action, most hypotheses dealing with the long iced shelf life of tropical fish species have centred around differences in bacterial flora. Shewan (1977) attributed the long iced shelf lives to the lower number of psychrotrophs on tropical fish. However, in 1977 only a very limited number of studies of the bacterial flora on tropical fish were published. During the last 10- 15 years several investigations have concluded that Gram-negative rod-shaped bacteria (e.g., *Pseudomonas, Moraxella* and *Acinetobacter)* dominate on many fish caught in tropical waters (Gram, 1989; Surendram *et al.,* 1989; Acuff *et*

al., 1984). Similarly, Sieburth (1967) concluded that the composition of the bacterial flora in Narragansett Bay did not change during a 2-year survey even though the water temperature fluctuated with 23°C on a year-round basis. Gram (1989) showed that 40-90% of the bacteria found on Nile perch were able to grow at 7°C. The number of psychrotrophic bacteria is within one log unit of the total count, and the level of psychrotrophic organisms is not *per se low* enough to account for the extended iced storage lives of tropical fish; Jorgensen *et al.* (1989) showed that a two log difference in number of spoilage bacteria only resulted in a difference of 3 days in the shelf life of iced cod.

As described in section 5, the bacterial flora on temperate water fish species resume growth immediately after the fish have been caught and rarely is a lag phase seen. Contrary to this, Gram (1989) concluded that a bacterial lag phase of 1-2 weeks is seen when tropical fish are stored in ice. Also, the subsequent growth of psychrotrophic bacteria is often slower on iced tropical than on iced temperate water fish. This is in agreement with Liston (1980) who attributed differences in shelf life to differences in bacterial growth rates. Although a large part of the bacteria on tropical fish are capable of growth at chill temperatures, they will (as this has never been necessary) require a period of adaptation (i.e., the lag phase and slow growth phase). Gram (1989) illustrated this by investigating the growth rate at 0°C of fish spoilage bacteria that had either been pre-cultured at 20°C or at 5°C. For some strains, the same bacterial strain would grow more quickly at 0°C if pre-cultured at 5°C than if pre-cultured at 20°C (Table 6.8). Preculturing was done with several sub-culture steps at each temperature. Similarly, Sieburth (1967) showed that although the taxonomic composition of the bacterial flora in Narrangansett Bay did not change with fluctuating temperature, the growth profile of the bacteria fluctuated following the water temperature. However, the adaptation hypothesis does not explain why some tropical fish spoil at rates comparable to temperate water fish.

Table 6.8 Generation times at 0°C for fish spoilage bacteria pre-cultured at high (20°C) or low (5°C) temperatures

Species	Origin	Pre-culture temperature (°C)	Subsequent generation time (hours) at 0°C
Aeromonas spp.	spoiled chilled trout	5 20	11 20
Pseudomonas spp.	iced cod (Denmark)	5 20	9 14

	spoiled iced sardine (Senegal)	5 20	12 14
Shewanella spp.	iced cod (Denmark)	5 20	8 17
	iced sole (Senegal)	5 20	9 17

It can be concluded that many factors affect shelf life of fish and that differences in the physiology of the bacterial flora are likely to be of major importance.

Off flavours related to fishing ground

Occasionally fish with off-flavours are caught, and in certain localities this is a fairly common phenomenon. Several of these off-flavours can be attributed to their feeding on different compounds or organisms. The planktonic mollusc, *Spiratella helicina,* gives rise to an off-flavour described as "mineral oil" or "petrol". It is caused by dimthyl-B-propiothetin which is converted to dimethylsulphide in the fish (Connell, 1975). The larvae of *Mytilus* spp. cause a bitter taste in herring. A very well known off-flavour is the muddy-earthy taint in many freshwater fish. The flavour is mainly caused by two compounds: geosmin (1a, 10ß-dimethyl-9a-decalol) and 2-methylisoborneol, which also are part of the chemical profile of wine with cork flavour. Geosmin, the odour of which is detectable in concentrations of 0.01-0.1 µg/l, is produced by several bacterial taxa, notably the actinomycetes *Streptomyces* and *Actinomyces.*

An iodine-like flavour is found in some fish and shrimp species in the marine environment. This is caused by volatile bromophenolic compounds; and it has been suggested that the compounds are formed by marine algae, sponges and Bryozoa and become distributed through the food chain (Anthoni *et al.,* 1990).

Oil taint may be found in the fish flesh in areas of the world where off-shore exploitation of oil is intensive or in areas where large oil spills occur. The fraction of the crude oil that is soluble in water is responsible for the off-flavours. This is caused by the accumulation of various hydrocarbon compounds, where particularly the aromatic compounds are strong flavourants (Martinsen *et al.,* 1992).

Figure 6.13 The situation on a South American hake trawler. The fishermen have spent considerable time and effort gutting the fish, where rapid chilling of whole, ungutted fish would have been more beneficial to quality

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7. IMPROVED FRESH FISH HANDLING METHODS

7.1. Basics of fresh fish handling and use of ice
7.2. Fish handling in artisanal fisheries
7.3. Improved catch handling in industrial fisheries

7.1 Basics of fresh fish handling and use of ice

Throughout history, man has preferred to consume fresh fish rather than other types of fish products. However, fish spoil very quickly and man has had to develop methods to preserve fish very early in history.

Keeping and transporting live fish

The first obvious way of avoiding spoilage and loss of quality is to keep caught fish alive until consumption. Handling of live fish for trade and consumption has been practised in China with carp probably for more than three thousand years. Today, keeping fish alive for consumption is a common fish-handling practice both in developed and developing countries and at both artisanal and industrial level.

In the case of live fish handling, fish are first conditioned in a container with clean water, while the damaged, sick and dead fish are removed. Fish are put to starve and, if possible, water temperature is reduced in order to reduce metabolic rates and make fish less active. Low metabolic rates decrease the fouling of water with ammonia, nitrite and carbon dioxide that are toxic to fish and impair their ability to extract oxygen from water. Such toxic substances will tend to increase mortality rates. Less active fish allow for an increase in the packing density of fish in the container.

A large number of fish species are usually kept alive in holding basins, floating cages, wells and fish yards. Holding basins, normally associated with fish culture companies, can be equipped with oxygen control, water filtering and circulation and temperature control. However, more simple methods are also used in practice, for instance large palm woven baskets acting as floating cages in rivers (China), or simple fish yards constructed in a backwater of a river or rivulet for large "surubi" (Platystoma spp.), "pacu" (Colossoma spp.) and "pirarucu" (Arapalma gigas) in the Amazonian and Parana basins in South America.

Methods of transporting live fish range from very sophisticated systems installed on trucks that regulate temperature, filter and recycle water and add oxygen (Schoemaker, 1991), to very simple artisanal systems of transporting fish in plastic bags with an oxygen supersaturated

atmosphere (Berka, 1986). There are trucks that can transport up to 50 t of live salmon; however, there is also the possibility of transporting a few kilo-grammes of live fish relatively easily in a plastic bag.

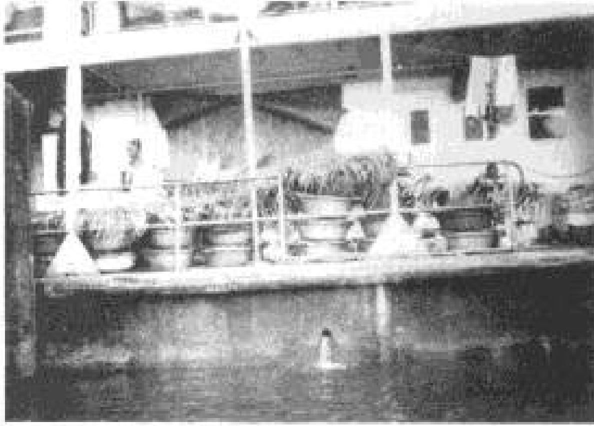
By now a large number of species, inter alia, salmon, trout, carp, eel, seabream, flounder, turbot, catfish, Clarias, tilapias, mussels, oysters, cockles, shrimp, crab and lobster are kept alive and transported, very often from one country to another.

There are wide differences in the behaviour and resistance of the various species. Therefore the method of keeping and transporting live fish should be tailored according to the particular species and the length of time it needs to be kept outside its natural habitat before slaughtering. For instance, the lungfish (Protopterus spp.) can be transported and kept alive out of water for long periods, merely by keeping its skin moist.

Some species of fish, noticeably freshwater fish, are more resistant than others to changes in oxygen in solution and the presence of toxic substances. This is probably due to the fact that their biology is adapted to the wide yearly variations in water composition presented by some rivers (cycles of matter in suspension and dissolved oxygen). In these cases, live fish are kept and transported just by changing the water from time to time in the transport containers (See Figures 7.1 (a) and (b)). This method is widely used in the Amazonian, Parana and Orinoco basins in South America; in Asia (particularly in the People's Republic of China, where also more sophisticated methods are used) and in Africa (N'Goma, 1993).

In the case presented in Figure 7.1 (a), aluminium containers with live freshwater fish are stored in the aisles of a public transport vessel. Containers are covered with palm leaves and water hyacinth to prevent the fish from jumping out of the containers and to reduce evaporation. The water in the containers is changed from time to time and an almost continuous visual control is kept on fish. Dead fish are immediately put to smoke-drying (African style) in drum smokers, also transported in the vessels or transporting barges.

In the case presented in Figure 7. 1(b), carp is kept in a metal container drawn by a bicycle. This is a rather common practice in China, and other Asian countries; for instance in Bangkok, live catfish is sold daily by street vendors.



(a) (b)

Figure 7.1 (a) Transport of live freshwater fish in Congo (Cuvette Congolaise) (N'Goma, 1993); (b) street vendor of live fish in China today (Suzhou, 1993, photo H. Lupin)

The most recent development is the keeping and transporting of fish in a state of hibernation. In this method, the body temperature of live fish is reduced drastically in order to reduce fish metabolism and to eliminate fish movement completely. The method greatly reduces death rates and increases package density, but careful temperature control should be exercised to maintain the hibernation temperature. There is an appropriate hibernation temperature for each species. Although the method is already utilized for instance to transport live "kuruma" shrimp *(Penaeus japonicus)* and lobster in pre-chilled wet sawdust, it should be considered an experimental technique for most of the species.

Although keeping and transporting live fish is becoming more and more important, it is not a viable solution for most of the bulk fish captures in the world.

Chilling fish with ice

Historical evidence proves that the Ancient Chinese utilized natural ice to preserve fish more than three thousand years ago. Natural ice mixed with seaweed was also used by the Ancient Romans to keep fish fresh. However, it was the development of mechanical refrigeration which made ice readily available for use in fish preservation.

In developed countries, particularly in USA and some European countries, the tradition of chilling fish with ice dates back more than a century. The practical advantages of utilizing ice in fresh fish handling are therefore well established. However, it is worthwhile for young generations of fish technologists and newcomers to the field, to review them, paying attention to the main points of this technique.

Ice is utilized in fish preservation for one or more of the following reasons:

(i) ***Temperature reduction.*** By reducing temperature to about 0°C the growth of spoilage and pathogenic micro-organisms (see section 6) is reduced, thus reducing the spoilage rate and

reducing or eliminating some safety risks.

Temperature reduction also reduces the rate of enzymatic reactions, in particular those linked to early *post mortem* changes extending, if properly applied, the *rigor mortis* period.

Fish temperature reduction is by far the most important effect of ice utilization. Therefore, the quicker the ice chills the better. Although cold-shock reactions have been reported in a few tropical species when iced, leading to a loss of yield of fillets (Curran *et* al., 1986), the advantage of quick chilling usually outweighs other considerations. The development of ad hoc fish handling methods is of course not ruled out in the case of species that could present cold-shock behaviour.

(ii) ***Melting ice keeps fish moist.*** This action mainly prevents surface dehydration and reduces weight losses. Melting water also increases the heat transport between fish and ice surfaces (water conducts heat better than air): the quickest practical chilling rate is obtained in a slurry of water and ice (e.g., the CSW system).

If, for some reason, ice is not utilized immediately after catching the fish, it is worthwhile keeping the fish moist. Evaporative cooling usually reduces the surface temperature of fish below the optimum growth temperature of common spoilage and pathogenic bacteria; although it does not prevent spoiling.

Ice should also be utilized in relation with chilling rooms to keep fish moist. It is advisable to keep chilling room temperature slightly above 0°C (e.g., 3-4°C).

However, water has a leaching effect and may drain away colour pigments from fish skin and gills. Ice melting water can also leach micronutrients in the case of fillets and extract relatively large amounts of soluble substances in some species (e.g., squid).

Depending on the species, severity of leaching and market requirements, an ad *hoc* handling procedure may be justified. In general, it has been found that drainage of ice meltwater is advisable in boxes and containers and that permanence of fish in chilled sea water (CSW) and refrigerated seawater (RSW) should be carefully assessed if leaching and other effects (e.g., uptake of salt from the seawater, whitening of fish eyes and gills) are to be avoided.

During the past there was much discussion about allowing drainage from one fish box to another, and consequent reduction or increase of bacterial load by washing with drainage water. Today, apart from the fact that in many cases box design allows for external drainage of each box in a stack, it is recognized that these aspects have less importance when compared with the need for quick reduction in temperature.

(iii) ***Advantageous physical properties.*** Ice has some advantages when compared with other cooling methods, including refrigeration by air. The properties can be listed as follows:

(a) *Ice has a large cooling capacity.* The latent heat of fusion of ice is about 80 kcal/kg. This means that a comparatively small amount of ice will be needed to cool 1 kg of fish.

For example, for I kg of lean fish at 25°C, about 0.25 kg of melted ice will be needed to reduce its temperature to 0°C (see Equation 7.c). The reason why more ice is needed in practice is mainly because ice melting should compensate for thermal losses.

The correct understanding of this ice characteristic is the main reason for the introduction of insulated fish containers in fish handling, particularly in tropical climates. The rationale is: ice keeps fish and the insulated container keeps ice. The possibility to handle fish with reduced amounts of ice improves the efficiency and economics of fresh fish handling (more volume available for fish in containers, trucks and cold storage rooms, less weight to transport and handle, reduction in ice consumption, less water consumed and less water drained).

(b) *Ice melting is a self-contained temperature control system.* Ice melting is a change in the physical state of ice (from solid to liquid), and in current conditions it occurs at a constant temperature (0°C).

This is a very fortunate property without which it would be impossible to put fresh fish of uniform quality on the market. Ice that melts around a fish has this property on all contact points. In the case of mechanical refrigeration systems (e.g., air and RSW) a mechanical or electronic control system (properly tuned) is needed; nevertheless, controlled temperature will be always an average temperature.

Depending on the volume, design and control scheme of mechanical refrigeration systems, different temperature gradients may appear in chill storage rooms and RSW holds, with fish slow freezing in one comer and maybe above 4°C in another comer. Although the need for proper records and control of temperature of chill storage rooms has been emphasized recently in connection with the application of HACCP (Hazard Analysis Critical Control Point) to fresh fish handling, it is clear that the only system that can assure accurate temperature control at the local level (e.g., in any box within a chill storage room) is ice melting.

Ice made of sea water melts at a lower temperature than fresh water ice, depending on the salt content. Theoretically with 3.5 % of salt content (the average salt content of seawater) seawater ice will melt at about - 2.1°C However, as ice made out of seawater is physically unstable (ice will tend to separate from salt), brine will leach out during storage lowering the overall temperature (and this is the reason why sea water ice always seems wet). In these conditions, fish may become partially frozen in storage conditions and there may be some intake of salt by the fish muscle. Therefore, it cannot be said that ice made out of seawater has a proper self-controlled temperature system.

There is a narrow range of temperature below 0°C before fish muscle starts to freeze. The freezing point of fish muscle depends on the concentration of different solutes in the tissue fluids: for cod and haddock, it is in the range of -0.8 to - 1 °C, for halibut -1 to -1.2°C, and for herring about -1.4°C (Sikorski, 1990).

The process of keeping fish below 0°C and above the freezing point is called superchilling, and it allows achievement of dramatic increases in overall keeping times. In principle it could be obtained using seawater ice or mixtures of seawater and freshwater ice, or ice made out of a 2% brine and/or mechanical refrigeration. However, in large volumes it is very difficult to control temperature so precisely and temperature gradients, partial freezing of fish in some pockets and hence lack of uniformity in quality are unavoidable (see section 6. 1).

(iv) ***Convenience***. Ice has a number of practical properties that makes its use advantageous. They are:

(a) It is *a portable cooling method.* It can be easily stored, transported and used. Depending on

the type of ice, it can be distributed uniformly around fish.

(b) *Raw material to produce ice is widely available.* Although clean, pure water is becoming increasingly difficult to find, it is still possible to consider it a widely available raw material. When there is no assurance that freshwater to produce ice will be up to the standard of drinking water, it should be properly treated, e.g., chlorination.

Clean seawater can also be utilized to produce ice. Ice from seawater is usually produced where freshwater is expensive or in short supply. However, it should be remembered that harbour waters are hardly suitable for this purpose.

(c) Ice can be a relatively cheap method of preserving fish. This is particularly true if ice is properly produced (avoiding wastage of energy at ice plant level), stored (to avoid losses) and utilized properly (not wasted).

(d) *Ice is a safe food-grade substance. If* produced properly and utilizing drinking water, ice is a safe food substance and does not entail any harm either to consumers or those handling it. Ice should be handled as food.

(v) **Extended shelf life.** The overall reason for icing fish is to extend fresh fish shelf life in a relative simple way as compared to storage of un-iced fish at ambient temperatures above 0°C (see Chapter 6). However, extension of shelf life is not an end in itself, it is a means for producing safe fresh fish of acceptable quality.

Most landed fish can be considered a commodity, that is, an article of trade. Unlike other food commodities, it is usually highly perishable and it is thus in the interest of the seller and the buyer to ensure fish safety at least until it is consumed or further processed into a less perishable product. Ice and refrigeration in general, by making possible extension of fish shelf life, convert fresh fish into a true trade commodity, both at local and international level.

Ice is used to make fish safe and of better quality to consumers. It is also used because otherwise the current fish trade at local and international level would be impossible. Shelf life is extended because there is a strong economic reason to do so. Fishermen and fish processors who fail to handle fresh fish appropriately ignore the essence of their business. The inability to recognize fresh fish also as a trade commodity is at the root of misunderstandings and difficulties linked to the improvement of fish handling methods and prevention of post-harvest losses.

Types of ice

Ice can be produced in different shapes; the most commonly utilized in fish utilization are flake, plate, tube and block. Block ice is ground before being utilized to chill fish.

Ice from freshwater, of whatever source, is always ice and small differences in salt content or water hardness do not have any practical influence, even if compared with ice made out of distilled water. The physical characteristics of the different types of ice are given in Table 7.1.

Cooling capacity is expressed by weight of ice (80 kcal/kg); therefore it is clear from Table 7.1 that the same volume of two different types of ice will not have the same cooling capacity. Ice volume per unit of weight can be more than twice that of water, and this is important when ice stowage and volume occupied by ice in a box or container are considered. Ice necessary to cool

fish to 0°C or to compensate for thermal losses is always expressed in kilogrammes.

Under tropical conditions ice starts to melt very quickly. Part of the melted water drains away but part is retained on the ice surface. The larger the ice surface per unit of weight the larger the amount of water retained on the ice surface. Direct calorimetric determinations show that at 27°C the water on the surface of flake ice at steady conditions is around 12-16% of the total weight and in crushed ice, 10-14% (Boeri et al., 1985). To avoid this problem, ice may be subcooled; however, under tropical conditions this effect is quickly lost. Therefore a given weight of wet ice will not have the same cooling capacity as the same weight of dry (or subcooled) ice, and this should be taken into account when making estimations of ice consumption.

Table 7.1 Physical characteristics of ice utilized in chilling fish. Adapted from Myers (1981)

Types	Approximate Dimensions (1)	Specific volume (m^3/t) (2)	Specific weight (t/ m^3)
Flake	10/20 - 2/3 mm	2.2 -2.3	0.45-0.43
Plate	30/50 - 8/15 mm	1.7 - 1.8	0.59-0.55
Tube	50(D)- 10/12 mm	1.6 - 2.0	0.62-0.5
Block	Variable (3)	1.08	0.92
Crushed block	Variable	1.4 - 1.5	0.71 -0.66

Notes:

(1) They depend on the type and adjustment of the ice machine.
(2) Indicative values, it is advisable to determine them in practice for each type of ice plant.
(3) Usually in blocks of 25 or 50 kg each.

There is always the question of which is the "best" ice to chill fish. There is no single answer. In general, flake ice will allow for an easier, more uniform and gentle distribution of ice around fish and in the box or container and will produce very little or no mechanical damage to fish and will chill fish rather more quickly than the other types of ice (see Figure 7.2). On the other hand, flake ice will tend to occupy more volume of the box or container for the same cooling capacity and if wet, its cooling capacity will be reduced more than the other types of ice (since it has a higher area per unit of weight).

With crushed ice there is always the risk of large and sharp pieces of ice that can damage fish physically. However, crushed ice usually contains fines that melt quickly on the fish surface and large pieces of ice that tend to last longer and compensate for thermal losses. Block ice requires less stowage volume for transport, melts slowly, and contains less water at the time it is crushed than flake or plate ice. For these reasons, many artisanal fishermen utilize block ice (e.g., in Colombia, Senegal and the Philippines).

Probably tube ice and crushed ice are more suitable for use in CSW systems if ice is wet (as it normally is under tropical conditions), since they will contain less water on their surfaces.

There are also economic and maintenance aspects that may play a role in deciding for one type of ice or another. The fish technologist should be prepared to analyze the different aspects involved.

Cooling rates

Cooling rates depend mainly on the surface per unit of weight of fish exposed to ice or chilled ice/water slurry. The larger the area per unit of weight the quicker the cooling rate and the shorter the time required to reach a temperature around 0°C at the thermal centre of the fish. This concept is also expressed as "the thicker the fish the lower the cooling rate".

Small species such as shrimp, sardines, anchovies and jack mackerels cool very quickly if properly handled (e.g., in CSW or CW). Large fish (e.g., tuna, bonito, large sharks) could take considerable time to cool. Fish with fat layers and thick skin will take longer to cool than lean fish and fish with thin skin of the same size.

In the case of large fish, it is advisable to gut them and to put ice into the empty belly as well as around it. In large sharks, gutting alone may not be enough to prevent spoilage during chilling, and therefore it is advisable to gut the shark, to skin it and to cut the flesh into sizeable portions (e.g., 2-3 cm thick) and to chill them as soon as possible. Chilled sea water (CSW) has in this case the advantage of extracting some of the urea present in shark muscle (see section 4.4). However, this is an extreme case, since in current situations fillets kept in ice will last less time than gutted fish or whole fish (because of the unavoidable microbial invasion of the flesh) and will lose soluble substances.

Typical curves for cooling fish in ice, using different types of ice and chilled water (CW) are shown in Figure 7.2.

From Figure 7.2 it is clear that the quickest method to chill fish is with chilled water (CW) or chilled sea water (CSW), although the practical difference with flake ice is not great. There are, however, noticeable differences after the quick initial drop in temperature with crushed block ice and tube ice, due to differences in contact areas between fish and ice and flow of melt-water.

Cooling curves may also be affected by the type of container and external temperature. Since ice will melt to cool fish and simultaneously to compensate for thermal losses, temperature gradients may appear in actual boxes and containers. This type of temperature gradient could affect the cooling rate, particularly in boxes at the top or side of the stacks, and more likely with tube and block crushed ice.

Curves such as those shown in Figure 7.2 are useful to determine the critical limit of chilling rates when applying HACCP to fresh fish handling. For instance, in specifying a critical limit for chilling fish "to be at 4.5°C in the thermal centre in no more than 4 hours", in the case of Figure 7.2 it could be achieved only by using flake ice or CW (or CSW).

In most cases the delay in reaching 0°C in the thermal centre of the fish may not have much practical influence because the surface temperature of the fish will be at 0°C. On the other hand, warming-up of the fish is much riskier because the fish surface temperature (which is actually the

riskiest point) will almost immediately be at the external temperature, and therefore ready for spoilage. As large fish will take longer than small fish to warm up and also have less surface area (where spoilage starts) per unit of volume than small fish, they usually take a little longer to spoil than small fish. This circumstance has been widely used (and abused) in practice in the handling of large species (e.g., tuna and Nile perch).

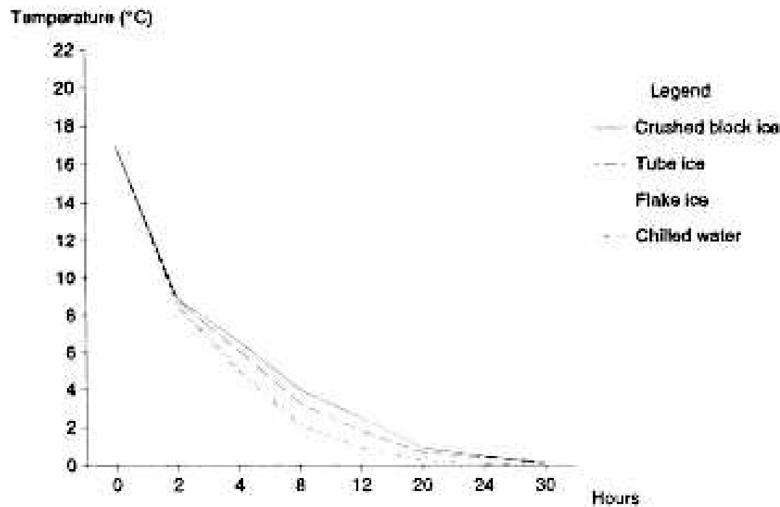


Figure 7.2 Chilling of large yellow croaker *(Pseudosciaena crocea)* with three different types of ice and chilled water (CW). Ice-to-fish ratio 1: 1; the same type of insulated containers (with drainage) was used in a parallel experiment (data obtained at the FAO/DANIDA National Workshop on Advances in Chilling and Processing Technology of Fish, Shanghai, China, June 1986)

Small species will warm up very quickly and definitely more quickly than large species (warming-up the same reason for which they cool faster). Although warming-up studies of fresh fish have received little attention in the past, they are necessary within an HACCP scheme, to determine critical limits (e.g., maximum time fish can be handled without ice in a fish processing line).

With application of HACCP and HACCP-based systems, thermometers including electronic thermometers, should be a standard tool in fish processing plants. Therefore, it is advisable to perform fish cooling and warming-up trials on actual conditions.

Ice consumption

Ice consumption can be assessed as the sum of two components: the ice necessary to cool fish to 0°C and the ice to compensate for thermal losses through the sides of the box or container.

Ice necessary to cool fish to 0°C

The amount of ice theoretically necessary to cool down fish from a temperature Tf to 0°C using ice can easily be calculated from the following energy balance:

$$L \cdot mi = mf \cdot cpf \cdot (Tf - 0) \qquad 7.a$$

where:

L = latent heat of fusion of ice (80 kcal/kg)
mi = mass of ice to be melted (kg)
mf = mass of fish to be cooled (kg)
cpf = specific heat capacity of fish (kcal/kg · °C)

From (7.a) it emerges that:

$$mi = mf \cdot cpf \cdot Tf / L \qquad 7.b$$

The specific heat capacity of lean fish is approximately 0. 8 (kcal/kg · °C). This means that as a first approximation:

$$mi = mf \cdot Tf / 100 \qquad 7.c$$

This is a very convenient formula, easily remembered, to quickly estimate the quantity of ice needed to cool fish to 0°C.

Fatty fish have lower cpf values than lean fish and, in theory, require less ice per kilogramme than lean fish; however, for safety purposes it is advisable to make calculations as if fish were always lean. Refinements in the determination of cpf are possible; however, they do not drastically alter the results.

The theoretical quantity necessary to cool fish to 0°C is relatively small and in practice much more ice is used to keep chilled fish. If we relate the proper fish handling principle of surrounding middle and large sized fish with ice, to the approximate dimensions of ice pieces (see Table 7. 1), it is clear that with some types of ice (tube, crushed block and plate) greater quantities are required for physical considerations alone.

However, the main reason for using more ice is losses. There are losses due to wet ice and ice spilt during fish handling, but by far the most important losses are thermal losses.

Ice necessary to compensate for thermal losses

In principle, the energy balance between the energy taken by the melted ice to compensate heat from outside the box or container could be expressed as follows:

$$L \cdot (dMi/dt) = U \cdot A \cdot (Te - Ti) \qquad 7.\ d$$

where:

Mi = mass of ice melted to compensate for thermal losses (kg)
U = overall heat transfer coefficient (kcal/hour · m^2 · °C)
A = surface area of the container (m^2)
Te = external temperature
Ti = ice temperature (usually taken as 0 °C)
t = time (hours)

Equation (7.d) can be easily integrated (assuming Te = constant) and the result can be expressed as:

$$Mi = Mio - (U \cdot A \cdot Te / L) \cdot t \qquad 7.e$$

It is possible to estimate thermal losses, calculating U and measuring A. However, this type of calculation will seldom give an accurate indication of ice requirements, for a number of practical factors (lack of reliable data on materials and conditions, irregularities in the construction of containers, irregular geometric shape of boxes and containers, influence of lid and drainage, radiation effect, type of stack).

More accurate calculations of ice requirements can be made if meltage tests are used to determine the overall heat transfer coefficient of the box or container, under actual working conditions (Boeri *et al.,* 1985; Lupin, 1986 a).

Ice meltage tests are very easy to conduct and no fish are needed. Containers or boxes should be filled with ice and weighed before commencing the test. At given periods, the melted water is drained (if it has not already drained) and the container is weighed again. The reduction of weight is an indication of the ice lost due to thermal losses. In Figure 7.3 the results of two ice meltage tests obtained under field conditions are presented.

Initially, some ice will be melted to cool down the walls of the box or container; depending on the relative size and weight of the container, wall materials and thickness and entity of the thermal losses this amount may be negligible. If it is not, the container can be cooled down before starting the test, or the ice necessary to cool down the container can be calculated by the difference disregarding the first part of the meltage test. A constant air surrounding temperature would be preferable and it can be achieved during short periods (e.g., the testing of a plastic box in tropical conditions). However, reasonably constant temperatures may be achieved during the intervals between weight loss measurements and an average used in the calculations.

Results as shown by Figure 7.3 can be interpolated empirically by a straight line equation of the form:

$$Mi = Mio - K \cdot t \qquad 7.f$$

Comparing Equations 7.e and 7.f, it is clear that:

$$K = (Uef \cdot Aef \cdot Te / L)$$

where:

Uef = overall effective heat transfer coefficient
Aef = effective surface area

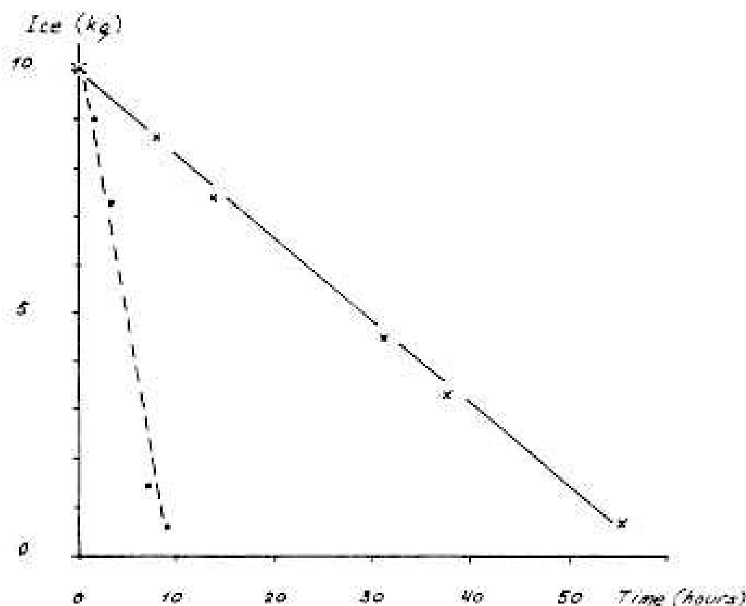


Figure 7.3 Results of ice meltage tests under field conditions. (·) standard plastic box (not insulated) 40 kg total capacity, (x) insulated plastic fish container (Metabox 70, DK). Both kept in the shade, un-stacked, flake ice, average external temperature (Te) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

From Expression 7.g it follows that:

$$K = K' - Te \qquad 7.h$$

and eventually K' could be determined, if experiments can be conducted at different controlled temperatures.

The advantage of meltage tests is that K can be obtained experimentally from the slope of straight lines, as appears in Figure 7.3, either graphically or by numerical regression (now found as sub-routine in common pocket scientific calculators). In the case of the straight lines appearing in Figure 7.3 the correlations found are as follows:

Plastic box:

$$Mi = 10.29 - 1.13 \cdot t, \quad r = -0.995 \qquad 7.i$$

K = 1. 13 kg of ice/hour

Insulated container:

$$Mi = 9.86 - 0.17 \cdot t, \quad r = 0.998 \qquad 7.j$$

K = 0. 17 kg of ice/hour

where r = correlation coefficient.

From 7.i and 7.j it follows that the ice consumption due to thermal losses in these conditions will be 6.6 times greater in the plastic box than in the insulated container. It is clear that under tropical conditions it will be practically impossible to handle fish in ice properly utilizing only non-insulated boxes, and that insulated containers will be needed, even if additional mechanical refrigeration is used.

The total amount of ice needed will be the result of adding mi (see Equations 7.b and 7.c) to Mi (according to expression 7.f) once t (the time fish should be kept chilled in the box or container in the particular case) has been estimated.

Under tropical conditions it may happen that, depending on the estimated t, total available volume in the box or container might not be enough even for ice to compensate for thermal losses, or the remaining volume for fish could be insufficient to make the chilling operation attractive.

In such cases it might be feasible to introduce one or more re-icing steps, or to resort to additional mechanical refrigeration (see Figure 7.5 to observe the effect of storage in a chill room on ice consumption). In practice, an indication of when re-icing is needed would be given to foremen or people in charge.

An analytical approach to this problem in connection with the estimation of the right ice-to-fish ratio in insulated containers can be found in Lupin (1986 b).

Ice consumption in the shade and in the sun

An important consideration, particularly in tropical countries, is the increased ice consumption in boxes and insulated containers when exposed to the sun. Figure 7.4 gives the results of a experimental meltage test conducted with a box in the shade and the same box (same colour) in the sun.

The plastic box in the shade is the same plastic box of Figure 7.3 (see Equation 7.i). The correlation for the plastic box in the sun is:

$$Mi = 9.62 - 3.126 \cdot t \qquad 7.\ k$$

This means that for this condition and this type of box, the ice consumption in the sun will be 2.75 times that in the shade (3.126/1.13). This considerable difference is due to the radiation effect. Depending on the surface material, type of material, colour of the surface and solar irradiation, it

will be a surface radiation temperature, that is higher than dry bulb temperature. Direct measurements on plastic surfaces of boxes and containers on field conditions, in tropical countries, have given values of surface radiation temperature up to 70°C.

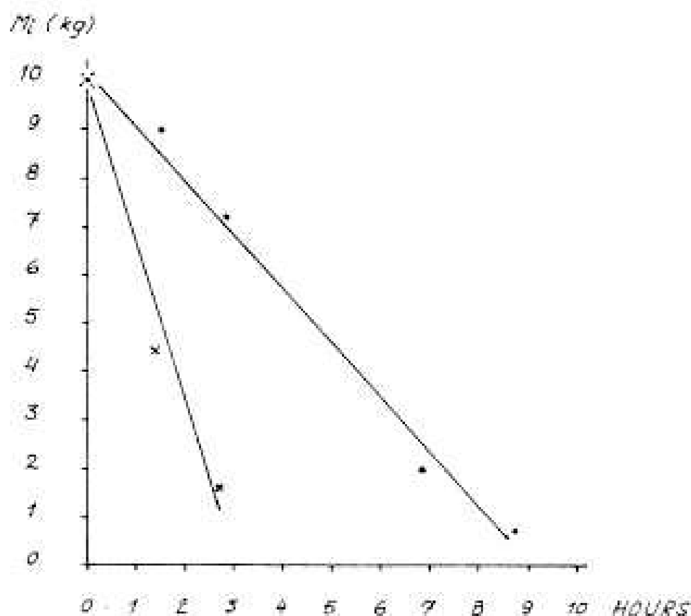


Figure 7.4 Results of ice meltage tests under field conditions. (·) plastic box in the shade, (x) plastic box in the sun. Plastic boxes, 40 kg capacity, red colour, unstacked, flake ice, external average temperature (dry bulb) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

It is clear that there is little practical possibility in tropical countries to handle chilled fish in plastic boxes exposed to the sun. An increase in ice consumption, even if less dramatic than in plastic boxes, can be measured in insulated containers exposed to the sun.

The obvious advice in this case is to keep and handle fish boxes and containers in the shade. This measure can be complemented by covering the boxes or containers with a wet tarpaulin. The wet tarpaulin will reduce the temperature of the air in contact with boxes and containers to the wet bulb temperature (some degrees below the dry bulb temperature, depending on the Equilibrium Relative Humidity - ERH - of the air), and will practically stop noticeable radiation effect (since there are always radiation effects between a body and its background).

Ice consumption in stacks of boxes and containers

In a stack of boxes or containers not all of them will lose ice in the same way. Figure 7.5 gives the results of an ice meltage test conducted on a stack of boxes. Boxes or containers at the top will consume more ice than boxes and containers at the bottom, and those in the middle will consume less than either.

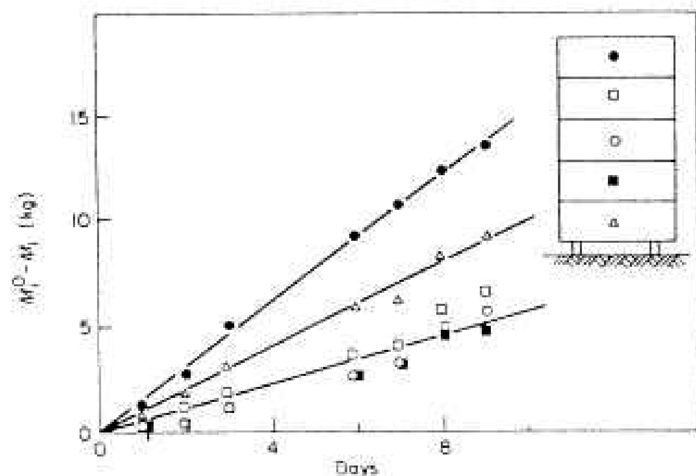


Figure 7.5 Results of ice meltage tests during storage in a stack of plastic boxes. Plastic boxes 35 kg in a chill storage room at 5°C, flake ice (from Boeri et al. (1985)

Jensen and Hansen (1973) and Hansen (1981) presented a system ("Icibox"), mainly for artisanal fisheries. In this system, a stack of plastic boxes were insulated by placing wooden frames, filled with polystyrene, at the top and at the bottom of the stack, and covering the whole with a case made out of canvas or oil skin. A similar system, composed of stacks of styropor boxes, accommodated in a pallet, and covered by an insulated mat of high reflective (Al) surface, is used in practice for shipment of fresh fish by air (e.g., it is utilized to ship fresh fillets of Nile perch from Lake Victoria to Europe).

Results of Figure 7.5 are also of interest to demonstrate the effect of a chill room on fresh fish handling. The use of chill rooms drastically reduces the ice consumption in plastic boxes, avoiding the need of re-icing. In a fish handling system chilling fish with ice, mechanical refrigeration is used to reduce the ice consumption and not to chill fish.

Although analytical models of ice consumption (e.g., Equations 7.a to 7.h) can be applied directly to estimate the ice consumption in simple and repetitive fish handling operations, their main importance is that they can help in arriving at solutions for the proper handling of chilled fish in rational. way (as seen from Figures 7.3, 7.4 and 7.5).

Ice consumption in the sides of boxes and containers

It is necessary to bear in mind that ice will not melt uniformly in the interior of a box or container, but meltage will follow the pattern of temperature gradients between the interior of the box/ container and the ambient. In Figure 7.6, a commercial plastic box with chilled hake shows the lack of ice in the sides due to the temperature gradients at the walls.

Following Figure 7.5, and supposing that a simple box could be devided into five subboxes, it is clear that the bottom and top of boxes and containers should receive more ice to compensate for thermal losses, the top receiving more ice than the bottom. However, in practice more ice should also be put in the sides of boxes and containers.

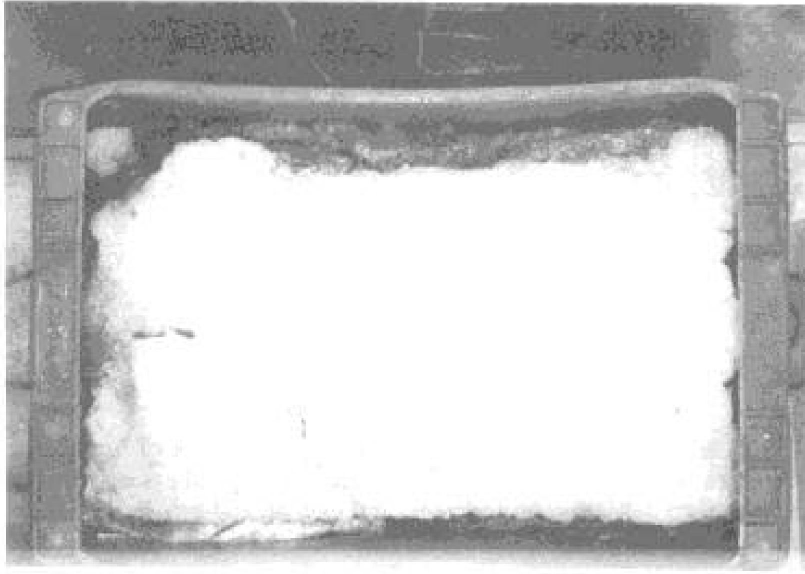


Figure 7.6 Commercial plastic box with chilled hake (M. *hubbsi)* showing the effects of lack of ice in the sides (photo H. Lupin)

The box of Figure 7.6 was initially prepared with enough ice, and it can be seen that ice is still abundant on topof the box. However, after a period of storage in a chill room, ice has melted, mainly on the sides, leaving some fish and parts of fish exposed to the air with a consequent rise in temperature and dehydration. In addition, ice and fish have formed a compact mass that can produce physical damage to exposed fish when the box is moved.

In chilled fish onboard fishing vessels or transported by truck, this problem may not exist if there is a continuous gentle movement which allows for ice melt water from the top to move to the sides. However, in chill rooms or storage rooms (insulated containers) it would be advisable to re-ice if this problem is observed. Under tropical conditions this effect is observed, even with insulated containers, in less than 24 hours of storage.

7.2 Fish handling in artisanal fisheries

Artisanal fisheries, existing both in developed and developing countries, encompass a very wide range of fishing boats from pirogues and canoes (large and small) to small outboard and onboard engine vessels, utilizing also a variety of fishing gears. It is difficult to find a common denominator; however, from a fish handling point of view, artisanal vessels handle relatively small amounts of fish (when compared with industrial vessels) and fishing journeys are usually short (usually less than one day and very often only a few hours).

In general, in tropical fisheries the artisanal fleet land a variety of species, although there are examples of the use of selective fishing gear. In temperate and cold climates artisanal fleets can focus more easily on specific species according to the period of the year; nevertheless, they may land a variety of species to respond to the market demand.

Although very often artisanal fisheries are seen as an unsophisticated practice, closer scrutiny will reveal that in many cases they are passing through a process change. There are many reasons for this process but very often the main driving forces are: urbanization, fish exports and competition with the industrial fleet.

This change in the scenario of artisanal fisheries is essential to understanding the fish handling problems faced by the artisanal and small sector of the fish industry, particularly in developing countries.

When the artisanal fleet was serving small villages, the amount of fish handled was very low; the customers usually bought the fish direct from the landing places, fishermen knew customers and their tastes, and fish was consumed within a few hours (e.g., fish caught at 06.00 h, landed and sold at 10.00 h, cooked and consumed by 13.00 h). In this situation, ice was not used, and gutting was unknown; very often fish arrived at landing places in rigor mortis (depending on fish species and fishing gear), and fish handling was at most reduced to covering the fish from the sun, keeping it moist and keeping off the flies. In Figure 7.7 two cases of landing un-iced fish by artisanal fishermen are shown.

(a) (b)

Figure 7.7 Landing by artisanal fishermen: (a) un-iced shrimp by artisanal fishermen (El Salvador, September 1987, photo H. Lupin); (b) un-iced fish (Bukova, Tanzania, 1994, photo S. P. Chen)

With urbanization and the request for safer and more quality products (as a result of exports and competition with industrial fish) conditions changed drastically. Large cities also demanded increased fish supplies, and thus middlemen and fish processors had to go to more distant landing places for fish. The amount of fish handled increased, fishing journeys lasted longer and/or passive fishing gears like gillnets were set to fish for longer times, a chain of middlemen and/or official fish markets replaced the direct buyer at the beach and, as a result of growing business (fish for income), in some places the catch effort also increased with a consequent increase in the number of fishing boats and an increase in the efficiency of the fishing gears.

In one way or another, each of the new circumstances added hours to the time which passed between catching the fish and eating or processing it (e.g., freezing). This increase in exposure of un- iced fish to ambient temperature (or water temperature for a dead fish in a gillnet), even though brief (e.g., an additional 6-12 hours), dramatically changed the situation regarding fish

spoilage and safety.

In the new situation, fish remained at ambient temperature some 13-19 or more hours. It could be already spoiled, at terminal quality and/or could present public health hazards (e.g., from the development of C. *botulinum* toxin to histamine formation). In addition to the safety and quality aspects, post-harvest losses, non-existent at subsistence level and very low at the village stage, become important. For instance, it is estimated that the post-harvest losses of Nile perch caught artisanally in Uganda amount to 25-30% of the total catch.

The situation described in previous paragraphs, and cases like those shown in Figure 7.7, moved extension services in developing countries and international technical assistance to focus on the problem of introducing improved fish handling methods at the artisanal level. The basic technical solution is the introduction of ice, proper fish handling methods and insulated containers, which is the approach utilized by most of the artisanal fleet in developed countries.

There are several examples where this approach was adopted by fishermen in developing countries and has become a self-sustained technology. Two very interesting cases to analyze are the introduction of insulated containers onboard of "navas", the traditional fishing vessels of Kakinada in Andhra Pradesh, India (Clucas, 1991) and the introduction of insulated fish containers in the pirogue fleet of Senegal (Coackley and Karnicki, 1984). The sketch of an insulated fish container for Senegalese pirogues is shown in Figure 7.8.

The insulated container of Figure 7.8 was designed to fit existing pirogues, according to the type of catch and needs expressed by fishermen. The materials and tools needed to construct the insulated container are available to fishermen in Senegal, even though some of them are imported (e.g., foam sheets and resin).

The example of Senegalese fishermen is now spreading steadily to similar fisheries in Gambia, Guinea-Bissau and Guinea which are adopting the use of insulated containers similar to those of Senegal. However, the process of diffusion and adoption of a technology, even if relatively simple, is not as straightforward as could be supposed. A pirogue with two insulated containers onboard is shown in Figure 7.9.

Once artisanal fishermen become aware of the rationale of insulated containers, they tend to favour large insulated fish containers rather than small ones. The reason is clear from Equations 7.e and 7.g, as for the same volume of fish and ice, large containers will present less external area than the area presented by several small containers. For example, a large cubic insulated fish container can be envisaged of a side measuring x m, and eight cubic insulated containers of sides equal to x/2 m presenting the same total volume as the large one. The eight containers will have an external area twice that of the big container, thus increasing the ice consumption by two, and decreasing the amount of fish that can be transported.

Other reasons are that small containers will cost more than a large one of the same total volume (simply because they need more material); small containers are not always easy to secure safely onboard small boats, and large containers allow for transport of large ice bars that can be crushed at sea (reducing stowage rate). However, large containers are difficult to handle and sometimes canoes and pirogues are very small or narrow and they cannot accommodate large insulated fish containers. This is the case for relatively small insulated fish containers. An example is shown in Figure 7. 10.

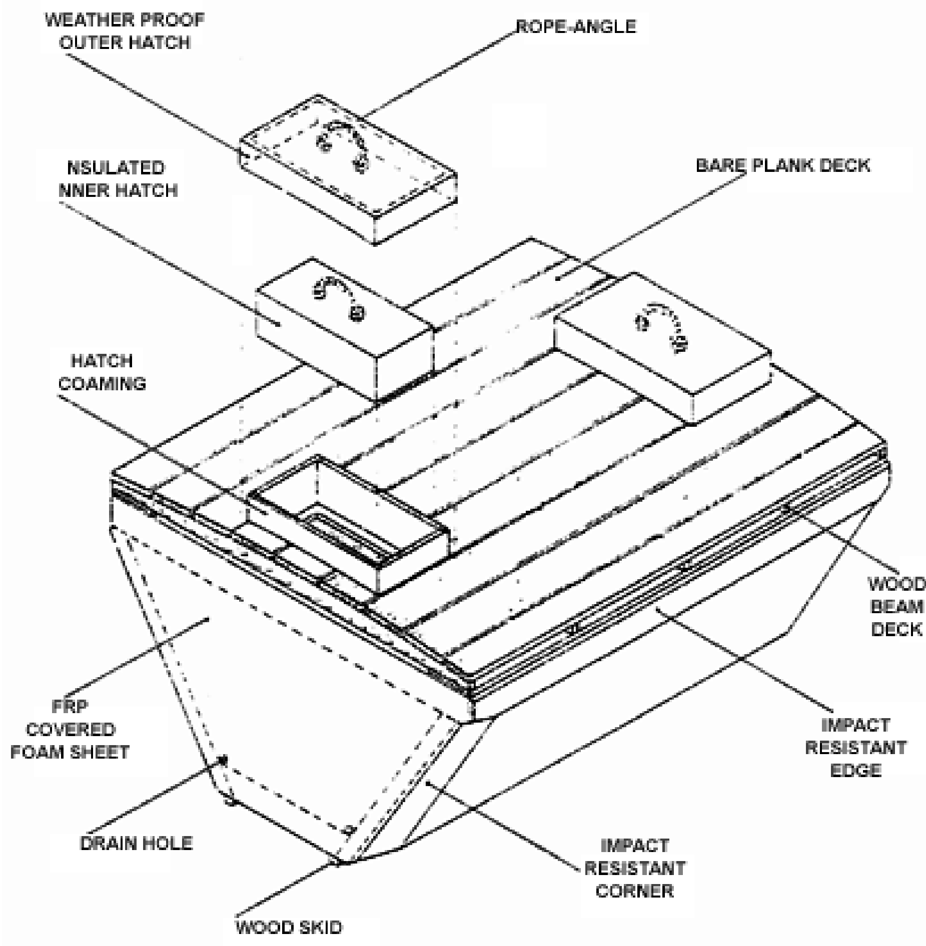


Figure 7.8 Sketch diagram of a two-hatch insulated container for Senegalese pirogues (after Coackley and Karnicki, 1985)

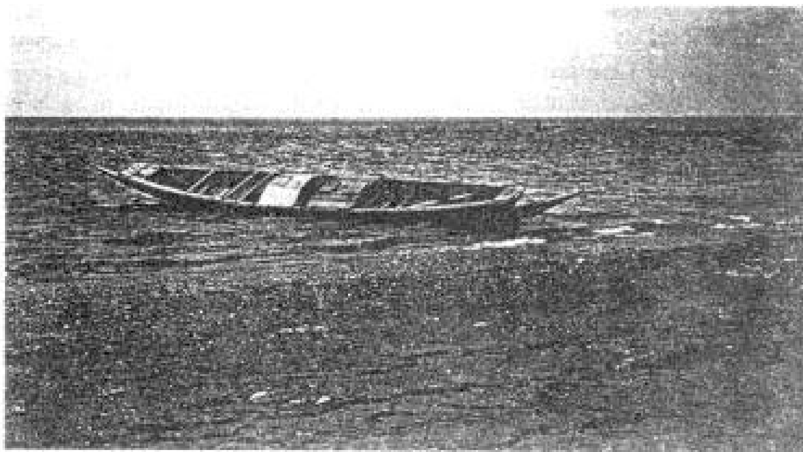


Figure 7.9 A Senegalese pirogue at the beach, carrying two insulated containers (photo B. Diakité, 1992)

Figure 7.10 Small insulated container installed onboard an artisanal fish catamaran (The Philippines, 1982, photo H. Lupin)

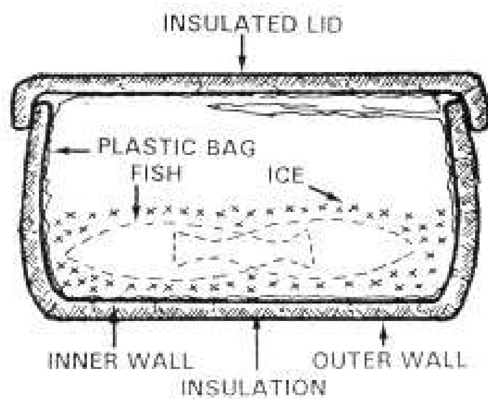
A serious constraint in many artisanal fisheries is the relatively high cost of industrial containers and the difficulty in finding appropriate industrial materials to construct them. For this reason, efforts have been made to develop artisanal containers made from locally available materials (Villadsen et al, 1979; Govindan, 1985; Clucas and Whitehead, 1987; Makene, Mgawe and Mlay, 1989; Wood and Cole, 1989; Johnson and Clucas, 1990; Lupin, 1994).

In some cases, the correct approach could be to add insulation to local fish containers; in other cases it could be necessary to develop a new container. In general, artisanal fish could be cheaper than industrial fish containers, but they will not last as long. An artisanal insulated container developed at Mbegani (Tanzania), based on the local basket container ("tenga") is shown in Figure 7.11.

A key factor in the construction of artisanal insulated containers is the selection of insulation material. There are a number of materials available: inter alia, sawdust, coconut fibre, straw, rice husks, dried grass, old tires and rejected cotton.

However, the use of such materials presents problems: the materials become wet very quickly (with the exception of old tires), losing their insulating capacity and increasing the weight of the container. When wet, most of them tend to rot very quickly. The solution is to put them inside a plastic bag (waterproof); however, in this case they tend to settle, leaving part of the walls without insulation.

With a view to overcoming these problems, the concept of "insulated pillows" was developed in various FAO/DANIDA fish technology workshops. This concept is very simple: the insulating material (e.g., coconut fibres) is placed inside one plastic tube of the type usually found to produce ordinary small polyethylene bags (10 cm in diameter); the insulating material is pressed before sealing the tube; the tube is sealed by heat at both ends (e.g., every 20 cm), and with some practice it is possible to produce a strip of "pillows". It is advisable to utilize a second tube to reduce the incidence of punctures due to fish spines and bones.



(a)

(b)

Figure 7.11 (a) Sketch of an artisanal insulated container (the "Mbegani fish container") developed and utilized in Tanzania; (b) The "Mbegani fish container" on a bicycle to distribute fresh fish. This container was initially developed at the FAO/DANIDA National Workshop on Fish Technology and Quality Control, held at Mbegani, Tanzania, May-June 1984

The strip of "insulated pillows" can then be placed between the internal and the external walls of the container. Once the container is finished with an insulated lid and handles, fish and ice can be put in a large resistant plastic bag, as shown in Figure 7.11 (a). The use of the plastic bag extends the lifespan of the container and improves fish quality.

This example indicates the type of practical problems found when developing an artisanal insulated fish container, and the possible solutions.

Why is ice not always used to chill fish when necessary

Despite the knowledge on the advantages of fish chilling, ice it is not as widely used as it should be, particularly at artisanal level in developing countries. Which are the main problems found in practice? Some of the problems that can be found are as follows:

(i) Ice should be produced mechanically

This obvious statement implies, *inter alia,* that it is not possible to produce ice artisanally for practical purposes (machines and energy are required). To produce ice under tropical conditions, from 55 to 85 kWh/l ton of ice (depending on the type of ice) are necessary whereas, in cold and temperate countries from 40 to 60 kWh are required for the same purpose. This may be a large power requirement for many locations in developing countries, particularly in islands and places relatively far from large cities or electricity networks. Ice plants require maintenance and hence trained people and spare parts (in many cases this requires access to hard currency).

A cold chain will also require chill rooms (onboard and on land), insulated containers, insulated trucks and other auxiliary equipment (e.g., water treatment units, electric generators). Besides increasing the cost, all this equipment will increase the technological difficulty associated with the fish cold chain.

(ii) Ice is produced and used within an economic context

In developed countries ice is very cheap and costs only a fraction of the price of fresh fish. In developing countries ice is very often expensive when compared with fresh fish prices.

A survey conducted in 1986 by the FAO/DANIDA Project on Training on Fish Technology and Quality Control on current fish and ice prices in fourteen African countries demonstrated that in all cases and for all the fish species, I kg of ice increased the fish price at least twice the rate recorded in developed countries. The cheaper the fish the worse the situation. For instance, in the case of small pelagics, the percentage of increase in the fish cost per kilogramme of ice added, was 40% for the "yaboy" of Senegal, 16-25% for the sardinella of Congo, and 66 % for the sardinella of Mauritania and the anchovy of Togo. The market price for fish, in this case, acts as a deterrent for the use of ice.

According to the relative cost of ice to fish, ice may or may not be used. For instance, in Accra, Ghana in 1992, it was found that using ice to chill small pelagics (Ghanian herring) in a proportion of 2 kg ice: 1 kg fish would increase the cost of fish by 32-40%. However, in the case of snapper, for the same ratio of ice to fish the cost increase would be in the range of 4.5-5.7%. The result is that ice chilling of snapper is relatively common in Accra, whereas ice is not utilized to chill small pelagics.

Very often fish compete with other sources of demand (soft drinks, beer), even if the ice machine was initially installed to supply ice for chilling fish. This and energy losses at the ice plants contribute to increase the market price of ice.

In addition to producing and utilizing ice on a sustainable basis, economic aspects must be considered (e.g., depreciation, reserves, investment). Moreover, in the case of ice manufacture there is a strong influence of the scale of production. Low ice prices in developed countries are also the result of large ice plants located at the fishing harbours that supply a large number of companies and fishing boats.

(iii) Practical constraints

Introduction of ice into fish handling systems that are not accustomed to using it can create practical problems. For instance, from Table 7.1 it is clear that the introduction of ice will increase the volume required for storage and distribution, and will reduce the effective fish hold in vessels. The use of ice will also increase the weight to be handled. This will have a number of implications such as an increased workload for the fishermen, fish processors and fishmongers, and an increase in costs and investment.

From Figures 7.3 and 7.4 it is clear that the total amount of ice needed per 1 kg of fish, in the complete cycle from the sea to the consumer will be much higher in tropical countries than in cold and temperate regions. As an indication, the average consumption of ice in the Cuban fishery industry was estimated at around 5 kg of ice per 1 kg of fish handled (including ice losses), although higher values (up to 8-10 kg of ice per 1 kg of fish) have been recorded in single industries in tropical countries; this necessitates large storage and transport capacities.

Freshwater or seawater utilized for producing ice should comply with standards (microbiological and chemical) for potable water and should be readily available in the volumes required. This is not always possible particularly in countries with energy problems (blackouts) and without (or with erratic) public tap- water distribution. If water has to be treated, this implies additional costs and additional equipment to operate and maintain.

Properly trained personnel are required to operate the ice plant and auxiliary equipment efficiently, and to handle ice and fish properly. Although many developing countries have made efforts to train people, in many cases there is a lack of technical personnel ranging from well trained fish technologists to refrigeration mechanics or electricians, or simply plant foremen.

Moreover, in many developing countries it is increasingly difficult to keep technical and professional schools operating in this field, thus jeopardizing the possibility of self-sustained training, and hence fishery industry developments.

(iv) Ice is not an additive

Knowledgeable people (e.g., fishmongers) are quickly aware of the fact that ice is not an additive. Therefore, when there is a delay in icing, ice is not usually utilized (even if available) because it will not improve fish quality. Consumers could also be intuitively aware of this fact, and they prefer to be presented with the fish as it is (e.g., at the terminal state of its quality) rather than in ice, because in this case ice will increase the price of fish but not enhance its quality. Due to the above and to the problems associated with the transition between artisanal and industrial or semi-industrial fisheries, already discussed, consumers in some countries (e.g., in Saint Lucia and Libya) tend to believe that iced fish is not fresh fish.

A need for chilled fish can develop if a market for iced fish (not just a market for "fresh fish") is developed, and to develop a market for iced fish where it does not already exist may be a very difficult and expensive endeavour as is the introduction of any other food product.

(v) Need for appropriate fish handling technologies

To chill and keep fish with ice is a very simple technique. A more complicated picture emerges when actual fish handling systems are analysed, including the economic aspect.

From a comparative study on the same fish handling operation, utilizing ice and insulated containers, carried out in both a developed and a developing country, it was seen that in developed countries, the more "appropriate" technology would aim at reducing wage costs (e.g., chutes to handle ice and fish, special tables to handle containers and boxes and conveyors to move them, machines that mix ice and fish automatically); in developing countries the main concern would be to reduce ice consumption, and to increase the fish : ice ratio in the containers (Lupin, 1986 b).

The same study found that a twentyfold difference in wage costs between developing countries and developed countries cannot offset a tenfold difference in the cost of ice. There is no "comparative advantage" in low wages in developing countries with regard to fresh fish handling. Advanced technology on fish handling from developed countries could make work easier for people in developing countries, but might not improve the economics of the operation as a whole.

There is obviously no single solution to the problems discussed above. However, it is clear that it is the problem to be solved in the coming decade in the field of fresh fish handling. With total catches having reached a plateau, losses due to the lack of ice utilization could be ill-afforded, and developing countries and artisanal fishermen in particular should not be deprived of potential market opportunities.

7.3 Improved catch handling in industrial fisheries

The aims of modern catch handling are the following:

- to maximize the quality of the landed fish raw material. It is of particular importance to provide a continuous flow in handling and to avoid any accumulation of unchilled fish, thereby bringing the important time-temperature phase under complete control.
- to improve working conditions onboard fishing vessels by eliminating those catch handling procedures which cause physical strain and fatigue to such a degree that no fishermen need to leave their occupation prematurely for health reasons.
- to give the fisherman the opportunity to concentrate almost exclusively on the quality aspects of fish handling.

To meet these aims, equipment and handling procedures that will eliminate heavy lifting, unsuitable working positions and rough handling of fish must be introduced. By doing so, the catch handling time is accelerated and the chilling process initiated much earlier than was previously the case (Olsen, 1992). The typical unit operations in catch handling are shown in Figure 7.12.

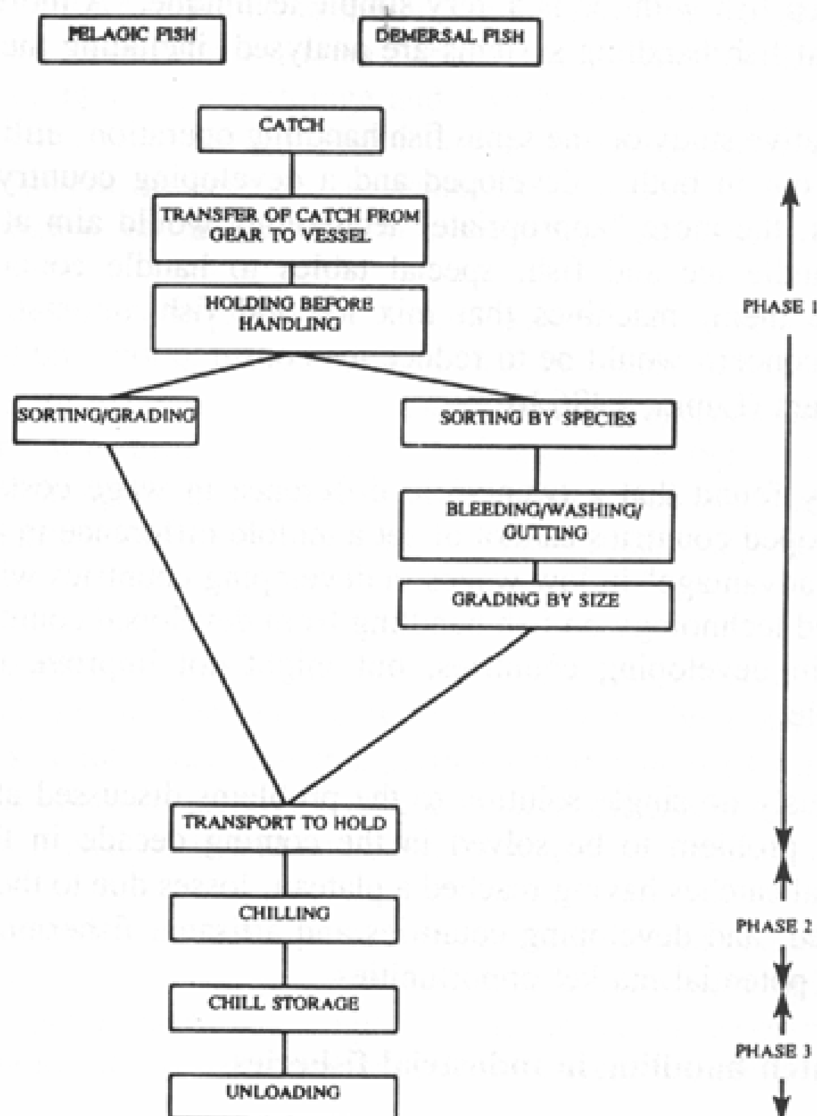


Figure 7.12 Typical unit operations in catch handling of pelagic and demersal fish

Important general aspects in modern catch handling are:

- phase one, which covers the time used for the necessary handling onboard, i.e., the time until the fish is placed in chilling medium, must be as short as possible. The fish temperature at time of capture can be high with consequent high spoilage rate.
- phase two - the chilling process - must be arranged so that a fast chilling rate is obtained for the whole catch. Maximum chilling rate will be obtained by a homogeneous mixing of fish and ice, where the individual fish is completely surrounded by ice and the heat transfer therefore is maximum, controlled by the conduction of heat through the meat to the surface. This ideal situation can be obtained during chilling of small pelagics in a chilled seawater (CSW) system; but by chilling demersal food fish in boxes with ice it is not always possible to obtain homogeneous fish/ice mixing. However, the appearance of fish completely surrounded by ice is often deteriorated due to discolorations and impression-marks. In practical life, icing is therefore often done by placing a single layer of fish on top of a layer of ice in the box even if it is bad practice from a temperature control and therefore shelf life point of view. Cooling is primarily achieved by melt-water dripping from the box stacked on top. This type of chilling will only function satisfactorily if fish boxes are shallow and have a perforated bottom.
- in phase three, which covers the chilled storage period, it is important that a homogeneous temperature at -1.5°-0°C is maintained in the fish until first hand sale. As this period may be extended for several days, this aspect has top priority.

Catch handling can be done in several ways ranging from manual methods to fully automated operations. How many operations will be used in practice and the order in which they are done depends on the fish species, the fishing gear used, vessel size, duration of the voyage and the market which has to be supplied.

Transferring catch from gear to vessel

Midwater trawlers and purse seiners fishing pelagic fish use tackling in lifts of up to 4 t, pumping or brailing for bringing the catch onboard. When lifting huge hauls (100 t or more) onboard by these methods, the danger of losing fish and gear always exists if the fish start to sink after having been brought to the surface. The speed of which the fish may sink depends on the species, catching depth and weather conditions during hauling.

Pumping the catch onboard using submersible pumps without bruising the fish can be difficult, as it is not easy to control the fish-to-water ratio during pumping.

In recent years, the so-called P/V pump (P/V - pressure/vacuum) has found increasing use. The P/V-pump principle is that an accumulation tank of 500-1500 1 size is alternately put under vacuum and pressure by a water-ring vacuum-pump (Figure 7.13). The fish, together with some water, are sucked through a hose and a valve into the tank of the system. When the tank is full, it is pressurized by changing the vacuum and pressure side connections from the tank to the pump and the fish/water mix flows through a valve and a hose into a strainer. The P/V-pump is claimed to handle the fish more gently than other fish pump types, but the capacity is generally lower, mostly because of the alternating way of operations. This problem can be solved by having two P/

V-tanks running in phase opposition using only one vacuum-pump.

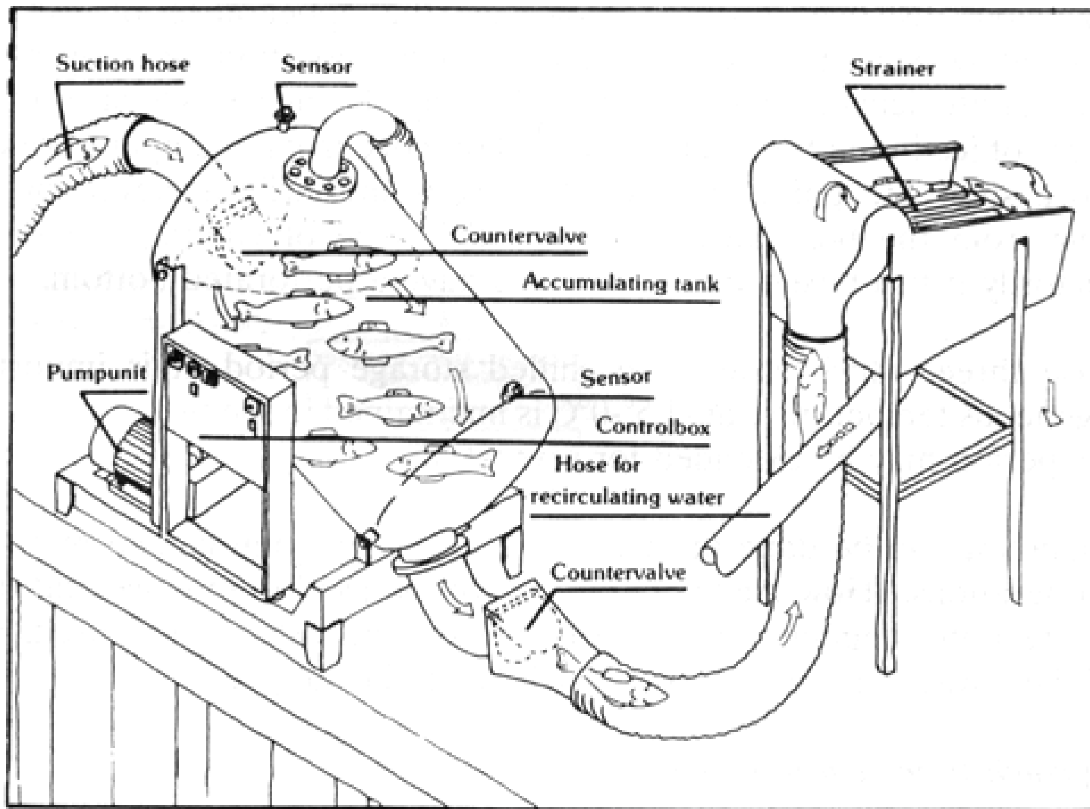


Figure 7.13 Working principle of a P/V pump

Small gillnetters (10-15 m) haul the nets with the net hauler, and very often store their catch in the net until landing. Here the net is drawn through a net shaker by two men in order to free the fish from the gear. It has been shown that the violent way in which the shaker works can be harmful to the men's hands, arms and shoulders. Ergonomic precautions have therefore been suggested to overcome this problem.

Trawlers and seiners (Danish and Scottish) tackle the catch into pounds. Commonly used pounds are those with a raised bottom which can be hoisted hydraulically. The purpose of these designs is to provide good working conditions for the crew (Figure 7.14). Also gillnetters may use a work-saving pound system, which is often connected with a conveyor to bring fish to the gutting-table.

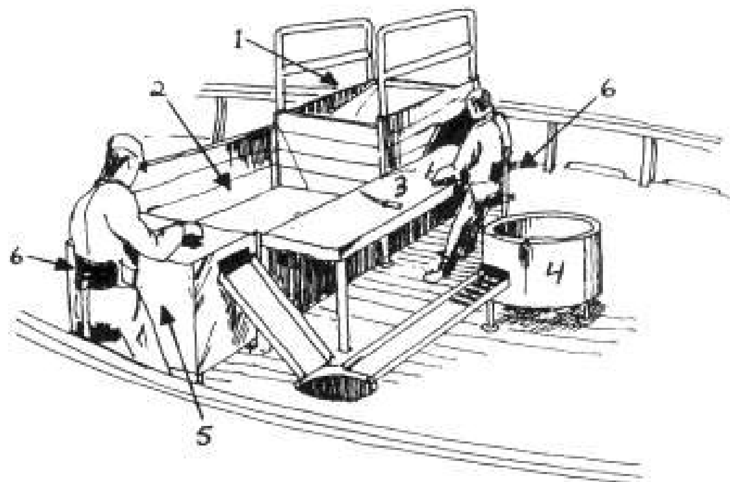


Figure 7.14 Deck lay-out for trawler using machine gutting of demersal fish
1. Tackle pound, 2. Hoisting pound, 3. Gutting table, 4. Bleeding/washing machine, 5. Gutting machine, 6. Chair.

Holding of catch before handling

When large catches are to be handled, or if for other reasons catch handling cannot start immediately, it is convenient and necessary to prechill the catch during holding in deck-pounds using ice or in tanks using Refrigerated Sea Water (RSW) or a mixture of ice and sea water (Chilled Sea Water, CSW).

Prechilling holding systems are mostly used on pelagic trawlers which grade their catches in size before storing in boxes or in portable CSW-containers. It is also essential to prechill when the pelagic fish are soft and feeding and therefore very prone to bellyburst. Prechilling tanks are unloaded by elevator or P/V-pumps. If no sorting is done onboard, the fish is conveyed directly for chilled storage in the hold. A system for holding demersal fish in tanks is shown in Figure 7.15.

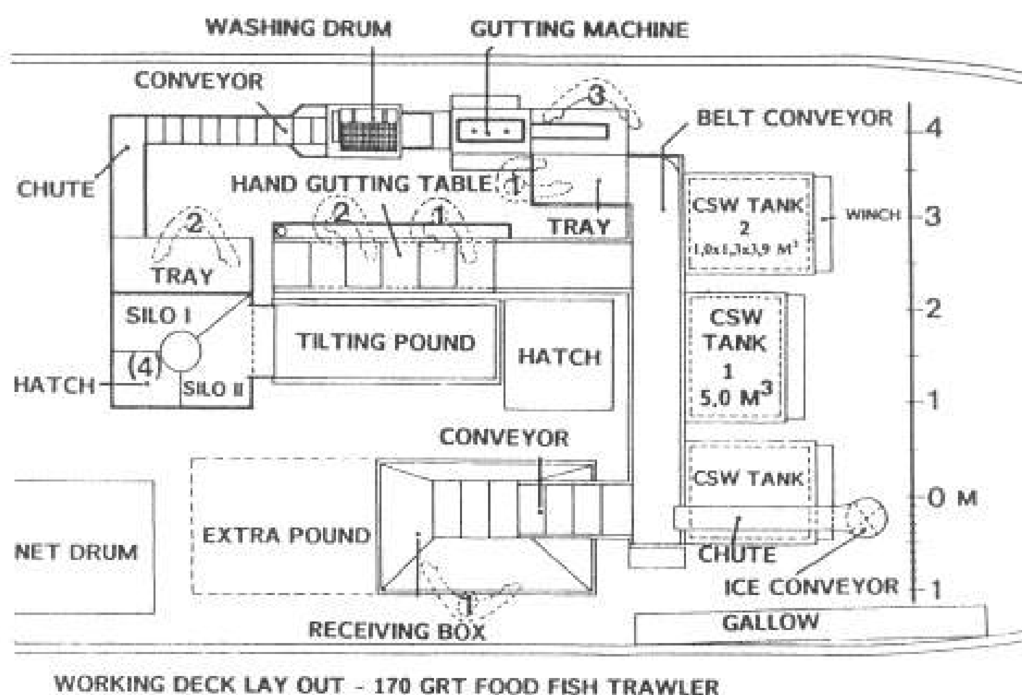


Figure 7.15 System comprising CSW raw material holding tanks before manual or machine gutting of fish

Sorting/grading

Pelagic fish are sometimes sorted or graded onboard according to size. The equipment used operates on the basis of thickness of fish using principles such as:

- vibrating, inclined diverging bars
- contrarotating, inclined, diverging rollers
- diverging conveyors where fish are being transported along a power driven V-belt.

Grading by thickness can meet the demand for the high capacity needed in pelagic fish handling, but it is generally accepted that the correlations between thickness and length or weight are not too good (Hewitt, 1980). The most important point, often forgotten, for making a grader function at its optimum is even feeding. This could be done with an elevator delivering to a (vibrating) water sprayed chute leading to the inlet guide chute of the grading machine.

Sometimes it is necessary to install a manual sorting conveyor before the grading machine for removal of larger fish and debris, e.g., in the fishery for argentine with by catch of grenadier.

Sorting and grading of demersal fish by species and by size is normally done by hand. However, some automatic systems of sorting according to width are in use. Static or dynamic weighing by marine weighing systems are also in use with good results. Research is under way using a computerized vision system for species and size grading.

Bleeding/gutting/washing

In order to obtain optimal quality in a white fillet, many white-fleshed demersal fish (but not all) need to be bled and gutted immediately after capture. The best procedures from an economic, biological and practical point of view are still under discussion (see section 3.2 on bleeding and section 6.4 on gutting).

The vast majority of fishermen are handling the fish in the easiest and also the fastest way, which means the fish are bled and gutted in one single operation. This may be done manually, but gutting machines have been introduced to obtain even more speed. The fish are transported to and from the fisherman by suitable conveyor systems. Using machines, round fish can be gutted with a speed of approximately 55 fish/minute for fish length up to 52 cm and 35 fish/minute for fish length up to 75 cm. Gutting by machine is 6-7 times faster than hand-gutting.

Existing gutting machines for round fish of the type using a circular saw blade for cutting and removing the guts destroy the valuable roe and liver. A new type of gutting machine which copies the manual gutting procedure is now available on the market. Gutting speed of this machine is 35-40 fish/minute and the roe and liver can be saved (Olsen, 1991). Flatfish can also be gutted by a recently developed machine. The speed of this machine is about 30 fish/minute.

After gutting, the fish are conveyed to the washing or bleeding operation. This may be done in pounds, often with raised bottom or in special bleeding tanks, frequently with a hydraulically-operated tilting system and rotating washing drums are also used (Figure 7.15); and special equipment such as the Norwegian and British fish washer may be used.

After catch handling (sorting, grading, gutting, etc.), the fish may be passed to an intermediate storage silo or batch holding system for the different sizes or grades before being dropped by chute to the hold, or the chutes may lead directly from the grading machines to the hold (Figure 7.16).

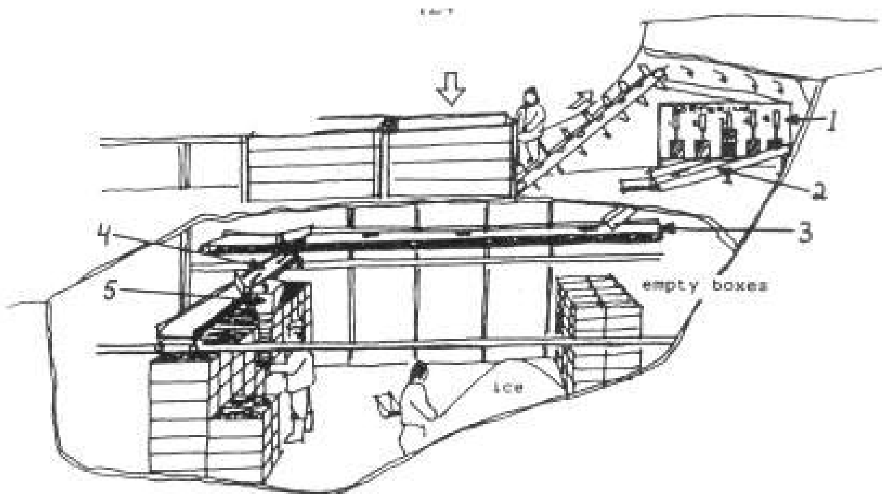


Figure 7.16 "Polar"-system. Mechanized sorting and boxing of herring 1. Herring sorting machine, 2,3,4. Conveyors, 5. Flexible dosing tube.

Chilling/chilled storage

Demersal fish have traditionally been stored on shelves or in boxes. Boxing has a big advantage over shelf storage as it reduces the static pressure on the fish and also facilitates unloading.

Shelf storage is done by alternating layers of ice and fish from one layer of ice and fish (single shelving 25 cm between shelves) up to ice/fish layers 100 cm deep. In practice, shelving often allows better temperature control than boxing and therefore also a longer storage life. Because excessive handling during unloading and excessive pressure on the fish have- a negative effect on quality, e.g., appearance, boxing is preferable to shelving, given proper icing.

In pelagic fisheries, boxed fish will be untouched until processed, but in demersal fisheries the catch is often only sorted by species onboard and not graded by size and weighed. These operations are carried out after landing before auction whereby some of the handling and quality advantages of boxing are lost.

In the near future when integrated quality assurance systems have been introduced, these unit operations will be carried out onboard and an informative label on each box will give details of factors of importance for first-hand sale (including freshness).

In general, two types of plastic fish boxes are used: stack-only and nest/stack boxes (Figures 17 a and 17 b).

To overcome some of the space problems in using stack-only boxes, the nest/stack type has been developed. These occupy only approximately a third of the space needed when stored empty compared to when the boxes are loaded with fish and ice.

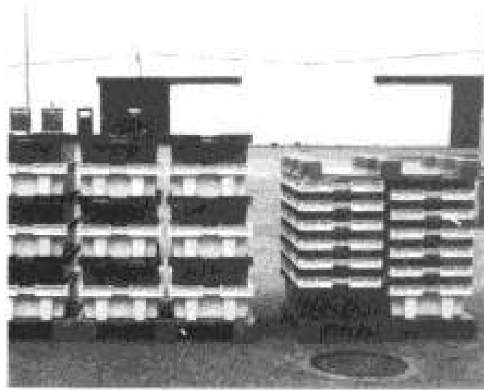


Fig. 7.17 a Stack-only boxes **Figure 7.17** b Nest/stack boxes

This type of box is widely used in France, the Netherlands and Germany and also in some Danish ports. When a system tailor-made for a certain type of plastic box is designed, the quality advantages of using boxes can be fully utilized onboard. The key points to consider are:

1. The handling rate necessary to prevent quality loss because of delayed icing. Prechilling can be of advantage to compensate lack in handling rate.
2. Handling methods which make it possible to guarantee that the icing procedure is sufficient to chill the fish to 0°C and maintain this temperature until landing.
3. The hold construction must be constructed such that safe and easy stacking of the boxes can take place.
4. Hold insulation of a relatively high quality should be considered. A small mechanical refrigeration plant can be of advantage. Air temperature in the hold should be + 1°-3°C

RSW-storage (Refrigerated Sea Water is a well established practice which has been refined both theoretically and practically since its introduction in the 1960s in Canada where it was developed for salmon and herring storage (Roach et al, 1967). At the beginning, most RSW vessels were salmon- packers and because of some failures in design which were attributed either to insufficient refrigeration or circulation systems, a standard for control of RSW-systems was established. Since vessels are different, the RSW-installation has to be studied carefully in every fishery to determine its real capability. Therefore, methods for rating each individual system and vessel and providing general specifications and guidelines for the proper installation have been suggested by the Canadian technicians (Gibbard and Roach, 1976).

In order to obtain maximum shelf life from RSW-systems, temperature homogeneity in the region of -1°C is very important. The factors affecting temperature homogeneity were recently studied in Denmark (Kraus, 1992). The most important conclusions were that the inflow of the chilled seawater in the bottom of the tank must take place over the whole tank bottom area, and that filling capacity for securing water circulation and temperature homogeneity is dependent on fish species. The necessary chilling rate was suggested to be: fish temperature must be below 3°C within four hours and below 0°C after 16 hours, and the temperature should be kept between -1.5°C and 0°C until unloading.

The CSW system has also been developed in Canada as a much cheaper means an investment point of view - to obtain rapid uniform chilling of fish. The most popular method used is the so-called "Champagne" method where rapid heat transfer between fish and ice is obtained by agitation with compressed air introduced at the bottom of the tanks, instead of using circulation

pumps as in RSW and some earlier CSW designs (Figure 7.18) (Kelmann, 1977; Lee, 1985). An indication of the chilling rate for herring could be: reduction of fish temperature from 15°C to 0°C within two hours. The concept of a CSW system is to load well insulated tanks at the harbour with the amount of ice necessary to chill the catch to between 0' and - 1°C and maintain this temperature until unloading.

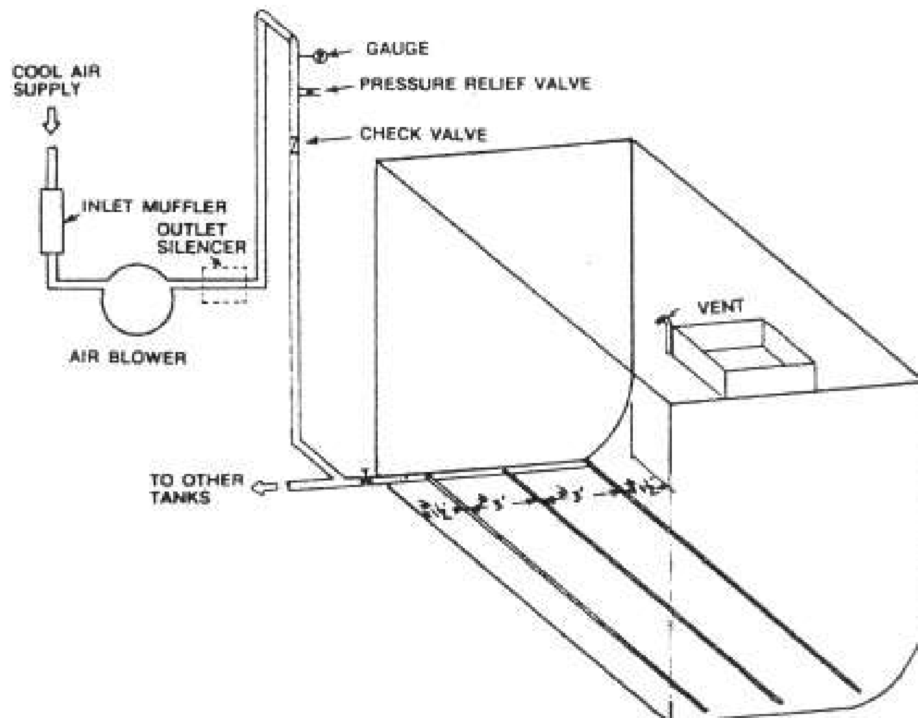


Figure 7.18 Chilled seawater system: piping layout

The Canadian west-coast fishermen are achieving this in practice by using a minimum of seawater when they start loading the tank and by forcing air through the ice-sea-water-fish-mixture only during loading, and stop forcing air immediately when the tankis full. Thereafter they will force the air only for 5-10 minutes with 3-4 hours' interval. The air agitation therefore only serves as a method to overcome local temperature differences in the tank. The objective is to obtain a uniform mixture of fish and ice in order to secure temperature homogeneity.

A proven rule-of-thumb for estimating the amount of ice necessary is simply to observe the amount of ice left in the tank at unloading, and compare it with temperature readings, which should be in the -1°C range measured in the landed fish. The starting situation should be conservative, which at sea-temperature around 12-14°C, for a trip lasting 7 days and with 10 cm polyurethane insulation, is 25% ice by weight of the tank capacity. The amount of ice is adjusted according to the observations on the following trips.

An analytical approach to estimate necessary ice quantities in a CSW tank system has been developed. The quantity of ice required takes into consideration tank size, catch volume, time at sea, water temperature, hold insulation and hold flooding strategy (Kolbe *et al.,* 1985).

CSW "Champagne" systems can also be used in small coastal vessels, e.g., in a fishery for small pelagic fish with vessels of 10-14 m length with a fish carrying capacity from 3 to 10 t fish (Roach, 1980).

Another way of loading a CSW tank, which is in practical use in Denmark, is to add the necessary amount of ice to the fish during loading by mixing a controlled stream of fish with a controlled stream of ice. The greatest amount of ice is added to the fish during loading. When the tank is full the voids are filled with seawater from a hose and the tank is left undisturbed, except for watercirculation by pumping or compressed air blowing for 5-10 minutes of 4-hour intervals. The ice is bulk-stored in the forward hold and the ice is shovelled into a conveyor flush with the floor. The conveyor then leads the ice to the mixing point at the deck.

The use of portable CSW containers for pelagic fish handling was tested in the early 1970s (Eddie and Hopper, 1974). The approximately 2 m^3 heat insulated containers were loaded with the necessary amount of ice from the harbour and agitated with compressed air in a similar way as for CSW-tanks. The main advantages with this method are that the fish will be undisturbed until processed and easily unloaded. The disadvantages are: marketing problems and reduced pay-load on existing vessels (Eddie, 1980). Portable 1.1m^3 CSW containers are used to a limited extent in combination with the earlier mentioned conveyor system originally laid-out for boxing without the above-mentioned reduced pay-load compared to boxing (Anon., 1986). Also, small coastal vessels can use insulated portable CSW containers (Figure 7.19).

Figure 7.19 Some of the 10 pieces of 200 l CSW containers placed on deck on a 15 GRT cod gillnet wooden boat

Unloading

Shelfed fish are unloaded, using baskets or boxes which are filled as the shelves are removed. The fish are tackled from the hold and emptied on a conveyor leading to the manual grading and weighing process.

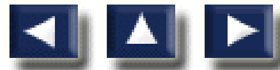
Boxed fish iced in 20 or 40 kg boxes at sea will normally be unloaded in pallet loads of, for instance, twelve 40 kg boxes per pallet. Swedish boats use hydraulic deck-mounted cranes and a special pallet fork during unloading. An unloading rate of approximately 30 t/h is possible by this method.

Danish coastal vessels, landing their pelagic catches daily, use quay mounted P/V-pumps for

unloading their catches, which often are iced in pens in layers up to approximatly 1 m height. It is necessary only to add small quantities of water to make the pump function properly. The fish is delivered to a strainer from where a conveyer leads the fish to a size grader. The strained water is recirculated to the hold. Grading machines with up to 30 t/h are often installed.

In Scandinavia the 30-50 in RSW/CSW vessels still use brailing to a limited extent when unloading their catches at a rate of 30 to 50 t/h. The main disadvantage of this method is that very big hatches are needed to obtain reasonable unloading rates.

P/V-pumps have recently been introduced for unloading herring and mackerel. Thus vessels with small tanks, e.g., 30 in , and small hatches can also be unloaded at a rate similar to or higher than the above-mentioned brailing rate. P/V-pumping rates will typically be around 40-50 t/h. The fish can be transported directly in a tube system into the factory where representative samples are taken for quality assessment.



7. IMPROVED FRESH FISH HANDLING METHODS

7.1. Basics of fresh fish handling and use of ice
7.2. Fish handling in artisanal fisheries
7.3. Improved catch handling in industrial fisheries

7.1 Basics of fresh fish handling and use of ice

Throughout history, man has preferred to consume fresh fish rather than other types of fish products. However, fish spoil very quickly and man has had to develop methods to preserve fish very early in history.

Keeping and transporting live fish

The first obvious way of avoiding spoilage and loss of quality is to keep caught fish alive until consumption. Handling of live fish for trade and consumption has been practised in China with carp probably for more than three thousand years. Today, keeping fish alive for consumption is a common fish-handling practice both in developed and developing countries and at both artisanal and industrial level.

In the case of live fish handling, fish are first conditioned in a container with clean water, while the damaged, sick and dead fish are removed. Fish are put to starve and, if possible, water temperature is reduced in order to reduce metabolic rates and make fish less active. Low metabolic rates decrease the fouling of water with ammonia, nitrite and carbon dioxide that are toxic to fish and impair their ability to extract oxygen from water. Such toxic substances will tend to increase mortality rates. Less active fish allow for an increase in the packing density of fish in the container.

A large number of fish species are usually kept alive in holding basins, floating cages, wells and fish yards. Holding basins, normally associated with fish culture companies, can be equipped with oxygen control, water filtering and circulation and temperature control. However, more simple methods are also used in practice, for instance large palm woven baskets acting as floating cages in rivers (China), or simple fish yards constructed in a backwater of a river or rivulet for large "surubi" (Platystoma spp.), "pacu" (Colossoma spp.) and "pirarucu" (Arapalma gigas) in the Amazonian and Parana basins in South America.

Methods of transporting live fish range from very sophisticated systems installed on trucks that regulate temperature, filter and recycle water and add oxygen (Schoemaker, 1991), to very simple artisanal systems of transporting fish in plastic bags with an oxygen supersaturated

atmosphere (Berka, 1986). There are trucks that can transport up to 50 t of live salmon; however, there is also the possibility of transporting a few kilo-grammes of live fish relatively easily in a plastic bag.

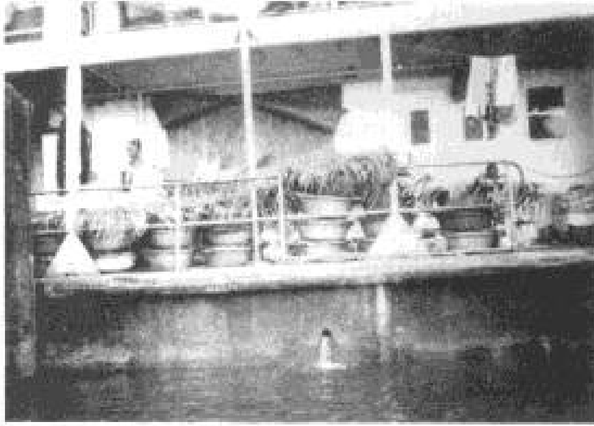
By now a large number of species, inter alia, salmon, trout, carp, eel, seabream, flounder, turbot, catfish, Clarias, tilapias, mussels, oysters, cockles, shrimp, crab and lobster are kept alive and transported, very often from one country to another.

There are wide differences in the behaviour and resistance of the various species. Therefore the method of keeping and transporting live fish should be tailored according to the particular species and the length of time it needs to be kept outside its natural habitat before slaughtering. For instance, the lungfish (Protopterus spp.) can be transported and kept alive out of water for long periods, merely by keeping its skin moist.

Some species of fish, noticeably freshwater fish, are more resistant than others to changes in oxygen in solution and the presence of toxic substances. This is probably due to the fact that their biology is adapted to the wide yearly variations in water composition presented by some rivers (cycles of matter in suspension and dissolved oxygen). In these cases, live fish are kept and transported just by changing the water from time to time in the transport containers (See Figures 7.1 (a) and (b)). This method is widely used in the Amazonian, Parana and Orinoco basins in South America; in Asia (particularly in the People's Republic of China, where also more sophisticated methods are used) and in Africa (N'Goma, 1993).

In the case presented in Figure 7.1 (a), aluminium containers with live freshwater fish are stored in the aisles of a public transport vessel. Containers are covered with palm leaves and water hyacinth to prevent the fish from jumping out of the containers and to reduce evaporation. The water in the containers is changed from time to time and an almost continuous visual control is kept on fish. Dead fish are immediately put to smoke-drying (African style) in drum smokers, also transported in the vessels or transporting barges.

In the case presented in Figure 7. 1(b), carp is kept in a metal container drawn by a bicycle. This is a rather common practice in China, and other Asian countries; for instance in Bangkok, live catfish is sold daily by street vendors.



(a) (b)

Figure 7.1 (a) Transport of live freshwater fish in Congo (Cuvette Congolaise) (N'Goma, 1993); (b) street vendor of live fish in China today (Suzhou, 1993, photo H. Lupin)

The most recent development is the keeping and transporting of fish in a state of hibernation. In this method, the body temperature of live fish is reduced drastically in order to reduce fish metabolism and to eliminate fish movement completely. The method greatly reduces death rates and increases package density, but careful temperature control should be exercised to maintain the hibernation temperature. There is an appropriate hibernation temperature for each species. Although the method is already utilized for instance to transport live "kuruma" shrimp *(Penaeus japonicus)* and lobster in pre-chilled wet sawdust, it should be considered an experimental technique for most of the species.

Although keeping and transporting live fish is becoming more and more important, it is not a viable solution for most of the bulk fish captures in the world.

Chilling fish with ice

Historical evidence proves that the Ancient Chinese utilized natural ice to preserve fish more than three thousand years ago. Natural ice mixed with seaweed was also used by the Ancient Romans to keep fish fresh. However, it was the development of mechanical refrigeration which made ice readily available for use in fish preservation.

In developed countries, particularly in USA and some European countries, the tradition of chilling fish with ice dates back more than a century. The practical advantages of utilizing ice in fresh fish handling are therefore well established. However, it is worthwhile for young generations of fish technologists and newcomers to the field, to review them, paying attention to the main points of this technique.

Ice is utilized in fish preservation for one or more of the following reasons:

(i) ***Temperature reduction.*** By reducing temperature to about 0°C the growth of spoilage and pathogenic micro-organisms (see section 6) is reduced, thus reducing the spoilage rate and

reducing or eliminating some safety risks.

Temperature reduction also reduces the rate of enzymatic reactions, in particular those linked to early *post mortem* changes extending, if properly applied, the *rigor mortis* period.

Fish temperature reduction is by far the most important effect of ice utilization. Therefore, the quicker the ice chills the better. Although cold-shock reactions have been reported in a few tropical species when iced, leading to a loss of yield of fillets (Curran *et* al., 1986), the advantage of quick chilling usually outweighs other considerations. The development of ad hoc fish handling methods is of course not ruled out in the case of species that could present cold-shock behaviour.

(ii) ***Melting ice keeps fish moist.*** This action mainly prevents surface dehydration and reduces weight losses. Melting water also increases the heat transport between fish and ice surfaces (water conducts heat better than air): the quickest practical chilling rate is obtained in a slurry of water and ice (e.g., the CSW system).

If, for some reason, ice is not utilized immediately after catching the fish, it is worthwhile keeping the fish moist. Evaporative cooling usually reduces the surface temperature of fish below the optimum growth temperature of common spoilage and pathogenic bacteria; although it does not prevent spoiling.

Ice should also be utilized in relation with chilling rooms to keep fish moist. It is advisable to keep chilling room temperature slightly above 0°C (e.g., 3-4°C).

However, water has a leaching effect and may drain away colour pigments from fish skin and gills. Ice melting water can also leach micronutrients in the case of fillets and extract relatively large amounts of soluble substances in some species (e.g., squid).

Depending on the species, severity of leaching and market requirements, an ad *hoc* handling procedure may be justified. In general, it has been found that drainage of ice meltwater is advisable in boxes and containers and that permanence of fish in chilled sea water (CSW) and refrigerated seawater (RSW) should be carefully assessed if leaching and other effects (e.g., uptake of salt from the seawater, whitening of fish eyes and gills) are to be avoided.

During the past there was much discussion about allowing drainage from one fish box to another, and consequent reduction or increase of bacterial load by washing with drainage water. Today, apart from the fact that in many cases box design allows for external drainage of each box in a stack, it is recognized that these aspects have less importance when compared with the need for quick reduction in temperature.

(iii) ***Advantageous physical properties.*** Ice has some advantages when compared with other cooling methods, including refrigeration by air. The properties can be listed as follows:

(a) *Ice has a large cooling capacity.* The latent heat of fusion of ice is about 80 kcal/kg. This means that a comparatively small amount of ice will be needed to cool 1 kg of fish.

For example, for I kg of lean fish at 25°C, about 0.25 kg of melted ice will be needed to reduce its temperature to 0°C (see Equation 7.c). The reason why more ice is needed in practice is mainly because ice melting should compensate for thermal losses.

The correct understanding of this ice characteristic is the main reason for the introduction of insulated fish containers in fish handling, particularly in tropical climates. The rationale is: ice keeps fish and the insulated container keeps ice. The possibility to handle fish with reduced amounts of ice improves the efficiency and economics of fresh fish handling (more volume available for fish in containers, trucks and cold storage rooms, less weight to transport and handle, reduction in ice consumption, less water consumed and less water drained).

(b) *Ice melting is a self-contained temperature control system.* Ice melting is a change in the physical state of ice (from solid to liquid), and in current conditions it occurs at a constant temperature (0°C).

This is a very fortunate property without which it would be impossible to put fresh fish of uniform quality on the market. Ice that melts around a fish has this property on all contact points. In the case of mechanical refrigeration systems (e.g., air and RSW) a mechanical or electronic control system (properly tuned) is needed; nevertheless, controlled temperature will be always an average temperature.

Depending on the volume, design and control scheme of mechanical refrigeration systems, different temperature gradients may appear in chill storage rooms and RSW holds, with fish slow freezing in one comer and maybe above 4°C in another comer. Although the need for proper records and control of temperature of chill storage rooms has been emphasized recently in connection with the application of HACCP (Hazard Analysis Critical Control Point) to fresh fish handling, it is clear that the only system that can assure accurate temperature control at the local level (e.g., in any box within a chill storage room) is ice melting.

Ice made of sea water melts at a lower temperature than fresh water ice, depending on the salt content. Theoretically with 3.5 % of salt content (the average salt content of seawater) seawater ice will melt at about - 2.1°C However, as ice made out of seawater is physically unstable (ice will tend to separate from salt), brine will leach out during storage lowering the overall temperature (and this is the reason why sea water ice always seems wet). In these conditions, fish may become partially frozen in storage conditions and there may be some intake of salt by the fish muscle. Therefore, it cannot be said that ice made out of seawater has a proper self-controlled temperature system.

There is a narrow range of temperature below 0°C before fish muscle starts to freeze. The freezing point of fish muscle depends on the concentration of different solutes in the tissue fluids: for cod and haddock, it is in the range of -0.8 to - 1 °C, for halibut -1 to -1.2°C, and for herring about -1.4°C (Sikorski, 1990).

The process of keeping fish below 0°C and above the freezing point is called superchilling, and it allows achievement of dramatic increases in overall keeping times. In principle it could be obtained using seawater ice or mixtures of seawater and freshwater ice, or ice made out of a 2% brine and/or mechanical refrigeration. However, in large volumes it is very difficult to control temperature so precisely and temperature gradients, partial freezing of fish in some pockets and hence lack of uniformity in quality are unavoidable (see section 6. 1).

(iv) ***Convenience****.* Ice has a number of practical properties that makes its use advantageous. They are:

(a) It is *a portable cooling method.* It can be easily stored, transported and used. Depending on

the type of ice, it can be distributed uniformly around fish.

(b) *Raw material to produce ice is widely available.* Although clean, pure water is becoming increasingly difficult to find, it is still possible to consider it a widely available raw material. When there is no assurance that freshwater to produce ice will be up to the standard of drinking water, it should be properly treated, e.g., chlorination.

Clean seawater can also be utilized to produce ice. Ice from seawater is usually produced where freshwater is expensive or in short supply. However, it should be remembered that harbour waters are hardly suitable for this purpose.

(c) Ice can be a relatively cheap method of preserving fish. This is particularly true if ice is properly produced (avoiding wastage of energy at ice plant level), stored (to avoid losses) and utilized properly (not wasted).

(d) *Ice is a safe food-grade substance. If* produced properly and utilizing drinking water, ice is a safe food substance and does not entail any harm either to consumers or those handling it. Ice should be handled as food.

(v) **Extended shelf life.** The overall reason for icing fish is to extend fresh fish shelf life in a relative simple way as compared to storage of un-iced fish at ambient temperatures above 0°C (see Chapter 6). However, extension of shelf life is not an end in itself, it is a means for producing safe fresh fish of acceptable quality.

Most landed fish can be considered a commodity, that is, an article of trade. Unlike other food commodities, it is usually highly perishable and it is thus in the interest of the seller and the buyer to ensure fish safety at least until it is consumed or further processed into a less perishable product. Ice and refrigeration in general, by making possible extension of fish shelf life, convert fresh fish into a true trade commodity, both at local and international level.

Ice is used to make fish safe and of better quality to consumers. It is also used because otherwise the current fish trade at local and international level would be impossible. Shelf life is extended because there is a strong economic reason to do so. Fishermen and fish processors who fail to handle fresh fish appropriately ignore the essence of their business. The inability to recognize fresh fish also as a trade commodity is at the root of misunderstandings and difficulties linked to the improvement of fish handling methods and prevention of post-harvest losses.

Types of ice

Ice can be produced in different shapes; the most commonly utilized in fish utilization are flake, plate, tube and block. Block ice is ground before being utilized to chill fish.

Ice from freshwater, of whatever source, is always ice and small differences in salt content or water hardness do not have any practical influence, even if compared with ice made out of distilled water. The physical characteristics of the different types of ice are given in Table 7.1.

Cooling capacity is expressed by weight of ice (80 kcal/kg); therefore it is clear from Table 7.1 that the same volume of two different types of ice will not have the same cooling capacity. Ice volume per unit of weight can be more than twice that of water, and this is important when ice stowage and volume occupied by ice in a box or container are considered. Ice necessary to cool

fish to 0°C or to compensate for thermal losses is always expressed in kilogrammes.

Under tropical conditions ice starts to melt very quickly. Part of the melted water drains away but part is retained on the ice surface. The larger the ice surface per unit of weight the larger the amount of water retained on the ice surface. Direct calorimetric determinations show that at 27°C the water on the surface of flake ice at steady conditions is around 12-16% of the total weight and in crushed ice, 10-14% (Boeri et al., 1985). To avoid this problem, ice may be subcooled; however, under tropical conditions this effect is quickly lost. Therefore a given weight of wet ice will not have the same cooling capacity as the same weight of dry (or subcooled) ice, and this should be taken into account when making estimations of ice consumption.

Table 7.1 Physical characteristics of ice utilized in chilling fish. Adapted from Myers (1981)

Types	**Approximate Dimensions (1)**	**Specific volume (m^3/t) (2)**	**Specific weight (t/ m^3)**
Flake	10/20 - 2/3 mm	2.2 -2.3	0.45-0.43
Plate	30/50 - 8/15 mm	1.7 - 1.8	0.59-0.55
Tube	50(D)- 10/12 mm	1.6 - 2.0	0.62-0.5
Block	Variable (3)	1.08	0.92
Crushed block	Variable	1.4 - 1.5	0.71 -0.66

Notes:

(1) They depend on the type and adjustment of the ice machine.
(2) Indicative values, it is advisable to determine them in practice for each type of ice plant.
(3) Usually in blocks of 25 or 50 kg each.

There is always the question of which is the "best" ice to chill fish. There is no single answer. In general, flake ice will allow for an easier, more uniform and gentle distribution of ice around fish and in the box or container and will produce very little or no mechanical damage to fish and will chill fish rather more quickly than the other types of ice (see Figure 7.2). On the other hand, flake ice will tend to occupy more volume of the box or container for the same cooling capacity and if wet, its cooling capacity will be reduced more than the other types of ice (since it has a higher area per unit of weight).

With crushed ice there is always the risk of large and sharp pieces of ice that can damage fish physically. However, crushed ice usually contains fines that melt quickly on the fish surface and large pieces of ice that tend to last longer and compensate for thermal losses. Block ice requires less stowage volume for transport, melts slowly, and contains less water at the time it is crushed than flake or plate ice. For these reasons, many artisanal fishermen utilize block ice (e.g., in Colombia, Senegal and the Philippines).

Probably tube ice and crushed ice are more suitable for use in CSW systems if ice is wet (as it normally is under tropical conditions), since they will contain less water on their surfaces.

There are also economic and maintenance aspects that may play a role in deciding for one type of ice or another. The fish technologist should be prepared to analyze the different aspects involved.

Cooling rates

Cooling rates depend mainly on the surface per unit of weight of fish exposed to ice or chilled ice/water slurry. The larger the area per unit of weight the quicker the cooling rate and the shorter the time required to reach a temperature around 0°C at the thermal centre of the fish. This concept is also expressed as "the thicker the fish the lower the cooling rate".

Small species such as shrimp, sardines, anchovies and jack mackerels cool very quickly if properly handled (e.g., in CSW or CW). Large fish (e.g., tuna, bonito, large sharks) could take considerable time to cool. Fish with fat layers and thick skin will take longer to cool than lean fish and fish with thin skin of the same size.

In the case of large fish, it is advisable to gut them and to put ice into the empty belly as well as around it. In large sharks, gutting alone may not be enough to prevent spoilage during chilling, and therefore it is advisable to gut the shark, to skin it and to cut the flesh into sizeable portions (e.g., 2-3 cm thick) and to chill them as soon as possible. Chilled sea water (CSW) has in this case the advantage of extracting some of the urea present in shark muscle (see section 4.4). However, this is an extreme case, since in current situations fillets kept in ice will last less time than gutted fish or whole fish (because of the unavoidable microbial invasion of the flesh) and will lose soluble substances.

Typical curves for cooling fish in ice, using different types of ice and chilled water (CW) are shown in Figure 7.2.

From Figure 7.2 it is clear that the quickest method to chill fish is with chilled water (CW) or chilled sea water (CSW), although the practical difference with flake ice is not great. There are, however, noticeable differences after the quick initial drop in temperature with crushed block ice and tube ice, due to differences in contact areas between fish and ice and flow of melt-water.

Cooling curves may also be affected by the type of container and external temperature. Since ice will melt to cool fish and simultaneously to compensate for thermal losses, temperature gradients may appear in actual boxes and containers. This type of temperature gradient could affect the cooling rate, particularly in boxes at the top or side of the stacks, and more likely with tube and block crushed ice.

Curves such as those shown in Figure 7.2 are useful to determine the critical limit of chilling rates when applying HACCP to fresh fish handling. For instance, in specifying a critical limit for chilling fish "to be at 4.5°C in the thermal centre in no more than 4 hours", in the case of Figure 7.2 it could be achieved only by using flake ice or CW (or CSW).

In most cases the delay in reaching 0°C in the thermal centre of the fish may not have much practical influence because the surface temperature of the fish will be at 0°C. On the other hand, warming-up of the fish is much riskier because the fish surface temperature (which is actually the

riskiest point) will almost immediately be at the external temperature, and therefore ready for spoilage. As large fish will take longer than small fish to warm up and also have less surface area (where spoilage starts) per unit of volume than small fish, they usually take a little longer to spoil than small fish. This circumstance has been widely used (and abused) in practice in the handling of large species (e.g., tuna and Nile perch).

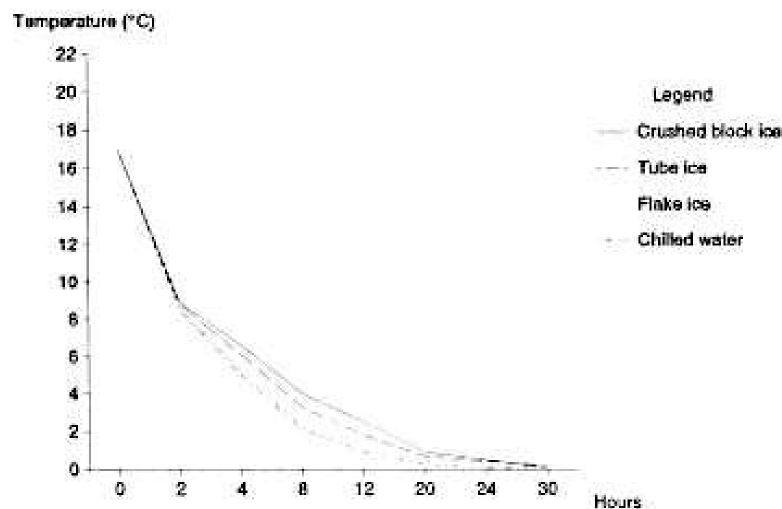


Figure 7.2 Chilling of large yellow croaker *(Pseudosciaena crocea)* with three different types of ice and chilled water (CW). Ice-to-fish ratio 1: 1; the same type of insulated containers (with drainage) was used in a parallel experiment (data obtained at the FAO/DANIDA National Workshop on Advances in Chilling and Processing Technology of Fish, Shanghai, China, June 1986)

Small species will warm up very quickly and definitely more quickly than large species (warming-up the same reason for which they cool faster). Although warming-up studies of fresh fish have received little attention in the past, they are necessary within an HACCP scheme, to determine critical limits (e.g., maximum time fish can be handled without ice in a fish processing line).

With application of HACCP and HACCP-based systems, thermometers including electronic thermometers, should be a standard tool in fish processing plants. Therefore, it is advisable to perform fish cooling and warming-up trials on actual conditions.

Ice consumption

Ice consumption can be assessed as the sum of two components: the ice necessary to cool fish to 0°C and the ice to compensate for thermal losses through the sides of the box or container.

Ice necessary to cool fish to 0°C

The amount of ice theoretically necessary to cool down fish from a temperature Tf to 0°C using ice can easily be calculated from the following energy balance:

$$L \cdot mi = mf \cdot cpf \cdot (Tf - 0) \qquad 7.a$$

where:

L = latent heat of fusion of ice (80 kcal/kg)
mi = mass of ice to be melted (kg)
mf = mass of fish to be cooled (kg)
cpf = specific heat capacity of fish (kcal/kg · °C)

From (7.a) it emerges that:

$$mi = mf \cdot cpf \cdot Tf / L \qquad 7.b$$

The specific heat capacity of lean fish is approximately 0. 8 (kcal/kg · °C). This means that as a first approximation:

$$mi = mf \cdot Tf / 100 \qquad 7.c$$

This is a very convenient formula, easily remembered, to quickly estimate the quantity of ice needed to cool fish to 0°C.

Fatty fish have lower cpf values than lean fish and, in theory, require less ice per kilogramme than lean fish; however, for safety purposes it is advisable to make calculations as if fish were always lean. Refinements in the determination of cpf are possible; however, they do not drastically alter the results.

The theoretical quantity necessary to cool fish to 0°C is relatively small and in practice much more ice is used to keep chilled fish. If we relate the proper fish handling principle of surrounding middle and large sized fish with ice, to the approximate dimensions of ice pieces (see Table 7. 1), it is clear that with some types of ice (tube, crushed block and plate) greater quantities are required for physical considerations alone.

However, the main reason for using more ice is losses. There are losses due to wet ice and ice spilt during fish handling, but by far the most important losses are thermal losses.

Ice necessary to compensate for thermal losses

In principle, the energy balance between the energy taken by the melted ice to compensate heat from outside the box or container could be expressed as follows:

$$L \cdot (dMi/dt) = U \cdot A \cdot (Te - Ti) \qquad 7.d$$

where:

Mi = mass of ice melted to compensate for thermal losses (kg)
U = overall heat transfer coefficient (kcal/hour · m^2 · °C)
A = surface area of the container (m^2)
Te = external temperature
Ti = ice temperature (usually taken as 0 °C)
t = time (hours)

Equation (7.d) can be easily integrated (assuming Te = constant) and the result can be expressed as:

$$Mi = Mio - (U \cdot A \cdot Te / L) \cdot t \qquad 7.e$$

It is possible to estimate thermal losses, calculating U and measuring A. However, this type of calculation will seldom give an accurate indication of ice requirements, for a number of practical factors (lack of reliable data on materials and conditions, irregularities in the construction of containers, irregular geometric shape of boxes and containers, influence of lid and drainage, radiation effect, type of stack).

More accurate calculations of ice requirements can be made if meltage tests are used to determine the overall heat transfer coefficient of the box or container, under actual working conditions (Boeri *et al.,* 1985; Lupin, 1986 a).

Ice meltage tests are very easy to conduct and no fish are needed. Containers or boxes should be filled with ice and weighed before commencing the test. At given periods, the melted water is drained (if it has not already drained) and the container is weighed again. The reduction of weight is an indication of the ice lost due to thermal losses. In Figure 7.3 the results of two ice meltage tests obtained under field conditions are presented.

Initially, some ice will be melted to cool down the walls of the box or container; depending on the relative size and weight of the container, wall materials and thickness and entity of the thermal losses this amount may be negligible. If it is not, the container can be cooled down before starting the test, or the ice necessary to cool down the container can be calculated by the difference disregarding the first part of the meltage test. A constant air surrounding temperature would be preferable and it can be achieved during short periods (e.g., the testing of a plastic box in tropical conditions). However, reasonably constant temperatures may be achieved during the intervals between weight loss measurements and an average used in the calculations.

Results as shown by Figure 7.3 can be interpolated empirically by a straight line equation of the form:

$$Mi = Mio - K \cdot t \qquad 7.f$$

Comparing Equations 7.e and 7.f, it is clear that:

$$K = (Uef \cdot Aef \cdot Te / L)$$

where:

Uef = overall effective heat transfer coefficient
Aef = effective surface area

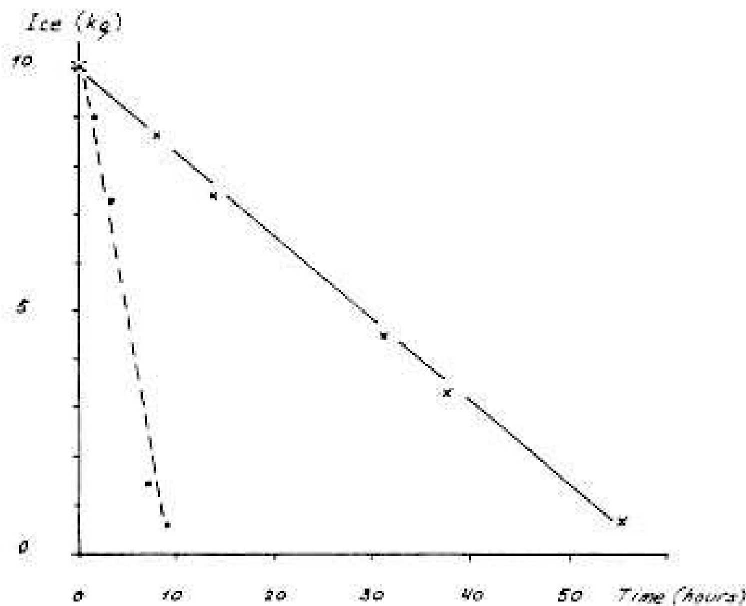


Figure 7.3 Results of ice meltage tests under field conditions. (·) standard plastic box (not insulated) 40 kg total capacity, (x) insulated plastic fish container (Metabox 70, DK). Both kept in the shade, un-stacked, flake ice, average external temperature (Te) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

From Expression 7.g it follows that:

$$K = K' - Te \qquad 7.h$$

and eventually K' could be determined, if experiments can be conducted at different controlled temperatures.

The advantage of meltage tests is that K can be obtained experimentally from the slope of straight lines, as appears in Figure 7.3, either graphically or by numerical regression (now found as sub-routine in common pocket scientific calculators). In the case of the straight lines appearing in Figure 7.3 the correlations found are as follows:

Plastic box:

$$Mi = 10.29 - 1.13 \cdot t, \quad r = -0.995 \qquad 7.i$$

K = 1. 13 kg of ice/hour

Insulated container:

$$Mi = 9.86 - 0.17 \cdot t, \quad r = 0.998 \qquad 7.j$$

K = 0. 17 kg of ice/hour

where r = correlation coefficient.

From 7.i and 7.j it follows that the ice consumption due to thermal losses in these conditions will be 6.6 times greater in the plastic box than in the insulated container. It is clear that under tropical conditions it will be practically impossible to handle fish in ice properly utilizing only non-insulated boxes, and that insulated containers will be needed, even if additional mechanical refrigeration is used.

The total amount of ice needed will be the result of adding mi (see Equations 7.b and 7.c) to Mi (according to expression 7.f) once t (the time fish should be kept chilled in the box or container in the particular case) has been estimated.

Under tropical conditions it may happen that, depending on the estimated t, total available volume in the box or container might not be enough even for ice to compensate for thermal losses, or the remaining volume for fish could be insufficient to make the chilling operation attractive.

In such cases it might be feasible to introduce one or more re-icing steps, or to resort to additional mechanical refrigeration (see Figure 7.5 to observe the effect of storage in a chill room on ice consumption). In practice, an indication of when re-icing is needed would be given to foremen or people in charge.

An analytical approach to this problem in connection with the estimation of the right ice-to-fish ratio in insulated containers can be found in Lupin (1986 b).

Ice consumption in the shade and in the sun

An important consideration, particularly in tropical countries, is the increased ice consumption in boxes and insulated containers when exposed to the sun. Figure 7.4 gives the results of a experimental meltage test conducted with a box in the shade and the same box (same colour) in the sun.

The plastic box in the shade is the same plastic box of Figure 7.3 (see Equation 7.i). The correlation for the plastic box in the sun is:

$$Mi = 9.62 - 3.126 \cdot t \qquad 7.\ k$$

This means that for this condition and this type of box, the ice consumption in the sun will be 2.75 times that in the shade (3.126/1.13). This considerable difference is due to the radiation effect. Depending on the surface material, type of material, colour of the surface and solar irradiation, it

will be a surface radiation temperature, that is higher than dry bulb temperature. Direct measurements on plastic surfaces of boxes and containers on field conditions, in tropical countries, have given values of surface radiation temperature up to 70°C.

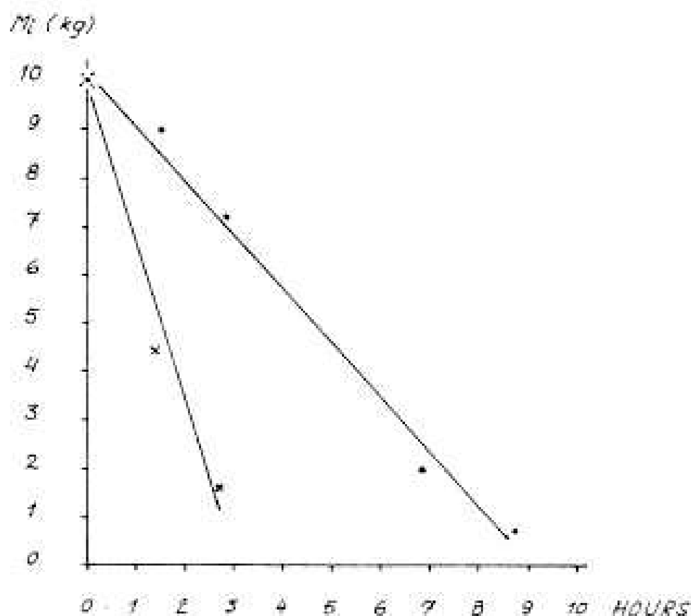


Figure 7.4 Results of ice meltage tests under field conditions. (·) plastic box in the shade, (x) plastic box in the sun. Plastic boxes, 40 kg capacity, red colour, unstacked, flake ice, external average temperature (dry bulb) 28°C. (Data obtained during the FAO/DANIDA National Workshop on Fish Technology and Quality Control, Bissau, Guinea-Bissau, March 1986)

It is clear that there is little practical possibility in tropical countries to handle chilled fish in plastic boxes exposed to the sun. An increase in ice consumption, even if less dramatic than in plastic boxes, can be measured in insulated containers exposed to the sun.

The obvious advice in this case is to keep and handle fish boxes and containers in the shade. This measure can be complemented by covering the boxes or containers with a wet tarpaulin. The wet tarpaulin will reduce the temperature of the air in contact with boxes and containers to the wet bulb temperature (some degrees below the dry bulb temperature, depending on the Equilibrium Relative Humidity - ERH - of the air), and will practically stop noticeable radiation effect (since there are always radiation effects between a body and its background).

Ice consumption in stacks of boxes and containers

In a stack of boxes or containers not all of them will lose ice in the same way. Figure 7.5 gives the results of an ice meltage test conducted on a stack of boxes. Boxes or containers at the top will consume more ice than boxes and containers at the bottom, and those in the middle will consume less than either.

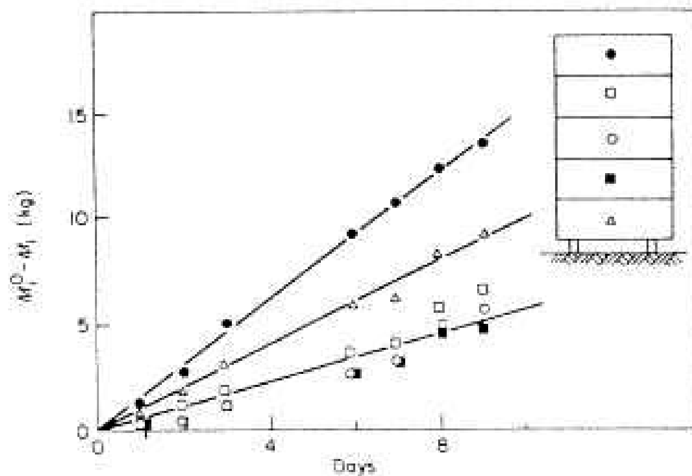


Figure 7.5 Results of ice meltage tests during storage in a stack of plastic boxes. Plastic boxes 35 kg in a chill storage room at 5°C, flake ice (from Boeri et al. (1985)

Jensen and Hansen (1973) and Hansen (1981) presented a system ("Icibox"), mainly for artisanal fisheries. In this system, a stack of plastic boxes were insulated by placing wooden frames, filled with polystyrene, at the top and at the bottom of the stack, and covering the whole with a case made out of canvas or oil skin. A similar system, composed of stacks of styropor boxes, accommodated in a pallet, and covered by an insulated mat of high reflective (Al) surface, is used in practice for shipment of fresh fish by air (e.g., it is utilized to ship fresh fillets of Nile perch from Lake Victoria to Europe).

Results of Figure 7.5 are also of interest to demonstrate the effect of a chill room on fresh fish handling. The use of chill rooms drastically reduces the ice consumption in plastic boxes, avoiding the need of re-icing. In a fish handling system chilling fish with ice, mechanical refrigeration is used to reduce the ice consumption and not to chill fish.

Although analytical models of ice consumption (e.g., Equations 7.a to 7.h) can be applied directly to estimate the ice consumption in simple and repetitive fish handling operations, their main importance is that they can help in arriving at solutions for the proper handling of chilled fish in rational. way (as seen from Figures 7.3, 7.4 and 7.5).

Ice consumption in the sides of boxes and containers

It is necessary to bear in mind that ice will not melt uniformly in the interior of a box or container, but meltage will follow the pattern of temperature gradients between the interior of the box/container and the ambient. In Figure 7.6, a commercial plastic box with chilled hake shows the lack of ice in the sides due to the temperature gradients at the walls.

Following Figure 7.5, and supposing that a simple box could be devided into five subboxes, it is clear that the bottom and top of boxes and containers should receive more ice to compensate for thermal losses, the top receiving more ice than the bottom. However, in practice more ice should also be put in the sides of boxes and containers.

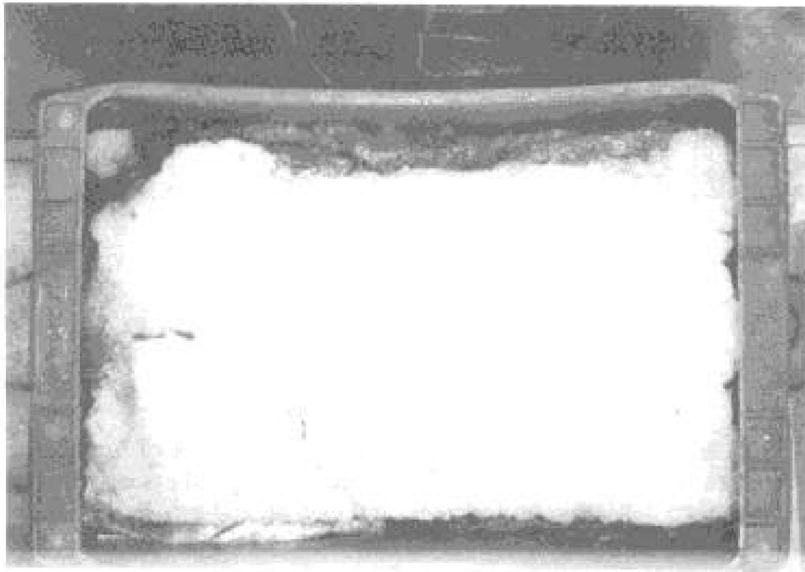


Figure 7.6 Commercial plastic box with chilled hake (M. *hubbsi)* showing the effects of lack of ice in the sides (photo H. Lupin)

The box of Figure 7.6 was initially prepared with enough ice, and it can be seen that ice is still abundant on topof the box. However, after a period of storage in a chill room, ice has melted, mainly on the sides, leaving some fish and parts of fish exposed to the air with a consequent rise in temperature and dehydration. In addition, ice and fish have formed a compact mass that can produce physical damage to exposed fish when the box is moved.

In chilled fish onboard fishing vessels or transported by truck, this problem may not exist if there is a continuous gentle movement which allows for ice melt water from the top to move to the sides. However, in chill rooms or storage rooms (insulated containers) it would be advisable to re-ice if this problem is observed. Under tropical conditions this effect is observed, even with insulated containers, in less than 24 hours of storage.

7.2 Fish handling in artisanal fisheries

Artisanal fisheries, existing both in developed and developing countries, encompass a very wide range of fishing boats from pirogues and canoes (large and small) to small outboard and onboard engine vessels, utilizing also a variety of fishing gears. It is difficult to find a common denominator; however, from a fish handling point of view, artisanal vessels handle relatively small amounts of fish (when compared with industrial vessels) and fishing journeys are usually short (usually less than one day and very often only a few hours).

In general, in tropical fisheries the artisanal fleet land a variety of species, although there are examples of the use of selective fishing gear. In temperate and cold climates artisanal fleets can focus more easily on specific species according to the period of the year; nevertheless, they may land a variety of species to respond to the market demand.

Although very often artisanal fisheries are seen as an unsophisticated practice, closer scrutiny will reveal that in many cases they are passing through a process change. There are many reasons for this process but very often the main driving forces are: urbanization, fish exports and competition with the industrial fleet.

This change in the scenario of artisanal fisheries is essential to understanding the fish handling problems faced by the artisanal and small sector of the fish industry, particularly in developing countries.

When the artisanal fleet was serving small villages, the amount of fish handled was very low; the customers usually bought the fish direct from the landing places, fishermen knew customers and their tastes, and fish was consumed within a few hours (e.g., fish caught at 06.00 h, landed and sold at 10.00 h, cooked and consumed by 13.00 h). In this situation, ice was not used, and gutting was unknown; very often fish arrived at landing places in rigor mortis (depending on fish species and fishing gear), and fish handling was at most reduced to covering the fish from the sun, keeping it moist and keeping off the flies. In Figure 7.7 two cases of landing un-iced fish by artisanal fishermen are shown.

(a) (b)

Figure 7.7 Landing by artisanal fishermen: (a) un-iced shrimp by artisanal fishermen (El Salvador, September 1987, photo H. Lupin); (b) un-iced fish (Bukova, Tanzania, 1994, photo S. P. Chen)

With urbanization and the request for safer and more quality products (as a result of exports and competition with industrial fish) conditions changed drastically. Large cities also demanded increased fish supplies, and thus middlemen and fish processors had to go to more distant landing places for fish. The amount of fish handled increased, fishing journeys lasted longer and/ or passive fishing gears like gillnets were set to fish for longer times, a chain of middlemen and/ or official fish markets replaced the direct buyer at the beach and, as a result of growing business (fish for income), in some places the catch effort also increased with a consequent increase in the number of fishing boats and an increase in the efficiency of the fishing gears.

In one way or another, each of the new circumstances added hours to the time which passed between catching the fish and eating or processing it (e.g., freezing). This increase in exposure of un- iced fish to ambient temperature (or water temperature for a dead fish in a gillnet), even though brief (e.g., an additional 6-12 hours), dramatically changed the situation regarding fish

spoilage and safety.

In the new situation, fish remained at ambient temperature some 13-19 or more hours. It could be already spoiled, at terminal quality and/or could present public health hazards (e.g., from the development of C. *botulinum* toxin to histamine formation). In addition to the safety and quality aspects, post-harvest losses, non-existent at subsistence level and very low at the village stage, become important. For instance, it is estimated that the post-harvest losses of Nile perch caught artisanally in Uganda amount to 25-30% of the total catch.

The situation described in previous paragraphs, and cases like those shown in Figure 7.7, moved extension services in developing countries and international technical assistance to focus on the problem of introducing improved fish handling methods at the artisanal level. The basic technical solution is the introduction of ice, proper fish handling methods and insulated containers, which is the approach utilized by most of the artisanal fleet in developed countries.

There are several examples where this approach was adopted by fishermen in developing countries and has become a self-sustained technology. Two very interesting cases to analyze are the introduction of insulated containers onboard of "navas", the traditional fishing vessels of Kakinada in Andhra Pradesh, India (Clucas, 1991) and the introduction of insulated fish containers in the pirogue fleet of Senegal (Coackley and Karnicki, 1984). The sketch of an insulated fish container for Senegalese pirogues is shown in Figure 7.8.

The insulated container of Figure 7.8 was designed to fit existing pirogues, according to the type of catch and needs expressed by fishermen. The materials and tools needed to construct the insulated container are available to fishermen in Senegal, even though some of them are imported (e.g., foam sheets and resin).

The example of Senegalese fishermen is now spreading steadily to similar fisheries in Gambia, Guinea-Bissau and Guinea which are adopting the use of insulated containers similar to those of Senegal. However, the process of diffusion and adoption of a technology, even if relatively simple, is not as straightforward as could be supposed. A pirogue with two insulated containers onboard is shown in Figure 7.9.

Once artisanal fishermen become aware of the rationale of insulated containers, they tend to favour large insulated fish containers rather than small ones. The reason is clear from Equations 7.e and 7.g, as for the same volume of fish and ice, large containers will present less external area than the area presented by several small containers. For example, a large cubic insulated fish container can be envisaged of a side measuring x m, and eight cubic insulated containers of sides equal to x/2 m presenting the same total volume as the large one. The eight containers will have an external area twice that of the big container, thus increasing the ice consumption by two, and decreasing the amount of fish that can be transported.

Other reasons are that small containers will cost more than a large one of the same total volume (simply because they need more material); small containers are not always easy to secure safely onboard small boats, and large containers allow for transport of large ice bars that can be crushed at sea (reducing stowage rate). However, large containers are difficult to handle and sometimes canoes and pirogues are very small or narrow and they cannot accommodate large insulated fish containers. This is the case for relatively small insulated fish containers. An example is shown in Figure 7. 10.

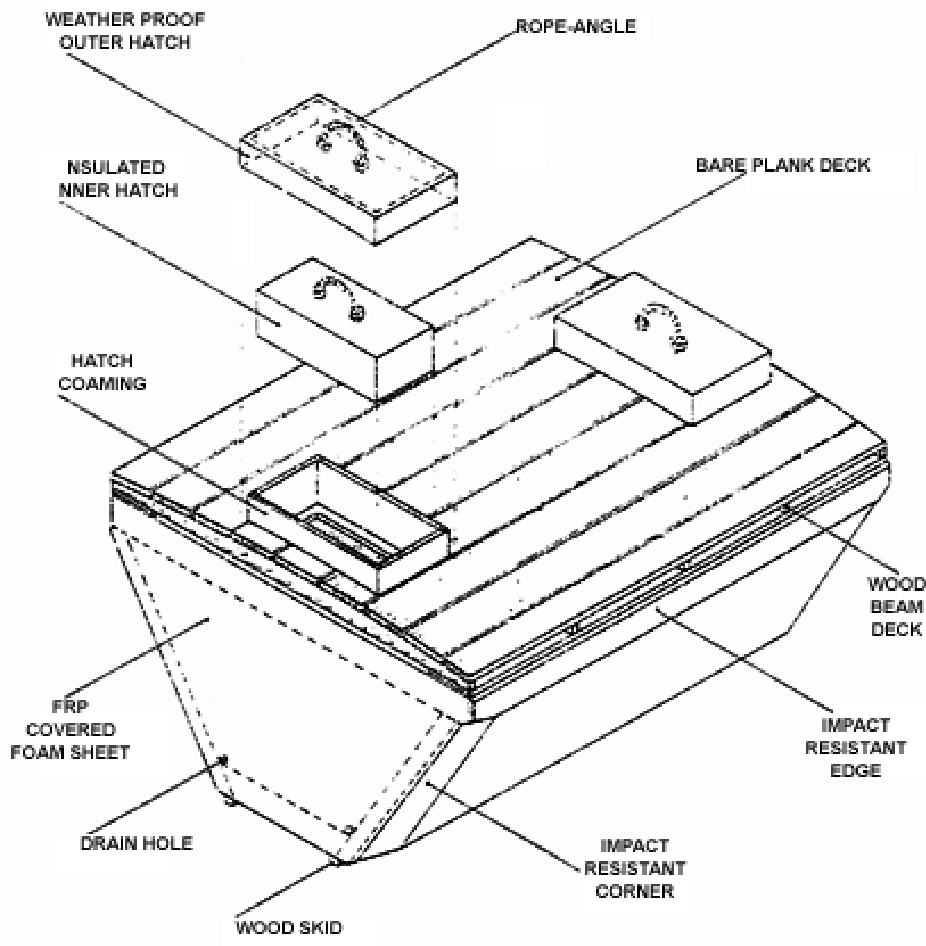


Figure 7.8 Sketch diagram of a two-hatch insulated container for Senegalese pirogues (after Coackley and Karnicki, 1985)

Figure 7.9 A Senegalese pirogue at the beach, carrying two insulated containers (photo B. Diakité, 1992)

Figure 7.10 Small insulated container installed onboard an artisanal fish catamaran (The Philippines, 1982, photo H. Lupin)

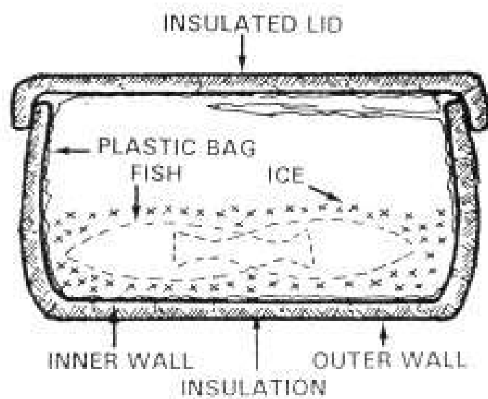
A serious constraint in many artisanal fisheries is the relatively high cost of industrial containers and the difficulty in finding appropriate industrial materials to construct them. For this reason, efforts have been made to develop artisanal containers made from locally available materials (Villadsen et al, 1979; Govindan, 1985; Clucas and Whitehead, 1987; Makene, Mgawe and Mlay, 1989; Wood and Cole, 1989; Johnson and Clucas, 1990; Lupin, 1994).

In some cases, the correct approach could be to add insulation to local fish containers; in other cases it could be necessary to develop a new container. In general, artisanal fish could be cheaper than industrial fish containers, but they will not last as long. An artisanal insulated container developed at Mbegani (Tanzania), based on the local basket container ("tenga") is shown in Figure 7.11.

A key factor in the construction of artisanal insulated containers is the selection of insulation material. There are a number of materials available: inter alia, sawdust, coconut fibre, straw, rice husks, dried grass, old tires and rejected cotton.

However, the use of such materials presents problems: the materials become wet very quickly (with the exception of old tires), losing their insulating capacity and increasing the weight of the container. When wet, most of them tend to rot very quickly. The solution is to put them inside a plastic bag (waterproof); however, in this case they tend to settle, leaving part of the walls without insulation.

With a view to overcoming these problems, the concept of "insulated pillows" was developed in various FAO/DANIDA fish technology workshops. This concept is very simple: the insulating material (e.g., coconut fibres) is placed inside one plastic tube of the type usually found to produce ordinary small polyethylene bags (10 cm in diameter); the insulating material is pressed before sealing the tube; the tube is sealed by heat at both ends (e.g., every 20 cm), and with some practice it is possible to produce a strip of "pillows". It is advisable to utilize a second tube to reduce the incidence of punctures due to fish spines and bones.



(a) (b)

Figure 7.11 (a) Sketch of an artisanal insulated container (the "Mbegani fish container") developed and utilized in Tanzania; (b) The "Mbegani fish container" on a bicycle to distribute fresh fish. This container was initially developed at the FAO/DANIDA National Workshop on Fish Technology and Quality Control, held at Mbegani, Tanzania, May-June 1984

The strip of "insulated pillows" can then be placed between the internal and the external walls of the container. Once the container is finished with an insulated lid and handles, fish and ice can be put in a large resistant plastic bag, as shown in Figure 7.11 (a). The use of the plastic bag extends the lifespan of the container and improves fish quality.

This example indicates the type of practical problems found when developing an artisanal insulated fish container, and the possible solutions.

Why is ice not always used to chill fish when necessary

Despite the knowledge on the advantages of fish chilling, ice it is not as widely used as it should be, particularly at artisanal level in developing countries. Which are the main problems found in practice? Some of the problems that can be found are as follows:

(i) Ice should be produced mechanically

This obvious statement implies, *inter alia,* that it is not possible to produce ice artisanally for practical purposes (machines and energy are required). To produce ice under tropical conditions, from 55 to 85 kWh/l ton of ice (depending on the type of ice) are necessary whereas, in cold and temperate countries from 40 to 60 kWh are required for the same purpose. This may be a large power requirement for many locations in developing countries, particularly in islands and places relatively far from large cities or electricity networks. Ice plants require maintenance and hence trained people and spare parts (in many cases this requires access to hard currency).

A cold chain will also require chill rooms (onboard and on land), insulated containers, insulated trucks and other auxiliary equipment (e.g., water treatment units, electric generators). Besides increasing the cost, all this equipment will increase the technological difficulty associated with the fish cold chain.

(ii) Ice is produced and used within an economic context

In developed countries ice is very cheap and costs only a fraction of the price of fresh fish. In developing countries ice is very often expensive when compared with fresh fish prices.

A survey conducted in 1986 by the FAO/DANIDA Project on Training on Fish Technology and Quality Control on current fish and ice prices in fourteen African countries demonstrated that in all cases and for all the fish species, I kg of ice increased the fish price at least twice the rate recorded in developed countries. The cheaper the fish the worse the situation. For instance, in the case of small pelagics, the percentage of increase in the fish cost per kilogramme of ice added, was 40% for the "yaboy" of Senegal, 16-25% for the sardinella of Congo, and 66 % for the sardinella of Mauritania and the anchovy of Togo. The market price for fish, in this case, acts as a deterrent for the use of ice.

According to the relative cost of ice to fish, ice may or may not be used. For instance, in Accra, Ghana in 1992, it was found that using ice to chill small pelagics (Ghanian herring) in a proportion of 2 kg ice: 1 kg fish would increase the cost of fish by 32-40%. However, in the case of snapper, for the same ratio of ice to fish the cost increase would be in the range of 4.5-5.7%. The result is that ice chilling of snapper is relatively common in Accra, whereas ice is not utilized to chill small pelagics.

Very often fish compete with other sources of demand (soft drinks, beer), even if the ice machine was initially installed to supply ice for chilling fish. This and energy losses at the ice plants contribute to increase the market price of ice.

In addition to producing and utilizing ice on a sustainable basis, economic aspects must be considered (e.g., depreciation, reserves, investment). Moreover, in the case of ice manufacture there is a strong influence of the scale of production. Low ice prices in developed countries are also the result of large ice plants located at the fishing harbours that supply a large number of companies and fishing boats.

(iii) Practical constraints

Introduction of ice into fish handling systems that are not accustomed to using it can create practical problems. For instance, from Table 7.1 it is clear that the introduction of ice will increase the volume required for storage and distribution, and will reduce the effective fish hold in vessels. The use of ice will also increase the weight to be handled. This will have a number of implications such as an increased workload for the fishermen, fish processors and fishmongers, and an increase in costs and investment.

From Figures 7.3 and 7.4 it is clear that the total amount of ice needed per 1 kg of fish, in the complete cycle from the sea to the consumer will be much higher in tropical countries than in cold and temperate regions. As an indication, the average consumption of ice in the Cuban fishery industry was estimated at around 5 kg of ice per 1 kg of fish handled (including ice losses), although higher values (up to 8-10 kg of ice per 1 kg of fish) have been recorded in single industries in tropical countries; this necessitates large storage and transport capacities.

Freshwater or seawater utilized for producing ice should comply with standards (microbiological and chemical) for potable water and should be readily available in the volumes required. This is not always possible particularly in countries with energy problems (blackouts) and without (or with erratic) public tap- water distribution. If water has to be treated, this implies additional costs and additional equipment to operate and maintain.

Properly trained personnel are required to operate the ice plant and auxiliary equipment efficiently, and to handle ice and fish properly. Although many developing countries have made efforts to train people, in many cases there is a lack of technical personnel ranging from well trained fish technologists to refrigeration mechanics or electricians, or simply plant foremen.

Moreover, in many developing countries it is increasingly difficult to keep technical and professional schools operating in this field, thus jeopardizing the possibility of self-sustained training, and hence fishery industry developments.

(iv) Ice is not an additive

Knowledgeable people (e.g., fishmongers) are quickly aware of the fact that ice is not an additive. Therefore, when there is a delay in icing, ice is not usually utilized (even if available) because it will not improve fish quality. Consumers could also be intuitively aware of this fact, and they prefer to be presented with the fish as it is (e.g., at the terminal state of its quality) rather than in ice, because in this case ice will increase the price of fish but not enhance its quality. Due to the above and to the problems associated with the transition between artisanal and industrial or semi-industrial fisheries, already discussed, consumers in some countries (e.g., in Saint Lucia and Libya) tend to believe that iced fish is not fresh fish.

A need for chilled fish can develop if a market for iced fish (not just a market for "fresh fish") is developed, and to develop a market for iced fish where it does not already exist may be a very difficult and expensive endeavour as is the introduction of any other food product.

(v) Need for appropriate fish handling technologies

To chill and keep fish with ice is a very simple technique. A more complicated picture emerges when actual fish handling systems are analysed, including the economic aspect.

From a comparative study on the same fish handling operation, utilizing ice and insulated containers, carried out in both a developed and a developing country, it was seen that in developed countries, the more "appropriate" technology would aim at reducing wage costs (e.g., chutes to handle ice and fish, special tables to handle containers and boxes and conveyors to move them, machines that mix ice and fish automatically); in developing countries the main concern would be to reduce ice consumption, and to increase the fish : ice ratio in the containers (Lupin, 1986 b).

The same study found that a twentyfold difference in wage costs between developing countries and developed countries cannot offset a tenfold difference in the cost of ice. There is no "comparative advantage" in low wages in developing countries with regard to fresh fish handling. Advanced technology on fish handling from developed countries could make work easier for people in developing countries, but might not improve the economics of the operation as a whole.

There is obviously no single solution to the problems discussed above. However, it is clear that it is the problem to be solved in the coming decade in the field of fresh fish handling. With total catches having reached a plateau, losses due to the lack of ice utilization could be ill-afforded, and developing countries and artisanal fishermen in particular should not be deprived of potential market opportunities.

7.3 Improved catch handling in industrial fisheries

The aims of modern catch handling are the following:

- to maximize the quality of the landed fish raw material. It is of particular importance to provide a continuous flow in handling and to avoid any accumulation of unchilled fish, thereby bringing the important time-temperature phase under complete control.
- to improve working conditions onboard fishing vessels by eliminating those catch handling procedures which cause physical strain and fatigue to such a degree that no fishermen need to leave their occupation prematurely for health reasons.
- to give the fisherman the opportunity to concentrate almost exclusively on the quality aspects of fish handling.

To meet these aims, equipment and handling procedures that will eliminate heavy lifting, unsuitable working positions and rough handling of fish must be introduced. By doing so, the catch handling time is accelerated and the chilling process initiated much earlier than was previously the case (Olsen, 1992). The typical unit operations in catch handling are shown in Figure 7.12.

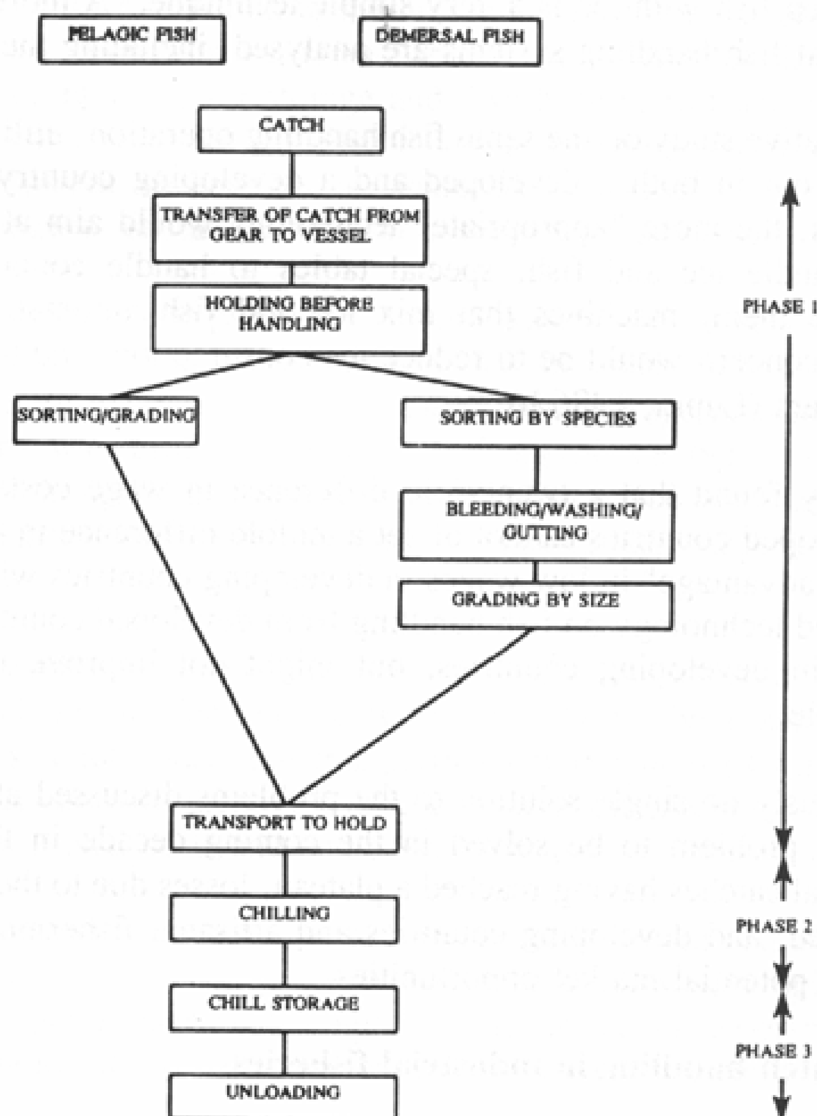


Figure 7.12 Typical unit operations in catch handling of pelagic and demersal fish

Important general aspects in modern catch handling are:

- phase one, which covers the time used for the necessary handling onboard, i.e., the time until the fish is placed in chilling medium, must be as short as possible. The fish temperature at time of capture can be high with consequent high spoilage rate.
- phase two - the chilling process - must be arranged so that a fast chilling rate is obtained for the whole catch. Maximum chilling rate will be obtained by a homogeneous mixing of fish and ice, where the individual fish is completely surrounded by ice and the heat transfer therefore is maximum, controlled by the conduction of heat through the meat to the surface. This ideal situation can be obtained during chilling of small pelagics in a chilled seawater (CSW) system; but by chilling demersal food fish in boxes with ice it is not always possible to obtain homogeneous fish/ice mixing. However, the appearance of fish completely surrounded by ice is often deteriorated due to discolorations and impression-marks. In practical life, icing is therefore often done by placing a single layer of fish on top of a layer of ice in the box even if it is bad practice from a temperature control and therefore shelf life point of view. Cooling is primarily achieved by melt-water dripping from the box stacked on top. This type of chilling will only function satisfactorily if fish boxes are shallow and have a perforated bottom.
- in phase three, which covers the chilled storage period, it is important that a homogeneous temperature at -1.5°-0°C is maintained in the fish until first hand sale. As this period may be extended for several days, this aspect has top priority.

Catch handling can be done in several ways ranging from manual methods to fully automated operations. How many operations will be used in practice and the order in which they are done depends on the fish species, the fishing gear used, vessel size, duration of the voyage and the market which has to be supplied.

Transferring catch from gear to vessel

Midwater trawlers and purse seiners fishing pelagic fish use tackling in lifts of up to 4 t, pumping or brailing for bringing the catch onboard. When lifting huge hauls (100 t or more) onboard by these methods, the danger of losing fish and gear always exists if the fish start to sink after having been brought to the surface. The speed of which the fish may sink depends on the species, catching depth and weather conditions during hauling.

Pumping the catch onboard using submersible pumps without bruising the fish can be difficult, as it is not easy to control the fish-to-water ratio during pumping.

In recent years, the so-called P/V pump (P/V - pressure/vacuum) has found increasing use. The P/V-pump principle is that an accumulation tank of 500-1500 1 size is alternately put under vacuum and pressure by a water-ring vacuum-pump (Figure 7.13). The fish, together with some water, are sucked through a hose and a valve into the tank of the system. When the tank is full, it is pressurized by changing the vacuum and pressure side connections from the tank to the pump and the fish/water mix flows through a valve and a hose into a strainer. The P/V-pump is claimed to handle the fish more gently than other fish pump types, but the capacity is generally lower, mostly because of the alternating way of operations. This problem can be solved by having two P/

V-tanks running in phase opposition using only one vacuum-pump.

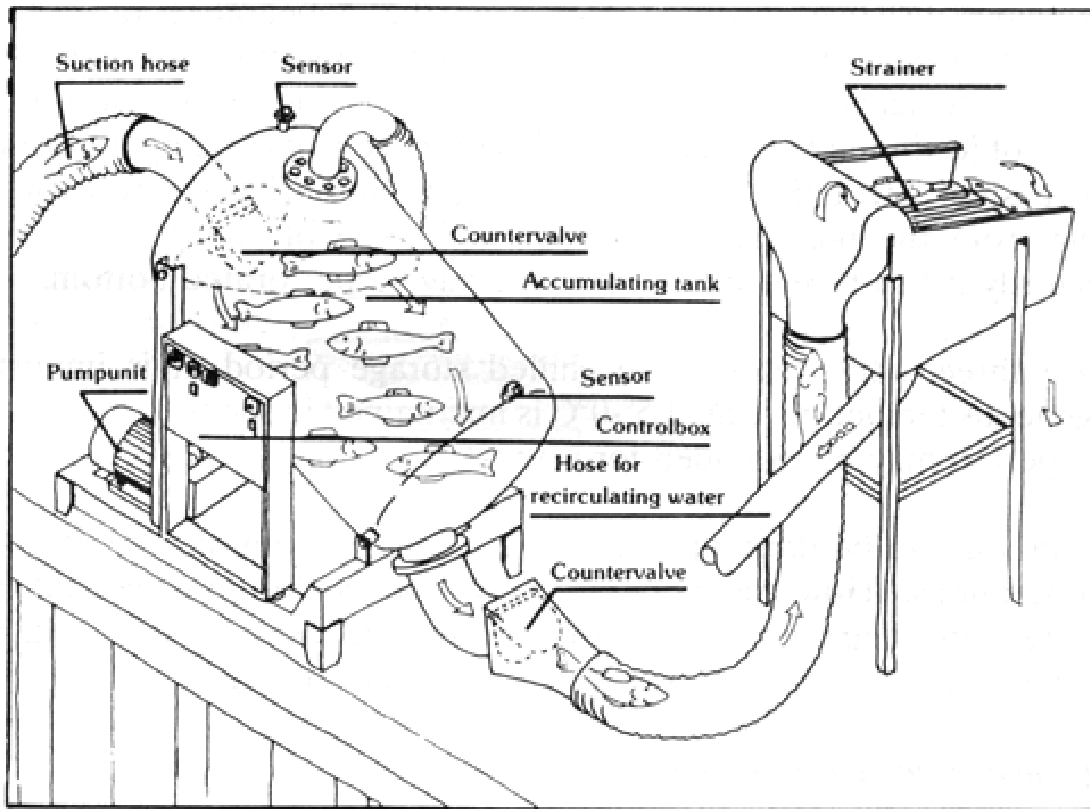


Figure 7.13 Working principle of a P/V pump

Small gillnetters (10-15 m) haul the nets with the net hauler, and very often store their catch in the net until landing. Here the net is drawn through a net shaker by two men in order to free the fish from the gear. It has been shown that the violent way in which the shaker works can be harmful to the men's hands, arms and shoulders. Ergonomic precautions have therefore been suggested to overcome this problem.

Trawlers and seiners (Danish and Scottish) tackle the catch into pounds. Commonly used pounds are those with a raised bottom which can be hoisted hydraulically. The purpose of these designs is to provide good working conditions for the crew (Figure 7.14). Also gillnetters may use a work-saving pound system, which is often connected with a conveyor to bring fish to the gutting-table.

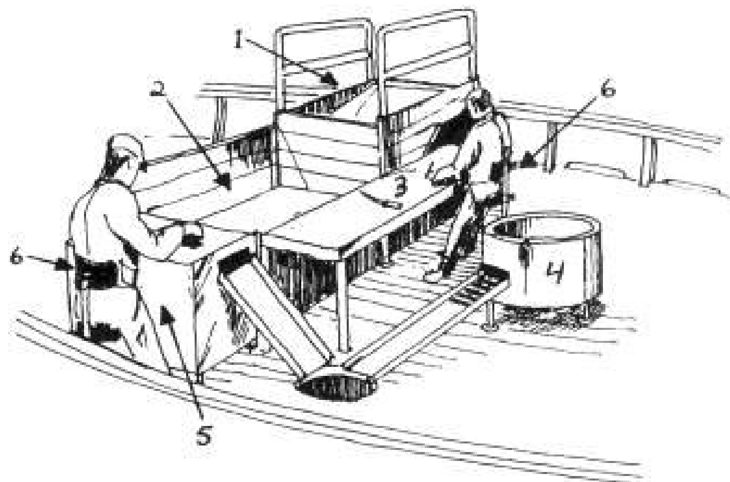


Figure 7.14 Deck lay-out for trawler using machine gutting of demersal fish
1. Tackle pound, 2. Hoisting pound, 3. Gutting table, 4. Bleeding/washing machine, 5. Gutting machine, 6. Chair.

Holding of catch before handling

When large catches are to be handled, or if for other reasons catch handling cannot start immediately, it is convenient and necessary to prechill the catch during holding in deck-pounds using ice or in tanks using Refrigerated Sea Water (RSW) or a mixture of ice and sea water (Chilled Sea Water, CSW).

Prechilling holding systems are mostly used on pelagic trawlers which grade their catches in size before storing in boxes or in portable CSW-containers. It is also essential to prechill when the pelagic fish are soft and feeding and therefore very prone to bellyburst. Prechilling tanks are unloaded by elevator or P/V-pumps. If no sorting is done onboard, the fish is conveyed directly for chilled storage in the hold. A system for holding demersal fish in tanks is shown in Figure 7.15.

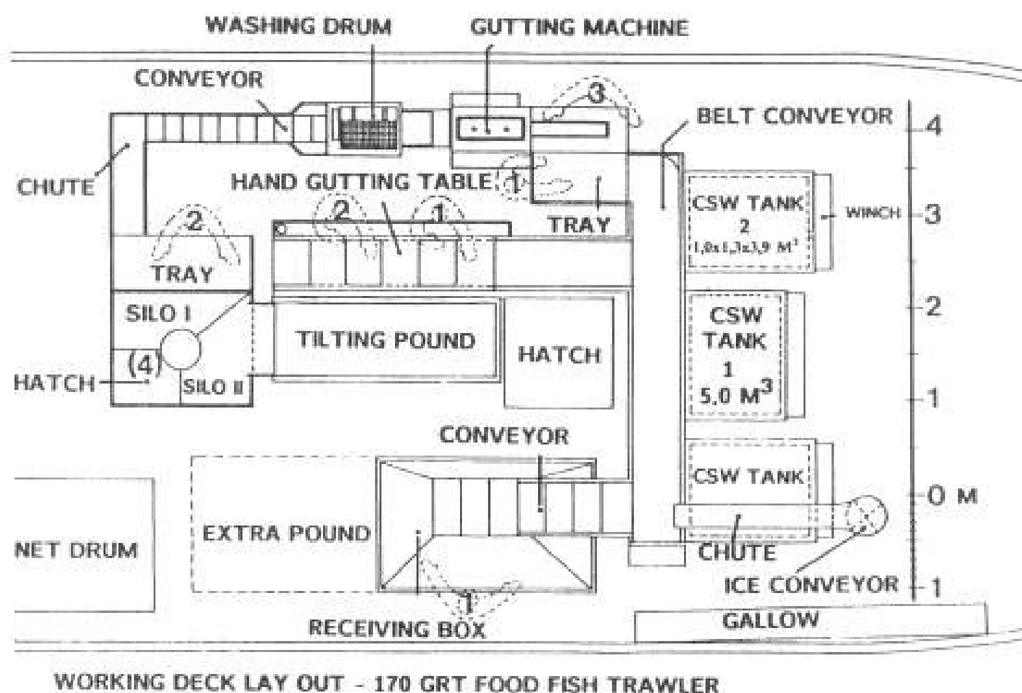


Figure 7.15 System comprising CSW raw material holding tanks before manual or machine gutting of fish

Sorting/grading

Pelagic fish are sometimes sorted or graded onboard according to size. The equipment used operates on the basis of thickness of fish using principles such as:

- vibrating, inclined diverging bars
- contrarotating, inclined, diverging rollers
- diverging conveyors where fish are being transported along a power driven V-belt.

Grading by thickness can meet the demand for the high capacity needed in pelagic fish handling, but it is generally accepted that the correlations between thickness and length or weight are not too good (Hewitt, 1980). The most important point, often forgotten, for making a grader function at its optimum is even feeding. This could be done with an elevator delivering to a (vibrating) water sprayed chute leading to the inlet guide chute of the grading machine.

Sometimes it is necessary to install a manual sorting conveyor before the grading machine for removal of larger fish and debris, e.g., in the fishery for argentine with by catch of grenadier.

Sorting and grading of demersal fish by species and by size is normally done by hand. However, some automatic systems of sorting according to width are in use. Static or dynamic weighing by marine weighing systems are also in use with good results. Research is under way using a computerized vision system for species and size grading.

Bleeding/gutting/washing

In order to obtain optimal quality in a white fillet, many white-fleshed demersal fish (but not all) need to be bled and gutted immediately after capture. The best procedures from an economic, biological and practical point of view are still under discussion (see section 3.2 on bleeding and section 6.4 on gutting).

The vast majority of fishermen are handling the fish in the easiest and also the fastest way, which means the fish are bled and gutted in one single operation. This may be done manually, but gutting machines have been introduced to obtain even more speed. The fish are transported to and from the fisherman by suitable conveyor systems. Using machines, round fish can be gutted with a speed of approximately 55 fish/minute for fish length up to 52 cm and 35 fish/minute for fish length up to 75 cm. Gutting by machine is 6-7 times faster than hand-gutting.

Existing gutting machines for round fish of the type using a circular saw blade for cutting and removing the guts destroy the valuable roe and liver. A new type of gutting machine which copies the manual gutting procedure is now available on the market. Gutting speed of this machine is 35-40 fish/minute and the roe and liver can be saved (Olsen, 1991). Flatfish can also be gutted by a recently developed machine. The speed of this machine is about 30 fish/minute.

After gutting, the fish are conveyed to the washing or bleeding operation. This may be done in pounds, often with raised bottom or in special bleeding tanks, frequently with a hydraulically-operated tilting system and rotating washing drums are also used (Figure 7.15); and special equipment such as the Norwegian and British fish washer may be used.

After catch handling (sorting, grading, gutting, etc.), the fish may be passed to an intermediate storage silo or batch holding system for the different sizes or grades before being dropped by chute to the hold, or the chutes may lead directly from the grading machines to the hold (Figure 7.16).

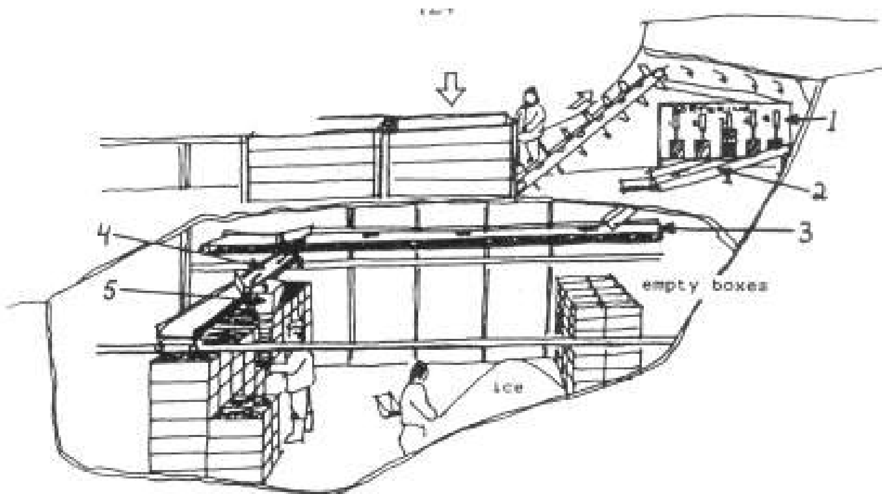


Figure 7.16 "Polar"-system. Mechanized sorting and boxing of herring 1. Herring sorting machine, 2,3,4. Conveyors, 5. Flexible dosing tube.

Chilling/chilled storage

Demersal fish have traditionally been stored on shelves or in boxes. Boxing has a big advantage over shelf storage as it reduces the static pressure on the fish and also facilitates unloading.

Shelf storage is done by alternating layers of ice and fish from one layer of ice and fish (single shelving 25 cm between shelves) up to ice/fish layers 100 cm deep. In practice, shelving often allows better temperature control than boxing and therefore also a longer storage life. Because excessive handling during unloading and excessive pressure on the fish have- a negative effect on quality, e.g., appearance, boxing is preferable to shelving, given proper icing.

In pelagic fisheries, boxed fish will be untouched until processed, but in demersal fisheries the catch is often only sorted by species onboard and not graded by size and weighed. These operations are carried out after landing before auction whereby some of the handling and quality advantages of boxing are lost.

In the near future when integrated quality assurance systems have been introduced, these unit operations will be carried out onboard and an informative label on each box will give details of factors of importance for first-hand sale (including freshness).

In general, two types of plastic fish boxes are used: stack-only and nest/stack boxes (Figures 17 a and 17 b).

To overcome some of the space problems in using stack-only boxes, the nest/stack type has been developed. These occupy only approximately a third of the space needed when stored empty compared to when the boxes are loaded with fish and ice.

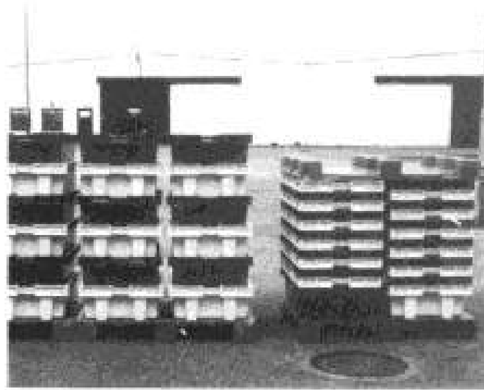


Fig. 7.17 a Stack-only boxes **Figure 7.17** b Nest/stack boxes

This type of box is widely used in France, the Netherlands and Germany and also in some Danish ports. When a system tailor-made for a certain type of plastic box is designed, the quality advantages of using boxes can be fully utilized onboard. The key points to consider are:

1. The handling rate necessary to prevent quality loss because of delayed icing. Prechilling can be of advantage to compensate lack in handling rate.
2. Handling methods which make it possible to guarantee that the icing procedure is sufficient to chill the fish to 0°C and maintain this temperature until landing.
3. The hold construction must be constructed such that safe and easy stacking of the boxes can take place.
4. Hold insulation of a relatively high quality should be considered. A small mechanical refrigeration plant can be of advantage. Air temperature in the hold should be + 1°-3°C

RSW-storage (Refrigerated Sea Water is a well established practice which has been refined both theoretically and practically since its introduction in the 1960s in Canada where it was developed for salmon and herring storage (Roach et al, 1967). At the beginning, most RSW vessels were salmon- packers and because of some failures in design which were attributed either to insufficient refrigeration or circulation systems, a standard for control of RSW-systems was established. Since vessels are different, the RSW-installation has to be studied carefully in every fishery to determine its real capability. Therefore, methods for rating each individual system and vessel and providing general specifications and guidelines for the proper installation have been suggested by the Canadian technicians (Gibbard and Roach, 1976).

In order to obtain maximum shelf life from RSW-systems, temperature homogeneity in the region of -1°C is very important. The factors affecting temperature homogeneity were recently studied in Denmark (Kraus, 1992). The most important conclusions were that the inflow of the chilled seawater in the bottom of the tank must take place over the whole tank bottom area, and that filling capacity for securing water circulation and temperature homogeneity is dependent on fish species. The necessary chilling rate was suggested to be: fish temperature must be below 3°C within four hours and below 0°C after 16 hours, and the temperature should be kept between -1.5°C and 0°C until unloading.

The CSW system has also been developed in Canada as a much cheaper means an investment point of view - to obtain rapid uniform chilling of fish. The most popular method used is the so-called "Champagne" method where rapid heat transfer between fish and ice is obtained by agitation with compressed air introduced at the bottom of the tanks, instead of using circulation

pumps as in RSW and some earlier CSW designs (Figure 7.18) (Kelmann, 1977; Lee, 1985). An indication of the chilling rate for herring could be: reduction of fish temperature from 15°C to 0°C within two hours. The concept of a CSW system is to load well insulated tanks at the harbour with the amount of ice necessary to chill the catch to between 0' and - 1°C and maintain this temperature until unloading.

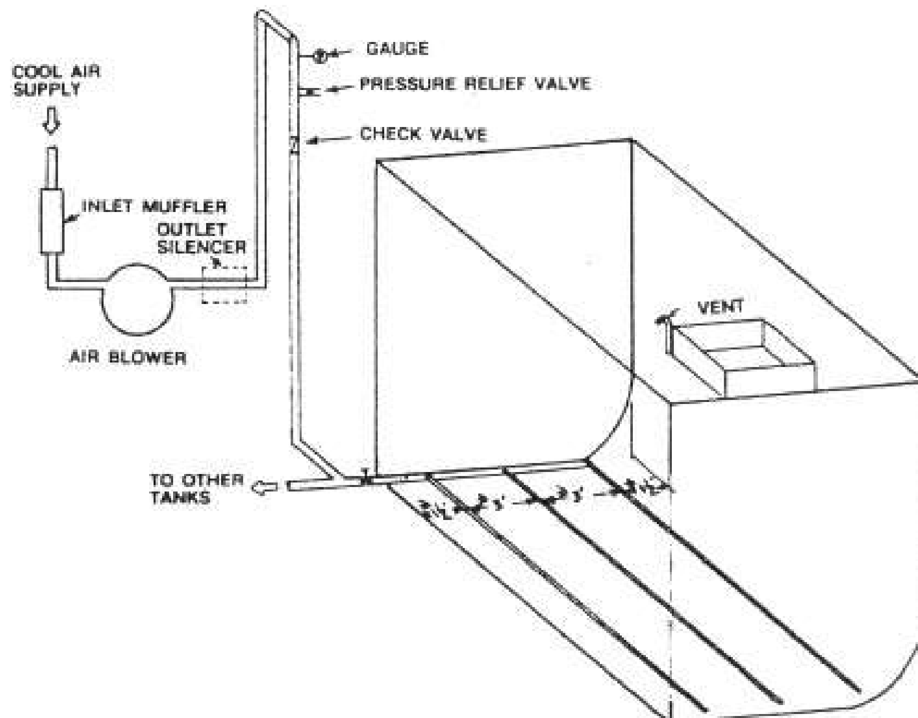


Figure 7.18 Chilled seawater system: piping layout

The Canadian west-coast fishermen are achieving this in practice by using a minimum of seawater when they start loading the tank and by forcing air through the ice-sea-water-fish-mixture only during loading, and stop forcing air immediately when the tankis full. Thereafter they will force the air only for 5-10 minutes with 3-4 hours' interval. The air agitation therefore only serves as a method to overcome local temperature differences in the tank. The objective is to obtain a uniform mixture of fish and ice in order to secure temperature homogeneity.

A proven rule-of-thumb for estimating the amount of ice necessary is simply to observe the amount of ice left in the tank at unloading, and compare it with temperature readings, which should be in the -1°C range measured in the landed fish. The starting situation should be conservative, which at sea-temperature around 12-14°C, for a trip lasting 7 days and with 10 cm polyurethane insulation, is 25% ice by weight of the tank capacity. The amount of ice is adjusted according to the observations on the following trips.

An analytical approach to estimate necessary ice quantities in a CSW tank system has been developed. The quantity of ice required takes into consideration tank size, catch volume, time at sea, water temperature, hold insulation and hold flooding strategy (Kolbe *et al.,* 1985).

CSW "Champagne" systems can also be used in small coastal vessels, e.g., in a fishery for small pelagic fish with vessels of 10-14 m length with a fish carrying capacity from 3 to 10 t fish (Roach, 1980).

Another way of loading a CSW tank, which is in practical use in Denmark, is to add the necessary amount of ice to the fish during loading by mixing a controlled stream of fish with a controlled stream of ice. The greatest amount of ice is added to the fish during loading. When the tank is full the voids are filled with seawater from a hose and the tank is left undisturbed, except for watercirculation by pumping or compressed air blowing for 5-10 minutes of 4-hour intervals. The ice is bulk-stored in the forward hold and the ice is shovelled into a conveyor flush with the floor. The conveyor then leads the ice to the mixing point at the deck.

The use of portable CSW containers for pelagic fish handling was tested in the early 1970s (Eddie and Hopper, 1974). The approximately 2 m^3 heat insulated containers were loaded with the necessary amount of ice from the harbour and agitated with compressed air in a similar way as for CSW-tanks. The main advantages with this method are that the fish will be undisturbed until processed and easily unloaded. The disadvantages are: marketing problems and reduced pay-load on existing vessels (Eddie, 1980). Portable 1.1m^3 CSW containers are used to a limited extent in combination with the earlier mentioned conveyor system originally laid-out for boxing without the above-mentioned reduced pay-load compared to boxing (Anon., 1986). Also, small coastal vessels can use insulated portable CSW containers (Figure 7.19).

Figure 7.19 Some of the 10 pieces of 200 l CSW containers placed on deck on a 15 GRT cod gillnet wooden boat

Unloading

Shelfed fish are unloaded, using baskets or boxes which are filled as the shelves are removed. The fish are tackled from the hold and emptied on a conveyor leading to the manual grading and weighing process.

Boxed fish iced in 20 or 40 kg boxes at sea will normally be unloaded in pallet loads of, for instance, twelve 40 kg boxes per pallet. Swedish boats use hydraulic deck-mounted cranes and a special pallet fork during unloading. An unloading rate of approximately 30 t/h is possible by this method.

Danish coastal vessels, landing their pelagic catches daily, use quay mounted P/V-pumps for

unloading their catches, which often are iced in pens in layers up to approximatly 1 m height. It is necessary only to add small quantities of water to make the pump function properly. The fish is delivered to a strainer from where a conveyer leads the fish to a size grader. The strained water is recirculated to the hold. Grading machines with up to 30 t/h are often installed.

In Scandinavia the 30-50 in RSW/CSW vessels still use brailing to a limited extent when unloading their catches at a rate of 30 to 50 t/h. The main disadvantage of this method is that very big hatches are needed to obtain reasonable unloading rates.

P/V-pumps have recently been introduced for unloading herring and mackerel. Thus vessels with small tanks, e.g., 30 in , and small hatches can also be unloaded at a rate similar to or higher than the above-mentioned brailing rate. P/V-pumping rates will typically be around 40-50 t/h. The fish can be transported directly in a tube system into the factory where representative samples are taken for quality assessment.

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8. ASSESSMENT OF FISH QUALITY

8.1. Sensory methods
8.2. Biochemical and chemical methods
8.3. Physical methods
8.4. Microbiological methods

Most often "quality" refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals. It is important to remember that "quality'' implies different things to different people and is a term which must be defined in association with an individual product type. For example, it is often thought that the best quality is found in fish which are consumed within the first few hours post mortem. However, very fresh fish which are in rigor mortis are difficult to fillet and skin and are often unsuitable for smoking. Thus, for the processor, slightly older fish which have passed through the rigor process are more desirable.

The methods for evaluation of fresh fish quality may be conveniently divided into two categories: sensory and instrumental. Since the consumer is the ultimate judge of quality, most chemical or instrumental methods must be correlated with sensory evaluation before being used in the laboratory. However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, etc., may be reduced.

8.1 Sensory methods

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing.

Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes.

Instruments capable of measuring parameters included in the sensory profile are the Instron, Bohlin Rheometer for measuring texture and other rheologic properties. Microscopic methods combined with image analysis are used to assess structural changes and "the artificial nose" to evaluate odour profile (Nanto et al., 1993).

Sensory process

In sensory analysis appearance, odour, flavour and texture are evaluated using the human senses. Scientifically, the process can be divided into three steps. Detection of a stimulus by the human sense organs; evaluation and interpretation by a mental process; and then the response of the assessor to the stimuli. Variations among individuals in the response of the same level of stimuli can vary and can contribute to a non-conclusive answer of the test. People can, for instance, differ widely in their response to colour (colour blindness) and also in their sensitivity to chemical stimuli. Some people cannot taste rancid flavour and some have a very low response to cold-storage flavour. It is very important to be aware of these differences when selecting and training judges for sensory analysis. Interpretation of the stimulus and response must be trained very carefully in order to receive objective responses which describe features of the fish being evaluated. It is very easy to give an objective answer to the question: is the fish in rigor (completely stiff), but more training is needed if the assessor has to decide whether the fish is *post* or pre-rigor. Subjective assessment, where the response is based on the assessor's preference for a product, can be applied in the fields like market research and product development where the reaction of the consumer is needed. Assessment in quality control must be objective.

Sensory methods

The analytical objective test used in quality control can be divided into two groups: discriminative tests and descriptive tests. Discriminative testing is used to determine if a difference exists between samples (triangle test, ranking test). Descriptive tests are used to determine the nature and intensity of the differences (profiling and quality tests). The subjective test is an affective test based on a measure of preference or acceptance.

Discriminative test
Is there a difference?

- **Triangle test**
- **Ranking**

Descriptive test
What is the difference or the absolute value and how big is it?

- **Quality index method**
- **Structured scaling**
- **Profiling**

Affective test
Is the difference of any significance?

- **Market test**

Figure 8.1 Methods of sensory analysis

In the following, examples of discriminative and descriptive testing will be given. For further information concerning market testing, see Meilgaard *et al.* (1991).

Quality assessment of fresh fish

Quality Index Method

During the last 50 years many schemes have been developed for sensory analysis of **raw fish.** The first modern and detailed method was developed by Torry Research Station (Shewan *et al.*, 1953). The fundamental idea was that each quality parameter is independent of other parameters. Later, the assessment was modified by collecting a group of characteristic features to be expressed in a score. This gives a single numerical value to a broad range of characteristics. In Europe today, the most commonly used method for quality assessment in the inspection service and in the fishing industry is the EU scheme, introduced in the council decision No. 103/76 January 1976 (Table 5.2). There are three quality levels in the EU scheme, E (Extra), A, B where E is the highest quality and below B is the level where fish is discarded for human consumption. The EU scheme is commonly accepted in the EU countries for sensory assessment. There is, however, still some discrepancy as the scheme

does not take account of differences between species into account as it only uses general parameters. A suggestion for renewal of the EU scheme can be seen in Multilingual Guide to EU Freshness Grades for Fishery Products (Howgate *et al.,* 1992),where special schemes for whitefish, dogfish, herring and mackerel are developed (Appendix E).

A new method, the Quality Index Method (QIM) originally developed by the Tasmanian Food Research unit (Bremner *et al.,* 1985)*,* is now used by the Lyngby Laboratory (Jonsdottir, 1992) for fresh and frozen cod, herring and saithe. In the Nordic countries and Europe it has also been developed for redfish, sardines and flounder.

Table 8.1 Quality assessment scheme used to identify the quality index demerit score (Larsen *et al.* 1992)

Quality parameter	Character	Score (ice/seawater)
General appearance	Skin	0 Bright, shining 1 Bright 2 Dull
	Bloodspot on gill cover	0 None 1 Small, 10-30% 2 Big, 30-50% 3 Very big, 50-100%
	Stiffness	0 Stiff, in *rigor mortis* 1 Elastic 2 Firm 3 Soft
	Belly	0 Firm 1 Soft 2 Belly burst
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Musty/sour 3 Stale meat/rancid
Eyes	Clarity	0 Clear 1 Cloudy

	Shape	0 Normal 1 Plain 2 Sunken
Gills	Colour	0 Characteristic, red 1 Faded, discoloured
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Sweaty/slightly rancid 3 Sour stink/stale, rancid
Sum of scores		(min. 0 and max. 20)

QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 4 demerit points (Jonsdottir, 1992). QIM is using a practical rating system, in which the fish is inspected and the fitting demerit point is recorded. The scores for all the characteristics are then summed to give an overall sensory score, the so-called quality index. QIM gives scores of zero for very fresh fish while increasingly larger totals result as fish deteriorate. The description of evaluation of each parameter is written in a guideline. For example, 0 demerit point for the appearance of the skin on herring means very bright skin only experienced in freshly caught herring. The appearance of the skin in a later state of decay turns less bright and dull and gives 2 demerit points. Most of the parameters chosen are equal to many other schemes. After the literal description, the scores are ranked for each description for all the parameters, giving scores 0-1, 0-2, 0-3 or 0-4. Parameters with less importance are given lower scores. The individual scores never exceed 4, so no parameter can excessively unbalance the score. A scheme for herring is shown in table 8.1; it is emphazised that it is neccessary to develop a new scheme for every species (the scheme for cod is seen in Appendix D).

There is a linear correlation between the sensory quality expressed as a demerit score and storage life on ice, which makes it possible to predict remaining storage life on ice. The theoretical demerit curve has a fixed point at (0,0) and its maximum has to be fixed as the point where the fish has been rejected by sensory evaluation of, e.g., the cooked product (see under structured scaling) or otherwise determined as the maximum keeping time. Using cooked evaluation the two parallel sensory tests demand an experienced sensory panel even though this is only required while developing the scheme, and later on it will not be necessary to assess cooked fish in order to predict the remaining shelf life. QIM does not follow the traditionally accepted S-curve pattern for deterioration of chilled fish during storage (Figure 5.1). The aim is a straight line which makes it possible to distiguish between fish at the start of the

plateau phase and fish near the end of the plateau phase (Figure 8.2).

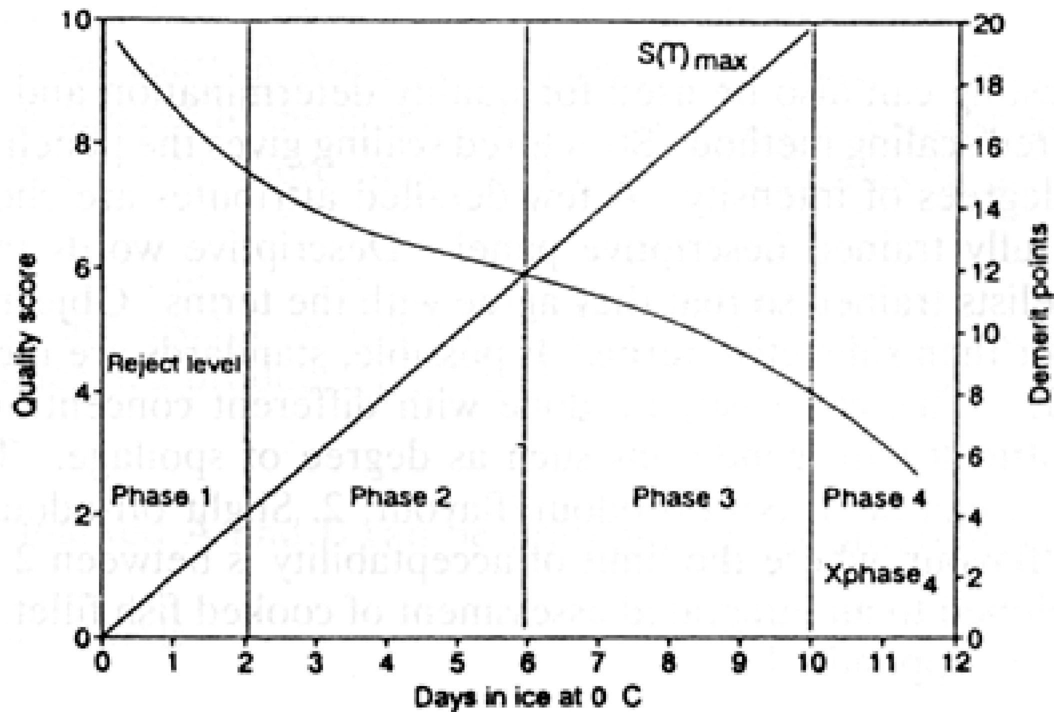


Figure 8.2 Combination of sensory curves for raw S(T) and cooked fish

When a batch of fish in Figure 8.2 reaches a sum of demerit points of 10, the remaining keeping time in ice will be 5 days. To predict remaining shelf life, the theoretical curve can be converted as shown in Figure 8.3.

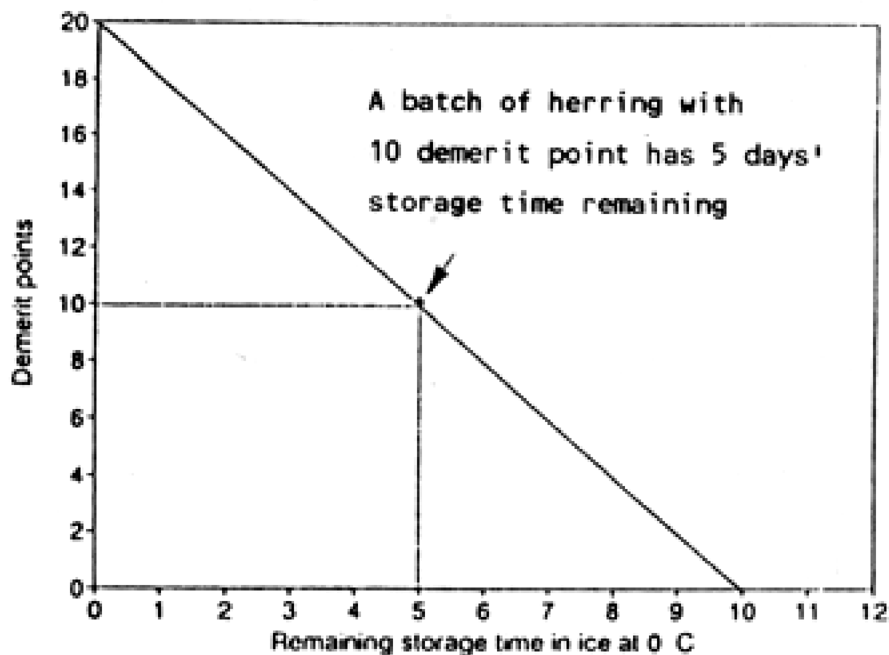


Figure 8.3 A curve to predict the storage time remaining for herring stored in ice or sea water at 0°C

A fish merchant may want to know how long his purchase will remain saleable if the fish are stored on ice immediately. A buyer at a fish market might be interested in the equivalent number of days on ice where the fish have been stored since they were caught, and thus how much marketable time on ice is left. These condition indicators can be extracted for a fish sample with a known rate of change in demerit points using the quality index method.

Structured Scaling

Descriptive testing can also be used for quality determination and shelf life studies applying a structured scaling method. Structured scaling gives the panelist an actual scale showing several degrees of intensity. A few detailed attributes are chosen often based on work from a fully trained descriptive panel. Descriptive words must be carefully selected, and panelists trained so that they agree with the terms. Objective terms should be preferred rather than subjective terms. If possible, standards are included at various points of the scale. This can easily be done with different concentrations of salt but might be more difficult with conditions such as degree of spoilage. The most simple method (Table 8.2) can be 1. No off-odour/flavour, 2. Slight off-odour/flavour and 3. Severe off-odour/flavour, where the limit of acceptability is between 2 and 3. This has been further developed to an integrated assessment of cooked fish fillet of lean and fatty fish (see example in Appendix E).

A 10-point scale is used as described under 5.1 Sensory changes, and an overall impression of odour, flavour and texture is evaluated in an integrated way. For statistics, t-test and analysis of variance can be used (see example in Appendix F).

Table 8.2 Evaluation of cooked fish

		Grade		Score
Acceptable	No off-odour/ flavour	I	Odour/flavour characteristic of species, very fresh, seaweedy Loss of odour/flavour Neutral	10 9 8 7 6
	Slight off-odour/ flavour	II	Slight off-odours/flavours such as mousy, garlic, bready, sour, fruity, rancid	5 4
Limit of acceptability				
Reject	Severe off-odour/ flavour	III	Strong off-odours/flavours such as stale cabbage, NH_3, H_2S or sulphides	3 2 1

Quality assessment of fish products

Assessment of **fishery products** can both be performed as a discriminative test and as a descriptive test.

Triangle test

The most used discriminative test in sensory analysis of fish is the triangle test (ISO standard 4120 1983), which indicates whether or not a detectable difference exists between two samples. The assessors receive three coded samples, are told that two of the samples are identical and one is different, and are asked to identify the odd sample.

Analysis of results from the triangle test is done by comparing the number of correct identifications with the number you would expect to obtain by chance

alone. In order to test this the statistical chart in Appendix A must be consulted. The number of correct identifications is compared to the number expected by use of a statistical table, e.g., if the number of responses is 12, there must be 9 correct responses to achieve a significant answer (1% level).

Triangle tests are useful in determining, e.g., if ingredient substitution gives a detectable difference in a product. Triangle tests are often used when selecting assessors to a taste panel.

The samples marked A and B can be presented in six different ways:

ABB	BBA	AAB
BAB	ABA	BAA

Equal numbers of the six possible combinations are prepared and served to the panel members. They must be served randomly, preferably as duplicates. The number of panel members should be no less than 12 (an example of a triangle test from the ISO standard can be seen in Appendix B).

Table 8.3 Example of score sheet: triangle test

TRIANGLE TEST	
Name:	Date:
Type of sample:	
Two of these three samples are identical, the third is different. Examine the samples from left to right and circle the number of the test sample which is different. It is essential you make a choice (guess if no difference is apparent).	
Test sample No.:	
Describe the difference:	

Ranking

In a ranking exercise, a number of samples are presented to the taste panel. Their task is to arrange them in order according to the degree to which they exhibit some specified characteristics, e.g, downward concentration of salt. Usually ranking can be done more quickly and with less training than evaluation by other methods. Thus ranking is often used for preliminary screening. The method gives no individual differences among samples and it is not suited for sessions where many criteria have to be judged simultaneously.

Profiling

Descriptive testing can be very simple and used for assessment of a single attribute of texture, flavour and appearance. Methods of descriptive analysis which can be used to generate a complete description of the fish product have also been developed. An excellent way of describing a product can be done by using flavour profiling (Meilgaard et al., 1991). Quantitative Descriptive Analysis provides a detailed description of all flavour characteristics in a qualitative and quantitative way. The method can also be used for texture. The panel members are handed a broad selection of reference samples and use the samples for creating a terminology that describes the product.

In Lyngby a descriptive sensory analysis for fish oil using QDA has been developed. A trained panel of 16 judges is used. Descriptive terms such as paint, nutty, grassy, metallic are used for describing the oil on an intensity scale. A moderately oxidized fish oil is given fixed scores and used as a reference.

Table 8.4 Profile of fish oil

Taste	Std				
Fresh fish	2				
Amine	1				
Oily	3				
Sweet	2				
Metallic	3				
Grassy	3				
Painty	2				
Fruity	2				

Remarks						
Taste as a whole (0 unacceptable - 9 neutral)	6					

Advanced multivariate analysis is used for statistics and makes it possible to correlate single attributes to oxidative deterioration in the fish oil. The results can be reported in a "spider's web" (se Figure 8.5). The panel uses an intensity scale normally ranging from 0 to 9.

Profiling can be used for all kinds of fishery products, even for fresh fish when special attention is placed on a single attribute.

The results of QDA can be analyzed statistically using analysis of variance or multivariate analysis (O'Mahony, 1986).

Statistics

In any experiment including sensory analysis the experimental design (e.g., number of panel members, number of samples, time aspects, hypotheses to test) and statistical principles should be planned beforehand. Failure to do so may often lead to insufficient data and non-conclusive experiments. A guide to the most used statistical methods can be seen in Meilgaard *et al.* (1991). A panel used for descriptive testing shall preferably consist of no less than 8-10 persons, and it should be remembered that the test becomes statistically much stronger if it is done in duplicate. This can often be difficult using sensory analysis on small fish. Thus the experiment must include a sufficient number ofsamples to remove the sources of variability, and the testing must be properly randomized. For further information see O'Mahony (1986) and Smith (1989).

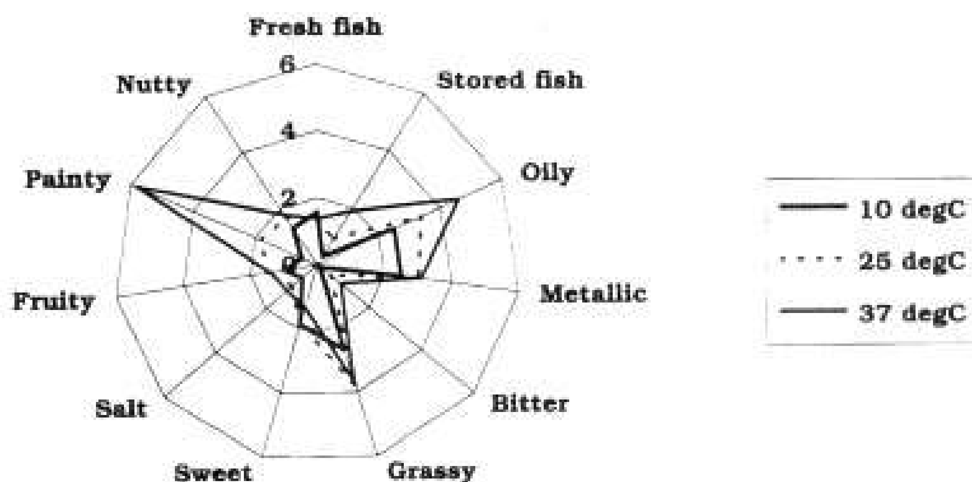


Figure 8.4 Flavour profiles of a fish oil after 2 weeks of storage at various temperatures (Rorbaek et al., 1993)

Training of assessors

Training of assessors for sensory evaluation is necessary in almost all sensory methods. The degree of training depends on the difficulty and complexity of the assessment. For example, for profiling a thorough training with presentation of a large range of samples is necessary in order to obtain proper definitions of the descriptors an equal use of the scoring system. The triangle test normally requires a minor degree of training.

Sensory quality control is often done by a few persons either at the fish market when buying fish or at quality inspection. The experience of these persons allows them to grade the fish. Starting as a fish inspector it is not necessary to know all the different methods of sensory assessment described in textbooks (Meilgaard et al., 1991), but some of the basic principles must be known. The assessor must be trained in basic tastes, the most common fish taste and must learn the difference between off- flavour and taints. This knowledge can be provided in a 2- day basic training course.

In bigger companies and for experimental work a further training of a sensory panel is necessary in order to have an objective panel. A laboratory panel must have 8-10 members, and the training and testing of panel members must be repeated regularly.

Facilities

The facilities required for sensory evaluation is described in textbooks on sensory evaluation.

The minimum requirement for evaluation is a preparation room and a room where the samples are served. The rooms should be well ventilated and provided with a good light (Howgate, 1994). There must be enough space on the tables for inspection of raw samples of fish.

Cooking and serving

The samples of fishery products should not be less than 50-100 g per person. Fillets can be served in loins and should be cooked to an internal temperature of 65°C. The samples should be kept warm when served, i.e., in insulated containers or on a hot plate. The fish can be heat treated by steaming in a

water bath, packed as boiled-in-the-bag in a plastic pouche or in alufoil. An oven (microwave or steam-oven) can also be used for heat treatment. The fish can be packed in plastic or put on a small porcelain plate covered with alufoil. For cod loins (2,5x1,5x6cm) on a porcelain plate covered with alufoil the heating time in a steam-oven (convectomate) at 100°C must be 10 minutes. The samples should be coded before serving.

8.2 Biochemical and chemical methods

The appeal of biochemical and chemical methods for the evaluation of seafood quality is related to the ability to set quantitative standards. The establishment of tolerance levels of chemical spoilage indicators would eliminate the need to base decisions regarding product quality on personal opinions. Of course, in most cases sensory methods are useful for identifying products of very good or poor quality. Thus, biochemical/ chemical methods may best be used in resolving issues regarding products of marginal quality. In addition, biochemical/ chemical indicators have been used to replace more time consuming microbiological methods. Such objective methods should however correlate with sensory quality evaluations and the chemical compound to be measured should increase or decrease with the level of microbial spoilage or autolysis. It is also important that the compounds to be measured must not be affected by processing (e.g., breakdown of amines or nucleotides in the canning process as a result of high temperatures).

The following is an overview of some of the most useful procedures for the objective measurement of seafood quality. Woyewoda *et al.* (1986) have produced a comprehensive manual of procedures (including proximate composition of seafood).

Amines - Total Volatile Basic Amines

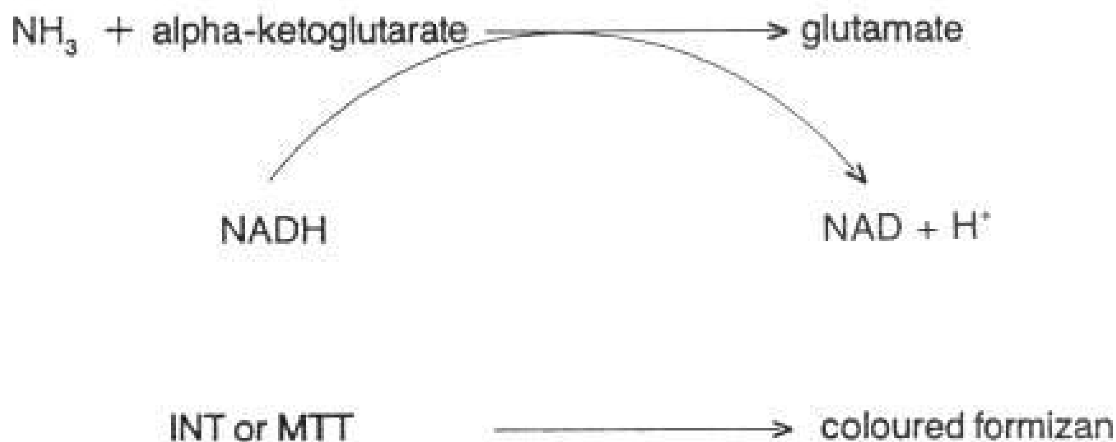
Total volatile basic amines (TVB) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of cod as well as several other species (Rehbein and Oehlenschlager, 1982). They are particularly useful for the measurement of quality in cephalopods such as squid (LeBlanc and Gill, 1984), industrial fish for meal and silage (Haaland and Njaa,

1988), and crustaceans (Vyncke, 1970). However, it should be kept in mind that TVB values do not reflect the mode of spoilage (bacterial or autolytic), and results depend to a great extent on the method of analysis. Botta *et al.* (1984) found poor agreement among six published TVB procedures. Most depend upon either steam distillation of volatile amines or microdiffusion of an extract (Conway, 1962); the latter method is the most popular in Japan. For a comparative examination of the most common procedures for TVB analysis, see Botta *et al.* (1984).

Ammonia

Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino- acids. It is also produced in the autolytic breakdown of adenosine monophosphate (AMP, Figure 5.4) in chilled seafood products. Although ammonia has been identified as a volatile component in a variety of spoiling fish, few studies have actually reported the quantification of this compound since it was impossible to determine its relative contribution to the overall increase in total volatile bases.

Recently, two convenient methods specifically for identifying ammonia have been made available. The first involves the use of the enzyme glutamate dehydrogenase, NADH and alpha-ketoglutarate. The molar reduction of NH_3 in a fish extract yields one mole of glutamic acid and NAD which can be monitored conveniently by absorbance measurements at 340 nm. Test kits for ammonia based on glutamate dehydrogenase are now available from Sigma (St. Louis, Missouri, USA) and Boehringer Mannheim (Mannheim, Germany). A third type of ammonia test kit is available in the form of a test strip (Merck, Darmstadt, Germany) which changes colour when placed in contact with aqueous extracts containing ammonia (ammonium ion). LeBlanc and Gill (1984) used a modification of the glutamate dehydrogenase procedure to determine the ammonia levels semi-quantitatively without the use of a spectrophotometer, but with a formazan dye, which changed colour according to the following reaction:



where INT is iodontrotetrazolium and MTT is 3 - [4,5-dimethylthiazol-2-yl] 2,5 diphenyl tetrazolium bromide

Ammonia has been found to be an excellent indicator of squid quality (LeBlanc and Gill, 1984) and comprised a major proportion of the TVB value for chilled short-finned squid (Figure 8.7). However, ammonia would appear to be a much better predictor of the latter changes in quality insofar as finfish are concerned. LeBlanc (1987) found that for iced cod, the ammonia levels did not increase substantially until the sixteenth day of storage. It would appear that at least for herring, the ammonia levels increase far more quickly than trimethylamine (TMA) levels which have traditionally been used to reflect the growth of spoilage bacteria on lean demersal fish species. Thus ammonia has potential as an objective quality indicator for fish which degrades autolytically rather than primarily through bacterial spoilage.

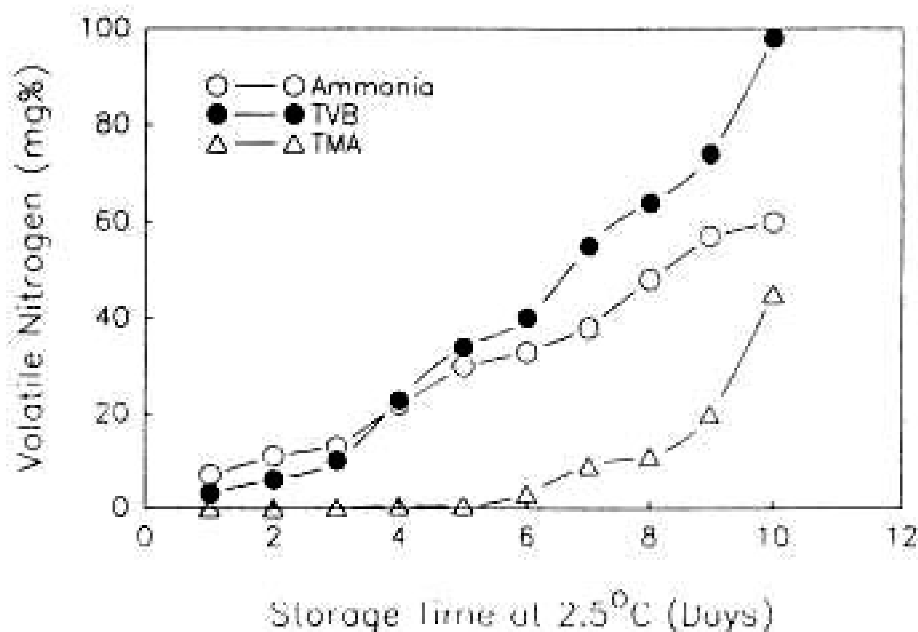


Figure 8.7 Effect of storage time on production of ammonia. TVB and TMA in

short finned squid *(Illex illecebrosus),* adapted from Gill (1990)

Trimethylainine (TMA)

Trimethylamine is a pungent volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. Although TMA is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good. This phenomenon is now thought to be due to the presence of small numbers of "specific spoilage" bacteria which do not always represent a large proportion of the total bacterial flora, but which are capable of producing large amounts of spoilage -related compounds such as TMA. One of these specific spoilage organisms, *Photobactetium phosphoreum,* generates approximately 10 - 100 fold the amount of TMA than that produced from the more commonly-known specific spoiler, *Shewanella putrefaciens* (Dalgaard, 1995) (in press).

As mentioned above, TMA is not a particularly good indicator of edibility of herring quality but is useful as a rapid means of objectively measuring the eating quality of many marine demersal fish. The correlations between TMA level or more preferably, TMA index (where TMA index = log (1 + TMA value)) and eating quality have been excellent in some cases (Hoogland, 1958; Wong and Gill, 1987). Figure 8.8 illustrates the relationship between odour score and TMA level for iced cod. The linear coefficient of determination was statistically significant at the P £ 0.05 level.

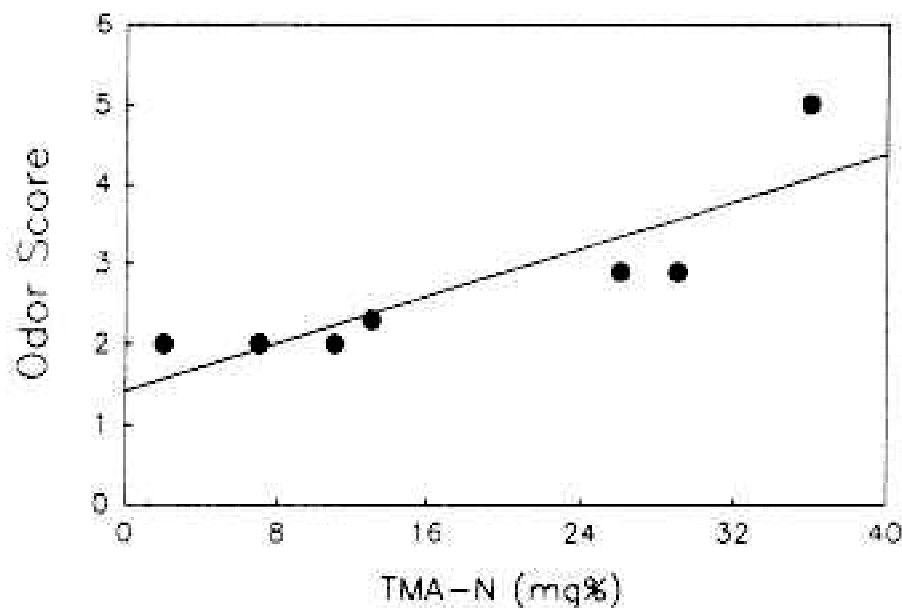


Figure 8.8 Relationship between odour score and TMA levels for iced cod. The

straight line was fitted by linear regression analysis (P£ 0.05) and all data points were averages of data obtained for three individual cod, adapted from Wong and Gill (1987)

The chief advantages of TMA analysis over the enumeration of bacterial numbers are that TMA determinations can be performed far more quickly and often reflect more accurately the degree of spoilage (as judged organoleptically) than do bacterial counts. For example, even high quality fillets cut with a contaminated filleting knife may have high bacterial counts. However, in such a case the bacteria have not had the opportunity to cause spoilage, thus TMA levels are bound to be low. The chief disadvantages of TMA analyses are that they do not reflect the earlier stages of spoilage and are only reliable for certain fish species. A word of caution should be given concerning the preparation of fish samples for amine analysis. TMA and many other amines become volatile at elevated pH. Most analytical methods proposed to date begin with a deproteinization step involving homogenization in perchloric or trichloroacetic acids. Volatilization of amines from stored samples may result in serious analytical errors. Therefore, samples should be neutralized to pH 7 immediately before analysis and should be left in their acidified form in sealed containers if being stored for extended time periods prior to analysis. It is also important to note that appropriate protection for hands and eyes be worn when handling perchloric and/or trichloroacetic acids. In addition, perchloric acid is a fire hazard when brought into contact with organic matter. Spills should be washed with copious quantities of water. Some of the methods of analysis reported to date include colorimetric (Dyer, 1945; Tozawa, 1971), chromatographic (Lundstrom and Racicot, 1983; Gill and Thompson, 1984) and enzymatic analysis (Wong and Gill, 1987; Wong *et al.,* 1988), to name but a few. For a more comprehensive review of the analytical techniques for TMA see the recent review articles: (Gill 1990, 1992).

Dimethylarnine (DMA)

As outlined in section 5.2, certain types of fish contain an enzyme, TMAO dimethylase (TMAO-ase), which converts TMAO into equimolar quantities of DMA and formaldehyde (FA). Thus for fish in the cod (gadoid) family, DMA is produced along with FA in frozen storage with the accompanying FA-induced toughening of the proteins. The amount of protein denaturation is roughly proportional to the amount of FA/DMA produced, but it is most common to monitor the quality of frozen-stored gadoid fish by measuring DMA rather than FA. Much of the FA becomes bound to the tissue and is thus not extractable and cannot be measured quantitatively. The most common method for DMA analysis is a colorimetric determination of the DMA in deproteinized fish extracts. The Dyer and Mounsey (1945) procedure is still in use today although

perhaps more useful is the colorimetric assay proposed by Castell *et al.* (1974) for the simultaneous determination of DMA and TMA, since both are often present in poor quality frozen fish. Unfortunately, many of the colorimetric methods proposed to date lack the specificity where mixtures of different amines are present in samples. The chromatographic methods including gas-liquid chromatography (Lundstrom and Racicot, 1983) and high performance liquid chromatography (Gill and Thompson, 1984) are somewhat more specific, and are not as prone to interferences as the spectrophotometric methods. Also, most of the methods proposed to date for the analysis of amines are destructive and not well suited for analyzing large numbers of samples. Gas chromatographic analysis of headspace volatiles has been proposed as a non-destructive alternative for amine determinations; however, none of the methods proposed to date are without serious practical limitations.

Dimethylamine is produced autolytically during frozen storage. For gadoid fish such as hake, it has been found to be a reliable indicator of FA-induced toughening (Gill et *al.,* 1979). Because it is associated with membranes in the muscle, its production is enhanced with rough handling and with temperature fluctuations in the cold storage facility. Dimethylamine has little or no effect on the flavour or texture of the fish *per se,* but is an indirect indicator of protein denaturation which is often traceable to improper handling before and/or during frozen storage.

BiogenicAmines

Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, presumably so that the organisms may raise the pH of the growth medium through the production of amines.

Histamine, putrescine, cadaverine and tyramine are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. Histamine has received most of the attention since it has been associated with incidents of scombroid poisoning in conjunction with the ingestion of tuna, mackerel, mahi-mahi (dolphinfish from Hawaii). However, the absence of histamine in scombroid fish (tuna, mackerel, etc.) does not ensure the wholesomeness of the product since spoilage at chill storage temperatures does not always result in the production of histamine. Mietz and Karmas (1977) proposed a chemical quality index based on biogenic amines which reflected the quality loss in canned tuna where:

$$\text{Quality Index} = \frac{\text{ppm histamine} + \text{ppm putrescine} + \text{ppm cadaverine}}{1 + \text{pm spermidine} + \text{ppm spermine}}$$

They found that as the quality index ratio increased, the sensory scores on the canned' product decreased. Later, Farn and Sims (1987) followed the production of histamine, cadaverine and putrescine in skipjack and yellowfin tuna at 20°C and found that cadaverine and histamine increased exponentially after an initial lag period of about 36 hours. However, putrescine increased slowly after an initial lag period of 48 hours. Levels of cadaverine and histamine increased to maximum levels of 5-6µg/g tuna but the authors reported that the absence of such amines in raw or cooked product did not necessarily mean that the products were not spoiled.

It is interesting to note that most of the biogenic amines are stable to thermal processing, so their presence in finished canned products is a good indication that the raw material was spoiled prior to heating.

Some of the methods for biogenic amine analysis include high pressure liquid chromatography (Mietz and Karmas, 1977), gas chromatography (Staruszkiewicz andBond, 1981), spectrofluorometric (Vidal-Carou *et al.,* 1990) and a newly-developed rapid enzymatic method for histamine using a microplate reader (Etienne and Bregeon, 1992).

Nucleotide Catabolites

A discussion of the analysis of nucleotide catabolites has been presented in section 5.2 -Autolytic Changes, although all of the catabolic changes are not due to autolysis alone. Most of the enzymes involved in the breakdown of adenosine triphosphate (ATP) to inosine monophosphate (IMP) are believed in most cases to be autolytic whereas the conversion of IMP to inosine (Ino) and then hypoxanthine (Rx) are believed mainly to be due to spoilage bacteria although Hx has been shown to accumulate slowly in sterile fish tissue. Since the levels of each of the catabolic intermediates rise and fall within the tissue as spoilage progresses, quality assessment must never be based upon levels of a single catabolite since the analyst has no way of knowing whether a single compound is increasing or decreasing. For example, if the IMP content of a fish sample were determined to be 5 µmoles/g tissue, the sample might well have been taken from a very fresh fish or a fish on the verge of spoilage, depending on whether or not AMP were present.

Thus, the analysis of the complete nucleotide catabolite profile is nearly always recommended. A complete analysis of nucleotide catabolites may be completed on a fish extract in 12-25 minutes using a high pressure liquid chromatographic (HPLC) system equipped with a single pump and spectrophotometric detector (wavelength 254 nm). Perhaps the simplest HPLC technique published to date is that proposed by Ryder (1985).

Several other approaches have been proposed for the analysis of individual or combination of nucleotide catabolites but none are more reliable than the HPLC approach. A word of caution is perhaps in order with regard to the quantitative analysis of nucleotide catabolites. Most methods proposed to date involve deproteinization of the fish samples by extraction with perchloric or trichloracetic acids. It is important that the acid extracts are neutralized with alkali (most often potassium hydroxide) as soon as possible after extraction to prevent nucleotide degradation in the extracts. Neutralized extracts appear to be quite stable even if held frozen for several weeks. One advantage of using perchloric acid is that the perchlorate ion is insoluble in the presence of potassium. Thus, neutralizing with KOH is a convenient method of sample "clean-up" before HPLC analysis and this procedure helps to extend the life of the HPLC column. Also, it should be noted that nucleotide determination on canned fish does not necessarily reflect the levels in the raw material. *Gill et al.* (1987) found recoveries of 50%, 75%, 64% and 92% for AMP, IMP, Ino and Hx standards which were spiked into canned tuna prior to thermal processing.

Several unusual but innovative approaches utilizing enzymatic assays have been proposed over the years and are presented in Table 8.3. All of the approaches to date rely on destructive sampling (tissue homogenization). It should be noted that regardless of the approach, enzymes denature with time and thus test kits, enzyme-coated strips, electrodes or sensors have a limited shelf life whereas the HPLC techniques do not.

Table 8.3 Fish Freshness Testing Using Enzyme Technology

Analyte (s)	**Principle**	**Advantages**	**Disadvantages**	**Reference**
Hx	enzymes (xanthine oxidase, X0) immobilized on a test strip	rapid simple to use outside the lab	semi -quantitative only capable of measuring Hx (later stages of spoilage)	Jahns *et al.* (1976)

Hx, Ino	test strip, with immobilized enzymes	rapid simple to use outside the lab	semi -quantitative poor reproducibility limited to Hx and Ino (later stages spoilage)	Ehira *et al.* (1986)
IMP, Ino, Hx	enzyme-coated oxygen electrode	rapid accurate	more complicated and time consuming than test strip technology	Karube *et al.* (1984)
K-index	coupled enzyme assay "KV-101 Freshness Meter"	rapid results comparable to HPLC	must purchase enzymes and reagents cost ?	commercially available from Orienta Electric, Niiza Saitama 352, Japan
K-index	enzyme-coated oxygen electrode "Microfresh"	rapid results comparable to HPLC	cost ?	commercially available from Pegasus Instruments, Agincourt, ON, Canada

The factors which have been shown to affect the nucleotide breakdown pattern include species, temperature of storage and physical disruption of the tissue. In addition, since nucleotide breakdown reflects the combined action of autolytic enzymes and bacterial action, the types of spoilage bacteria would no doubt affect the nucleotide patterns. The selection of which nucleotide or combination of nucleotide catabolites to be measured should be made carefully. For example, in certain cases one or two of the catabolites change rapidly with time of chilled storage, whereas the remaining components may change very little. The technical literature should be consulted for guidance on this matter. An excellent overview on the biological and technological factors affecting the nucleotide catabolites as quality indicators was presented by Frazer Hiltz *et al.* (1972).

Ethanol

Ethanol has been used for many years as an objective indicator for seafood quality although it is not nearly as common as the analysis of TMA. Since

ethanol can be derived from carbohydrates via anaerobic fermentation (glycolysis) and/or deamination and decarboxylation of amino-acids such as alanine, it is a common metabolite of a variety of bacteria. It has been used to objectively measure the quality of a variety of fish including canned tuna (Iida *et al.,* 1981 a, 1981b; Lerke and Huck, 1977), canned salmon (Crosgrove, 1978*;* Hollingworth and Throm, 1982)*,* raw tuna (Human and Khayat, 1981), redfish, pollock, flounder and cod (Kelleher and Zall, 1983).

To date, the simplest and perhaps most reliable means of measuring ethanol in fish tissue is the use of the commercial enzyme test kits available from Boehringer Mannheim (German) or Diagnostic Chemicals (Charlottetown, P.E. I., Canada). One advantage of using ethanol as a spoilage indicator is that it is heat-stable (although volatile) and may be used to assess the quality of canned fish products.

Measurements of oxidative rancidity

The highly unsaturated fatty acids found in fish lipids (section 4.2) are very susceptible to oxidation (section5.4).The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential to oxidize iodide to iodine or to oxidize iron(II) to iron(III). The concentration of the hydroperoxides may be determined by titrimetricor by spectrophotometric methods, giving the peroxide value (PV) as milliequivalents (mEq) peroxide per 1 kg of fat extracted from the fish. A method for PV- determination by iodometry has been described by Lea (1952)*,* and for determination by spectrophotometry of iron (III)thiocyanate by Stine *et al.* (1954).The methods for PV-determination are empirically based, and comparisons between PVs are only possible for results obtained using identical methods. For instance, the thiocyanate-method may give values 1.5 - 2 times higher than the iodine titration method (Barthel and Grosch, 1974).

For several reasons, interpretation of the PV as an index of quality is not straightforward. First, the hydroperoxides are odour- and flavour-less, thus the PV is not related to the actual sensory quality of the product analyzed. However, the peroxide value may indicate a potential for a later formation of sensorial-objectionable compounds. Second, lipid hydroperoxides break down with time, and a low PV at a certain point during the storage of a product can indicate both an early phase of autoxidation and a late stage of a severely oxidized product, where most hydroperoxides have been broken down (Kanner and Rosenthal, 1992),e.g., in dried, salted fish (Smith *et al.,* 1990).

In later stages of oxidation *secondary oxidation products will* usually be present and thus be indicative of a history of autoxidation. These products (section *5.4)*

comprise aldehydes, ketones, short chain fatty acid and others, many of which have very unpleasant odours and flavours, and which in combination yield the fishy and rancid character associated with oxidized fish lipid. Some of the aldehydic secondary oxidation products react with thiobarbituric acid, forming a reddish coloured product that can be determined spectrophotometrically. Using this principle, a measure of thiobarbituric acid-reactive substances (TBA-RS) can be obtained. Several method variations exist; one method for fish lipids is described by Ke and Woyewoda *(1979),* and for fish by Vyncke *(1975).* The results are expressed in terms of the standard (di-)aldehyde used, malonaldehyde, and reported as micromoles malonaldehyde present in 1 g of fat. *(A note of caution:* Sometimes the TBA-results may be expressed as mg malonaldehyde in 1 g of fat, or as amount of malonaldehyde (µmol or µg) in relation to amount of tissue analyzed.) Several reports (e.g., by Hoyland and Taylor *(1991)* and by Raharjo *et al. (1993))* speak of some correlation between TBA-RS and sensory assessments, but other authors fail to find a correlation (e. g., Boyd *et al., 1993).* Thus, caution is necessary in interpretation of TBA-RS values into measures of sensory quality.

Provided that the PV has not been lowered through extended storage or high temperature exposure, the PV (by iodometric titration) should not be above 10-20 meq/kg fish fat (Connell, *1975).*

Examples of guidelines for TBA-RS-values: foods with TBA-RS above 1-2 µmol MDA-equiv per g fat (Connell, *1975)* or above 10,µmol MDA-equiv per 1 kg fish (Ke *et al.,* 1976) will probably have rancid flavours.

Modern instrumental methods allow analysis of better defined oxidation products (specific hydroperoxides, actual content of malonaldehyde), but for general quality estimation, methods that determine a broader range of oxidation products (such as PV and TBA-RS) are to be preferred, although these methods have their limitations as discussed above. Headspace analysis of the volatile oxidation products gives results correlating well with sensory evaluation (e.g., in catfish (Freeman and Hearnsberger, *1993)),* but the method requires access to gas chromatographic equipment.

8.3 Physical methods

Electrical Properties

It has long been known that the electrical properties of skin and tissue change after death, and this has been expected to provide a means of measuring *post mortem* changes or degree of spoilage. However, many difficulties have been encountered in developing an instrument: for example, species variation;

variation within a batch of fish; different instrument readings when fish are damaged, frozen, filleted, bled or not bled; and a poor correlation between instrument reading and sensory analysis. Most of these problems, it is claimed, are overcome by the GR Torrymeter (Jason and Richards, 1975). However, the instrument is not able to measure quality or freshness of a single fish, although it may find application in grading batches of fish, as shown in Figure 8.9.

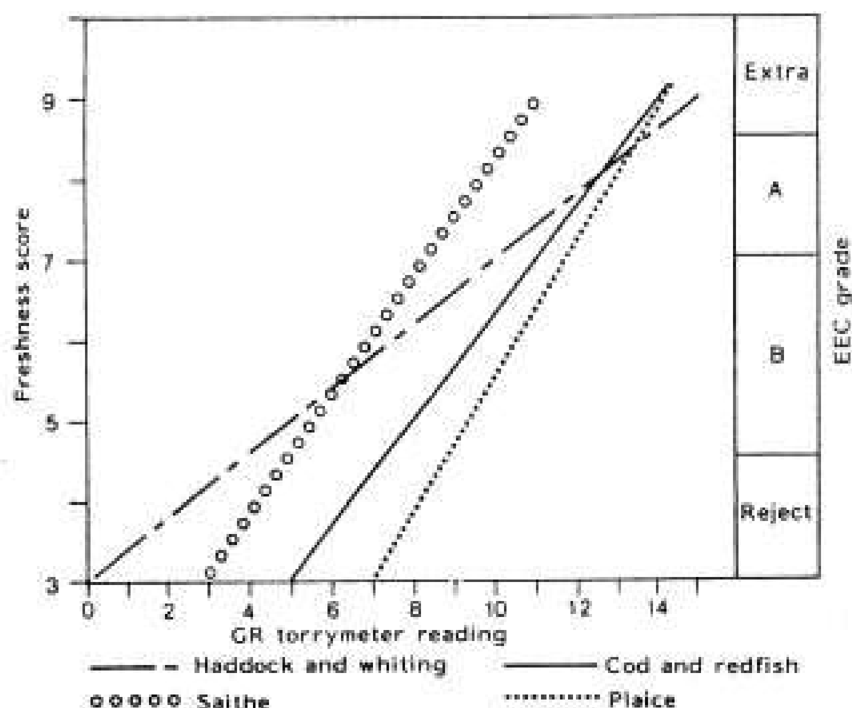


Figure 8.9 Relationship between GR Torrymeter readings of various species of fish and freshness, adapted from Cheyne (1975)

Until recently, no instruments have been capable of on-line determination of quality although this type of mechanized quality evaluation would be highly desirable on the processing floor. The RT Freshness Grader development began in 1982 and, by 1990, a production model capable of sorting 70 fish per minute over 4 channels was made available. The developer was Rafagnataekni Electronics (Reykjavik, Iceland) based the sensing unit on the GR Torrymeter.

pH and Eh

Knowledge about the pH of fish meat may give valuable information about its condition. Measurements are carried out with a pH-meter by placing the electrodes (glass-calomel) either directly into the flesh or into a suspension of fish flesh in distilled water. Measurements of Eh are not carried out routinely, but it is likely that a freshness test can be based on this principle.

Measuring Texture

Texture is an extremely important property of fish muscle, whether raw or cooked. Fish muscle may become tough as a result of frozen storage or soft and mushy as a result of autolytic degradation. Texture may be monitored organoleptically but there has for many years been a need for the development of a reliable objective rheological test which would accurately reflect the subjective evaluation of a well-trained panel of judges. Gill *et al.* (1979) developed a method for evaluating the formaldehyde-induced toughening of frozen fish muscle. The method utilized an Instron Model TM equipped with a Kramer shear cell with 4 cutting blades. This method correlated well with data obtained from a trained texture panel. A method for measuring hardness/ softness of fish flesh, designated as compressive deformability, has been reported by Johnson *et al.* (1980). An accuratelycut fish sample is compressed by a plunger, and the stress-strain curve recorded. A modulus of deformability is calculated from the recorded graph. The results from such measurements may, however, be difficult to interpret.

Another method, measuring the shear force of fish flesh, has been investigated by Dunajski (1980). From this work, it has been concluded that a thin-bladed shear force cell of the Kramer type can be applied.

These are but a few of the examples cited in the literature and until recently all involved expensive equipment and destructive sampling. Therefore, Botta (1991) developed a rapid non-destructive method for the measurement of cod fillet texture. It is a small, portable penetrometer which measures both firmness and resilience. Each test takes only 2-3 seconds to complete and results appear to correlate well with subjective texture grades.

8.4 Microbiological methods

The aim of microbiological examinations of fish products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality of the fish including temperature abuse and hygiene during handling and processing. Microbiological data will in general not give any information about eating quality and freshness. However, as outlined in sections 5 and 6, the number of specific spoilage bacteria will be related to the remaining shelf life and this can be predicted from such numbers (see Figure 5.8).

Traditional bacteriological examinations are laborious, time-consuming, costly and require skill in execution and interpretation of the results. It is recommended that such analyses be limited in number and extent. Various

rapid microbiological methods have been developed during the last decade and some of these automated procedures may be of use when large numbers of samples are to be analyzed.

Total counts

This parameter is synonymous with Total Aerobic Count (TAC) and Standard Plate Count (SPC). The total count represents, if carried out by traditional methods, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. This figure is seldom a good indicator of the sensoric quality or expected shelf life of the product (Huss *et al.,* 1974). In ice-stored Nile perch, the total count was 109 cfu/g for days before the fish was rejected (Gram *et al., 1989)* and in lightly preserved fish products high counts prevail for long time before rejection. If a count is made after systematic sampling and a thorough knowledge of the handling of the fish before sampling, temperature conditions, packaging etc., it may give a comparative measure of the overall degree of bacterial contamination and the hygiene applied. However, it should also be noted that there is no correlation between the total count and presence of any bacteria of public health significance. A summary of different methods used for fish and fish products is given in Table 8.4.

Common plate count agars (PCA) are still the substrates most widely used for determination of total counts. However, when examining several types of seafood a more nutrient rich agar (Iron Agar, Lyngby, Oxoid) gives significantly higher counts than PCA (Gram, 1990). Furthermore, the iron agar yields also the number of hydrogen sulphide producing bacteria, which in some fish products are the specific spoilage bacteria. Incubation temperature at and above 30°C are inappropriate when examining seafood products held at chill temperatures. Pour plating and a 3-4 day incubation at 25°C is relevant when examining products where psychrotrophs are the most important organisms, whereas products where the psychrophilic *Photobacterium phosphoreum* occurs should be examined by surface plating and incubation at maximum 15°C.

Several attempts have been made to ease the procedures for determination of the content of bacteria (Fung *et al., 1987).* Both *Redigel* (RCR Scientific) and *Petrifilm*™ SM (3M Company) are dried agars with a gelling agent to which the sample is added directly. The main advantage of Redigel and Petrifilm compared to conventional plate counts in addition to the costs, is the ease of handling. However, all agar-based methods share a common drawback in the lengthy incubation required.

Microscopic examination of foods is a rapid way of estimating bacterial levels. By phase contrast microscopy the level of bacteria in a sample can be determined within one log-unit. One cell per field of vision equals approximately 5 -10^5cfu/ml at 1000 X magnification. The staining of cells with acridine orange and detection by fluorescence microscopy has earned widespread acceptance as the direct epifluorescence filter technique (DEFT). Whilst microscopic methods are very rapid, the low sensitivity must be considered their major drawback.

Bacterial numbers have been estimated in foods by measuring the amount of bacterial adenosine triphosphate (ATP) (Sharpe *et al., 1970)* or by measuring the amount of endotoxin (Gram-negative bacteria) by the *Limulus* amoebocytes lysate (LAL) test (Gram, *1992).* The former is very rapid but difficulties exist in separating bacterial and somatic ATP.

Table 8.4 Methods for determination of the content of bacteria in seafood

Method	**Temperature, °C**	**Incubation**	**Sensitivity, cfu/g**
Plate count or Iron agar	15-25	3-5 days	10
"Redigel"/"Petrifilm™ SM"	15-25	3-5 days	10
Microcolony-DEFT	15-30	3-4 hours	10^4-10^5
DEFT	-	30 min.	10^4-10^5
ATP	-	1 hour	10^4-10^5
Limulus lysate test	-	2-3 hours	10^3-10^4
Microcalorimetry/Dye reduction Conductance/Capacitance	15-25	4-40 hours	10

Several methods (microcalorimetry, dye reduction, conductance and capacitance) used for rapid estimation of bacterial numbers are based on the withdrawal of a sample, incubation at high temperature (20-25°C) and detection of a given signal. In microcalorimetry the heat generated by the sample is compared to a sterile control, whereas in conductance and capacitance

measurements of the change in electrical properties of the sample, as compared to a sterile control, is registered. The time taken before a significant change occurs in the measured parameter (heat, conductance, etc.) is called the Detection Time (DT). The DT is inversely related to the initial number of bacteria, i.e., early reaction indicates a high bacterial count in the sample. However, although the signal obtained is reversely proportional to the bacterial count done by agar methods, it is only a small fraction of the microflora that give rise to the signal and care must be taken in selection of incubation temperature and substrate.

Spoilage bacteria

The total number of bacteria on fish rarely indicates sensoric quality or expected storage characteristics (Huss *et al.,* 1974). However, it is recognized that certain bacteria are the main cause of spoilage (see section 5.3). Different peptone-rich substrates containing ferric citrate have been used for detection of H_2S-producing bacteria such as *Shewanella putrefaciens,* which can be seen as black colonies due to precipitation of FeS (Levin, 1968; Gram *et al.,* 1987). Ambient spoilage is often caused by members of *Vibrionaceae* that also will form black colonies on an iron agar to which an organic sulphur source is added (e.g., Iron Agar, Lyngby). No selective or indicative medium exists for the *Pseudomonas spp.* that spoil some tropical and freshwater fish or for *Photobacterium phosphoreum* that spoil packed fresh fish. At the Technological Laboratory, Lyngby, a conductance- based method for specific detection of P. *phosphoreum is* currently being developed (Dalgaard, personal communication). The presence or absence of pathogenic bacteria is often evaluated by methods based on immuno- or molecular biology techniques. Such techniques may also be developed for specific spoilage bacteria, and the Technological Laboratory has been currently investigating the use of antibodies specific for S. *putrefaciens* (Fonnesbech *et al.,* 1993).Also, a gene-probe which is specific for S. *putrefaciens* has been developed but has not been tried on fish products (DiChristina and DeLong, *1993).*

Spoilage reactions

Several spoilage reactions can be used for evaluation of the bacteriological status of fish products. As described above, agars on which H_2S producing organisms are counted have been developed. During spoilage of white lean fish, one of the major spoilage reactions is the bacteriological reduction of trimethylamine oxide to trimethylamine (Liston, 1980; Hobbs and Hodgkiss, 1982).When TMAO is reduced to TMA several physical changes occur: the redox-potential decreases, the pH increases and the electrical conductance increases. The measurement of such changes in a TMAO containing substrate

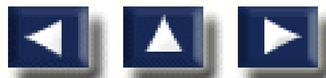
inoculated with the sample can be used to evaluate the level of organisms with spoilage potential; thus the more rapid the change occurs the higher the level of spoilage organisms.

Several authors have inoculated a known amount of sample in a TMAO-containing substrate and recorded the time taken until a significant change in conductivity occurs (Gibson *et al.,* 1984;Gram, *1985;* Jorgensen *et al.,* 1988). This time, the detection time, has been found to be inversely proportional to the number of hydrogen sulphide producing bacteria in fresh aerobically-stored fish, and rapid estimation of their numbers can be given within 8-36 hours.

The changes in redox-potential in a TMAO-containing substrate can be recorded either by electrodes or by observing the colour of a redox-indicator (Huss *et al.,* 1987).As with the conductimetric measurements, the time taken until a significant change is recorded is inversely proportional to the initial amount of bacteria.

Pathogenic bacteria

Several pathogenic bacteria may either be present in the environment or contaminate the fish during handling. A detailed description of these organisms, their importance, and detection methods is given by Huss (1994).



8. ASSESSMENT OF FISH QUALITY

8.1. Sensory methods
8.2. Biochemical and chemical methods
8.3. Physical methods
8.4. Microbiological methods

Most often "quality" refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals. It is important to remember that "quality'' implies different things to different people and is a term which must be defined in association with an individual product type. For example, it is often thought that the best quality is found in fish which are consumed within the first few hours post mortem. However, very fresh fish which are in rigor mortis are difficult to fillet and skin and are often unsuitable for smoking. Thus, for the processor, slightly older fish which have passed through the rigor process are more desirable.

The methods for evaluation of fresh fish quality may be conveniently divided into two categories: sensory and instrumental. Since the consumer is the ultimate judge of quality, most chemical or instrumental methods must be correlated with sensory evaluation before being used in the laboratory. However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, etc., may be reduced.

8.1 Sensory methods

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing.

Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes.

Instruments capable of measuring parameters included in the sensory profile are the Instron, Bohlin Rheometer for measuring texture and other rheologic properties. Microscopic methods combined with image analysis are used to assess structural changes and "the artificial nose" to evaluate odour profile (Nanto et al., 1993).

Sensory process

In sensory analysis appearance, odour, flavour and texture are evaluated using the human senses. Scientifically, the process can be divided into three steps. Detection of a stimulus by the human sense organs; evaluation and interpretation by a mental process; and then the response of the assessor to the stimuli. Variations among individuals in the response of the same level of stimuli can vary and can contribute to a non-conclusive answer of the test. People can, for instance, differ widely in their response to colour (colour blindness) and also in their sensitivity to chemical stimuli. Some people cannot taste rancid flavour and some have a very low response to cold-storage flavour. It is very important to be aware of these differences when selecting and training judges for sensory analysis. Interpretation of the stimulus and response must be trained very carefully in order to receive objective responses which describe features of the fish being evaluated. It is very easy to give an objective answer to the question: is the fish in rigor (completely stiff), but more training is needed if the assessor has to decide whether the fish is *post* or pre-rigor. Subjective assessment, where the response is based on the assessor's preference for a product, can be applied in the fields like market research and product development where the reaction of the consumer is needed. Assessment in quality control must be objective.

Sensory methods

The analytical objective test used in quality control can be divided into two groups: discriminative tests and descriptive tests. Discriminative testing is used to determine if a difference exists between samples (triangle test, ranking test). Descriptive tests are used to determine the nature and intensity of the differences (profiling and quality tests). The subjective test is an affective test based on a measure of preference or acceptance.

Discriminative test
Is there a difference?

- **Triangle test**
- **Ranking**

Descriptive test
What is the difference or the absolute value and how big is it?

- **Quality index method**
- **Structured scaling**
- **Profiling**

Affective test
Is the difference of any significance?

- **Market test**

Figure 8.1 Methods of sensory analysis

In the following, examples of discriminative and descriptive testing will be given. For further information concerning market testing, see Meilgaard *et al.* (1991).

Quality assessment of fresh fish

Quality Index Method

During the last 50 years many schemes have been developed for sensory analysis of **raw fish.** The first modern and detailed method was developed by Torry Research Station (Shewan *et al.*, 1953). The fundamental idea was that each quality parameter is independent of other parameters. Later, the assessment was modified by collecting a group of characteristic features to be expressed in a score. This gives a single numerical value to a broad range of characteristics. In Europe today, the most commonly used method for quality assessment in the inspection service and in the fishing industry is the EU scheme, introduced in the council decision No. 103/76 January 1976 (Table 5.2). There are three quality levels in the EU scheme, E (Extra), A, B where E is the highest quality and below B is the level where fish is discarded for human consumption. The EU scheme is commonly accepted in the EU countries for sensory assessment. There is, however, still some discrepancy as the scheme

does not take account of differences between species into account as it only uses general parameters. A suggestion for renewal of the EU scheme can be seen in Multilingual Guide to EU Freshness Grades for Fishery Products (Howgate *et al.,* 1992),where special schemes for whitefish, dogfish, herring and mackerel are developed (Appendix E).

A new method, the Quality Index Method (QIM) originally developed by the Tasmanian Food Research unit (Bremner *et al.,* 1985), is now used by the Lyngby Laboratory (Jonsdottir, 1992) for fresh and frozen cod, herring and saithe. In the Nordic countries and Europe it has also been developed for redfish, sardines and flounder.

Table 8.1 Quality assessment scheme used to identify the quality index demerit score (Larsen *et al.* 1992)

Quality parameter	Character	Score (ice/seawater)
General appearance	Skin	0 Bright, shining 1 Bright 2 Dull
	Bloodspot on gill cover	0 None 1 Small, 10-30% 2 Big, 30-50% 3 Very big, 50-100%
	Stiffness	0 Stiff, in *rigor mortis* 1 Elastic 2 Firm 3 Soft
	Belly	0 Firm 1 Soft 2 Belly burst
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Musty/sour 3 Stale meat/rancid
Eyes	Clarity	0 Clear 1 Cloudy

	Shape	0 Normal 1 Plain 2 Sunken
Gills	Colour	0 Characteristic, red 1 Faded, discoloured
	Smell	0 Fresh, seaweed/ metallic 1 Neutral 2 Sweaty/slightly rancid 3 Sour stink/stale, rancid
Sum of scores		(min. 0 and max. 20)

QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 4 demerit points (Jonsdottir, 1992). QIM is using a practical rating system, in which the fish is inspected and the fitting demerit point is recorded. The scores for all the characteristics are then summed to give an overall sensory score, the so-called quality index. QIM gives scores of zero for very fresh fish while increasingly larger totals result as fish deteriorate. The description of evaluation of each parameter is written in a guideline. For example, 0 demerit point for the appearance of the skin on herring means very bright skin only experienced in freshly caught herring. The appearance of the skin in a later state of decay turns less bright and dull and gives 2 demerit points. Most of the parameters chosen are equal to many other schemes. After the literal description, the scores are ranked for each description for all the parameters, giving scores 0-1, 0-2, 0-3 or 0-4. Parameters with less importance are given lower scores. The individual scores never exceed 4, so no parameter can excessively unbalance the score. A scheme for herring is shown in table 8.1; it is emphazised that it is neccessary to develop a new scheme for every species (the scheme for cod is seen in Appendix D).

There is a linear correlation between the sensory quality expressed as a demerit score and storage life on ice, which makes it possible to predict remaining storage life on ice. The theoretical demerit curve has a fixed point at (0,0) and its maximum has to be fixed as the point where the fish has been rejected by sensory evaluation of, e.g., the cooked product (see under structured scaling) or otherwise determined as the maximum keeping time. Using cooked evaluation the two parallel sensory tests demand an experienced sensory panel even though this is only required while developing the scheme, and later on it will not be necessary to assess cooked fish in order to predict the remaining shelf life. QIM does not follow the traditionally accepted S-curve pattern for deterioration of chilled fish during storage (Figure 5.1). The aim is a straight line which makes it possible to distiguish between fish at the start of the

plateau phase and fish near the end of the plateau phase (Figure 8.2).

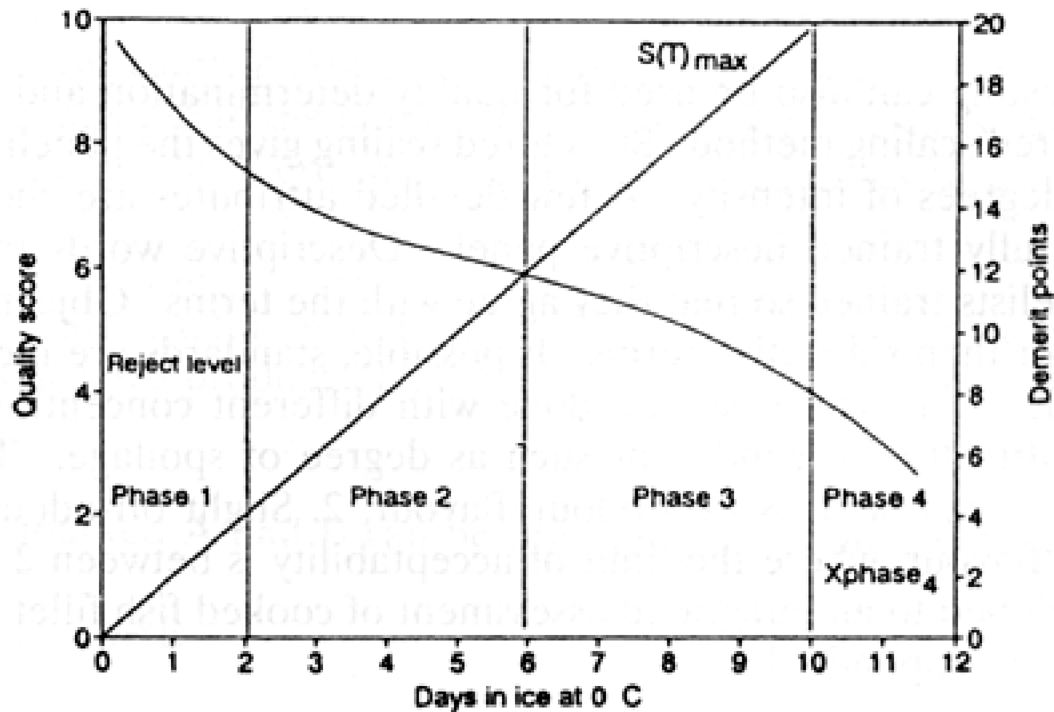


Figure 8.2 Combination of sensory curves for raw S(T) and cooked fish

When a batch of fish in Figure 8.2 reaches a sum of demerit points of 10, the remaining keeping time in ice will be 5 days. To predict remaining shelf life, the theoretical curve can be converted as shown in Figure 8.3.

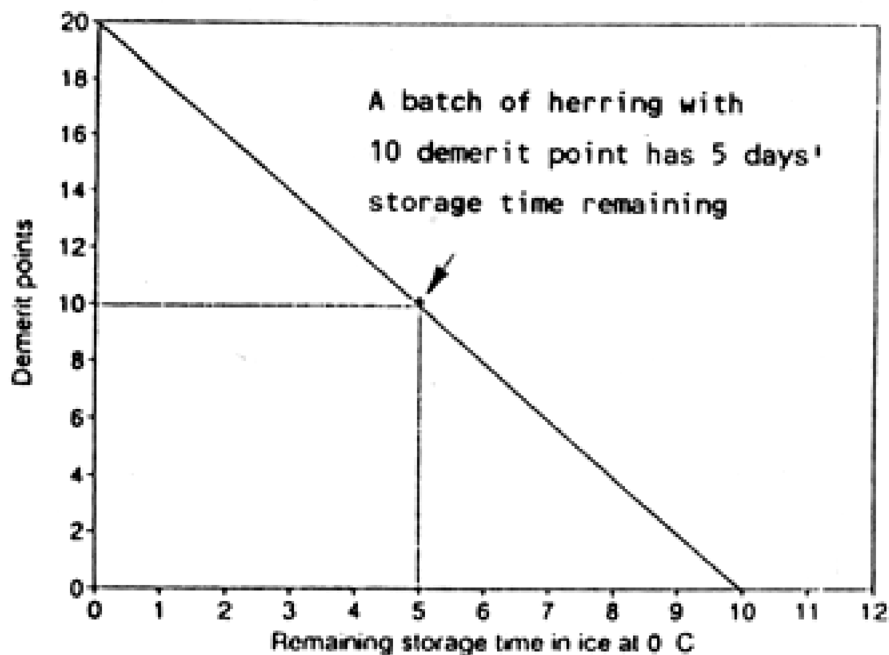


Figure 8.3 A curve to predict the storage time remaining for herring stored in ice or sea water at 0°C

A fish merchant may want to know how long his purchase will remain saleable if the fish are stored on ice immediately. A buyer at a fish market might be interested in the equivalent number of days on ice where the fish have been stored since they were caught, and thus how much marketable time on ice is left. These condition indicators can be extracted for a fish sample with a known rate of change in demerit points using the quality index method.

Structured Scaling

Descriptive testing can also be used for quality determination and shelf life studies applying a structured scaling method. Structured scaling gives the panelist an actual scale showing several degrees of intensity. A few detailed attributes are chosen often based on work from a fully trained descriptive panel. Descriptive words must be carefully selected, and panelists trained so that they agree with the terms. Objective terms should be preferred rather than subjective terms. If possible, standards are included at various points of the scale. This can easily be done with different concentrations of salt but might be more difficult with conditions such as degree of spoilage. The most simple method (Table 8.2) can be 1. No off-odour/flavour, 2. Slight off-odour/flavour and 3. Severe off-odour/flavour, where the limit of acceptability is between 2 and 3. This has been further developed to an integrated assessment of cooked fish fillet of lean and fatty fish (see example in Appendix E).

A 10-point scale is used as described under 5.1 Sensory changes, and an overall impression of odour, flavour and texture is evaluated in an integrated way. For statistics, t-test and analysis of variance can be used (see example in Appendix F).

Table 8.2 Evaluation of cooked fish

		Grade		Score
Acceptable	No off-odour/ flavour	I	Odour/flavour characteristic of species, very fresh, seaweedy Loss of odour/flavour Neutral	10 9 8 7 6
	Slight off-odour/ flavour	II	Slight off-odours/flavours such as mousy, garlic, bready, sour, fruity, rancid	5 4
Limit of acceptability				
Reject	Severe off-odour/ flavour	III	Strong off-odours/flavours such as stale cabbage, NH_3, H_2S or sulphides	3 2 1

Quality assessment of fish products

Assessment of **fishery products** can both be performed as a discriminative test and as a descriptive test.

Triangle test

The most used discriminative test in sensory analysis of fish is the triangle test (ISO standard 4120 1983), which indicates whether or not a detectable difference exists between two samples. The assessors receive three coded samples, are told that two of the samples are identical and one is different, and are asked to identify the odd sample.

Analysis of results from the triangle test is done by comparing the number of correct identifications with the number you would expect to obtain by chance

alone. In order to test this the statistical chart in Appendix A must be consulted. The number of correct identifications is compared to the number expected by use of a statistical table, e.g., if the number of responses is 12, there must be 9 correct responses to achieve a significant answer (1% level).

Triangle tests are useful in determining, e.g., if ingredient substitution gives a detectable difference in a product. Triangle tests are often used when selecting assessors to a taste panel.

The samples marked A and B can be presented in six different ways:

ABB BBA AAB
BAB ABA BAA

Equal numbers of the six possible combinations are prepared and served to the panel members. They must be served randomly, preferably as duplicates. The number of panel members should be no less than 12 (an example of a triangle test from the ISO standard can be seen in Appendix B).

Table 8.3 Example of score sheet: triangle test

TRIANGLE TEST	
Name:	Date:
Type of sample:	
Two of these three samples are identical, the third is different. Examine the samples from left to right and circle the number of the test sample which is different. It is essential you make a choice (guess if no difference is apparent).	
Test sample No.:	
Describe the difference:	

Ranking

In a ranking exercise, a number of samples are presented to the taste panel. Their task is to arrange them in order according to the degree to which they exhibit some specified characteristics, e.g, downward concentration of salt. Usually ranking can be done more quickly and with less training than evaluation by other methods. Thus ranking is often used for preliminary screening. The method gives no individual differences among samples and it is not suited for sessions where many criteria have to be judged simultaneously.

Profiling

Descriptive testing can be very simple and used for assessment of a single attribute of texture, flavour and appearance. Methods of descriptive analysis which can be used to generate a complete description of the fish product have also been developed. An excellent way of describing a product can be done by using flavour profiling (Meilgaard et al., 1991). Quantitative Descriptive Analysis provides a detailed description of all flavour characteristics in a qualitative and quantitative way. The method can also be used for texture. The panel members are handed a broad selection of reference samples and use the samples for creating a terminology that describes the product.

In Lyngby a descriptive sensory analysis for fish oil using QDA has been developed. A trained panel of 16 judges is used. Descriptive terms such as paint, nutty, grassy, metallic are used for describing the oil on an intensity scale. A moderately oxidized fish oil is given fixed scores and used as a reference.

Table 8.4 Profile of fish oil

Taste	Std					
Fresh fish	2					
Amine	1					
Oily	3					
Sweet	2					
Metallic	3					
Grassy	3					
Painty	2					
Fruity	2					

Remarks						
Taste as a whole (0 unacceptable - 9 neutral)	6					

Advanced multivariate analysis is used for statistics and makes it possible to correlate single attributes to oxidative deterioration in the fish oil. The results can be reported in a "spider's web" (se Figure 8.5). The panel uses an intensity scale normally ranging from 0 to 9.

Profiling can be used for all kinds of fishery products, even for fresh fish when special attention is placed on a single attribute.

The results of QDA can be analyzed statistically using analysis of variance or multivariate analysis (O'Mahony, 1986).

Statistics

In any experiment including sensory analysis the experimental design (e.g., number of panel members, number of samples, time aspects, hypotheses to test) and statistical principles should be planned beforehand. Failure to do so may often lead to insufficient data and non-conclusive experiments. A guide to the most used statistical methods can be seen in Meilgaard *et al.* (1991). A panel used for descriptive testing shall preferably consist of no less than 8-10 persons, and it should be remembered that the test becomes statistically much stronger if it is done in duplicate. This can often be difficult using sensory analysis on small fish. Thus the experiment must include a sufficient number ofsamples to remove the sources of variability, and the testing must be properly randomized. For further information see O'Mahony (1986) and Smith (1989).

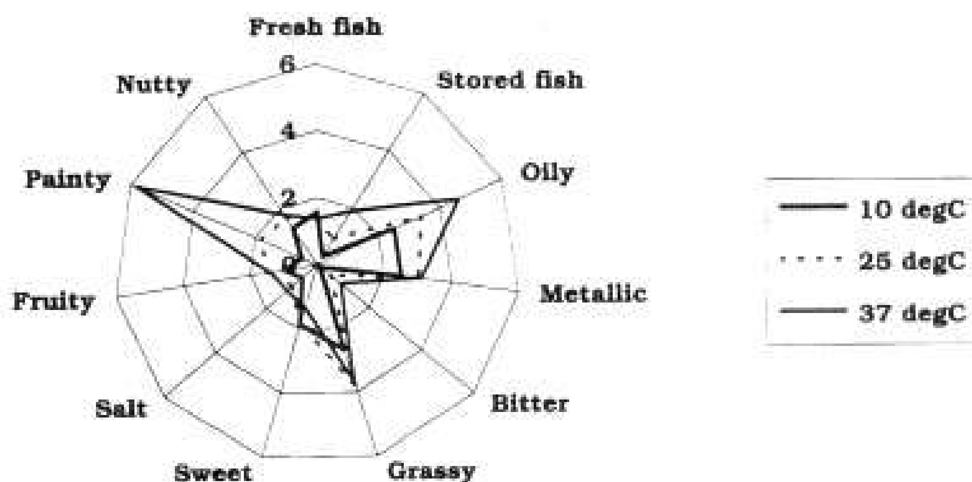


Figure 8.4 Flavour profiles of a fish oil after 2 weeks of storage at various temperatures (Rorbaek et al., 1993)

Training of assessors

Training of assessors for sensory evaluation is necessary in almost all sensory methods. The degree of training depends on the difficulty and complexity of the assessment. For example, for profiling a thorough training with presentation of a large range of samples is necessary in order to obtain proper definitions of the descriptors an equal use of the scoring system. The triangle test normally requires a minor degree of training.

Sensory quality control is often done by a few persons either at the fish market when buying fish or at quality inspection. The experience of these persons allows them to grade the fish. Starting as a fish inspector it is not necessary to know all the different methods of sensory assessment described in textbooks (Meilgaard et al., 1991), but some of the basic principles must be known. The assessor must be trained in basic tastes, the most common fish taste and must learn the difference between off- flavour and taints. This knowledge can be provided in a 2- day basic training course.

In bigger companies and for experimental work a further training of a sensory panel is necessary in order to have an objective panel. A laboratory panel must have 8-10 members, and the training and testing of panel members must be repeated regularly.

Facilities

The facilities required for sensory evaluation is described in textbooks on sensory evaluation.

The minimum requirement for evaluation is a preparation room and a room where the samples are served. The rooms should be well ventilated and provided with a good light (Howgate, 1994). There must be enough space on the tables for inspection of raw samples of fish.

Cooking and serving

The samples of fishery products should not be less than 50-100 g per person. Fillets can be served in loins and should be cooked to an internal temperature of 65°C. The samples should be kept warm when served, i.e., in insulated containers or on a hot plate. The fish can be heat treated by steaming in a

water bath, packed as boiled-in-the-bag in a plastic pouche or in alufoil. An oven (microwave or steam-oven) can also be used for heat treatment. The fish can be packed in plastic or put on a small porcelain plate covered with alufoil. For cod loins (2,5x1,5x6cm) on a porcelain plate covered with alufoil the heating time in a steam-oven (convectomate) at 100°C must be 10 minutes. The samples should be coded before serving.

8.2 Biochemical and chemical methods

The appeal of biochemical and chemical methods for the evaluation of seafood quality is related to the ability to set quantitative standards. The establishment of tolerance levels of chemical spoilage indicators would eliminate the need to base decisions regarding product quality on personal opinions. Of course, in most cases sensory methods are useful for identifying products of very good or poor quality. Thus, biochemical/ chemical methods may best be used in resolving issues regarding products of marginal quality. In addition, biochemical/ chemical indicators have been used to replace more time consuming microbiological methods. Such objective methods should however correlate with sensory quality evaluations and the chemical compound to be measured should increase or decrease with the level of microbial spoilage or autolysis. It is also important that the compounds to be measured must not be affected by processing (e.g., breakdown of amines or nucleotides in the canning process as a result of high temperatures).

The following is an overview of some of the most useful procedures for the objective measurement of seafood quality. Woyewoda *et al.* (1986) have produced a comprehensive manual of procedures (including proximate composition of seafood).

Amines - Total Volatile Basic Amines

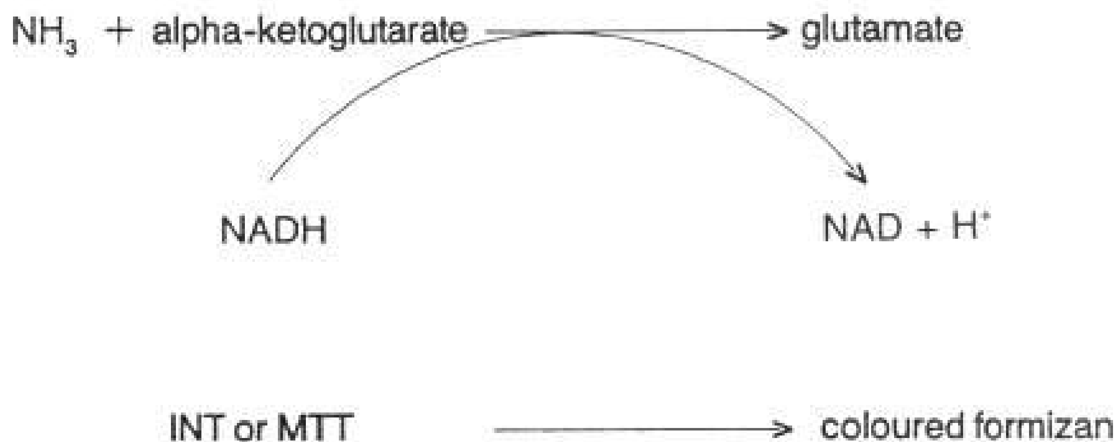
Total volatile basic amines (TVB) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of cod as well as several other species (Rehbein and Oehlenschlager, 1982). They are particularly useful for the measurement of quality in cephalopods such as squid (LeBlanc and Gill, 1984), industrial fish for meal and silage (Haaland and Njaa,

1988), and crustaceans (Vyncke, 1970). However, it should be kept in mind that TVB values do not reflect the mode of spoilage (bacterial or autolytic), and results depend to a great extent on the method of analysis. Botta *et al.* (1984) found poor agreement among six published TVB procedures. Most depend upon either steam distillation of volatile amines or microdiffusion of an extract (Conway, 1962); the latter method is the most popular in Japan. For a comparative examination of the most common procedures for TVB analysis, see Botta *et al.* (1984).

Ammonia

Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino- acids. It is also produced in the autolytic breakdown of adenosine monophosphate (AMP, Figure 5.4) in chilled seafood products. Although ammonia has been identified as a volatile component in a variety of spoiling fish, few studies have actually reported the quantification of this compound since it was impossible to determine its relative contribution to the overall increase in total volatile bases.

Recently, two convenient methods specifically for identifying ammonia have been made available. The first involves the use of the enzyme glutamate dehydrogenase, NADH and alpha-ketoglutarate. The molar reduction of NH_3 in a fish extract yields one mole of glutamic acid and NAD which can be monitored conveniently by absorbance measurements at 340 nm. Test kits for ammonia based on glutamate dehydrogenase are now available from Sigma (St. Louis, Missouri, USA) and Boehringer Mannheim (Mannheim, Germany). A third type of ammonia test kit is available in the form of a test strip (Merck, Darmstadt, Germany) which changes colour when placed in contact with aqueous extracts containing ammonia (ammonium ion). LeBlanc and Gill (1984) used a modification of the glutamate dehydrogenase procedure to determine the ammonia levels semi-quantitatively without the use of a spectrophotometer, but with a formazan dye, which changed colour according to the following reaction:



where INT is iodontrotetrazolium and MTT is 3 - [4,5-dimethylthiazol-2-yl] 2,5 diphenyl tetrazolium bromide

Ammonia has been found to be an excellent indicator of squid quality (LeBlanc and Gill, 1984) and comprised a major proportion of the TVB value for chilled short-finned squid (Figure 8.7). However, ammonia would appear to be a much better predictor of the latter changes in quality insofar as finfish are concerned. LeBlanc (1987) found that for iced cod, the ammonia levels did not increase substantially until the sixteenth day of storage. It would appear that at least for herring, the ammonia levels increase far more quickly than trimethylamine (TMA) levels which have traditionally been used to reflect the growth of spoilage bacteria on lean demersal fish species. Thus ammonia has potential as an objective quality indicator for fish which degrades autolytically rather than primarily through bacterial spoilage.

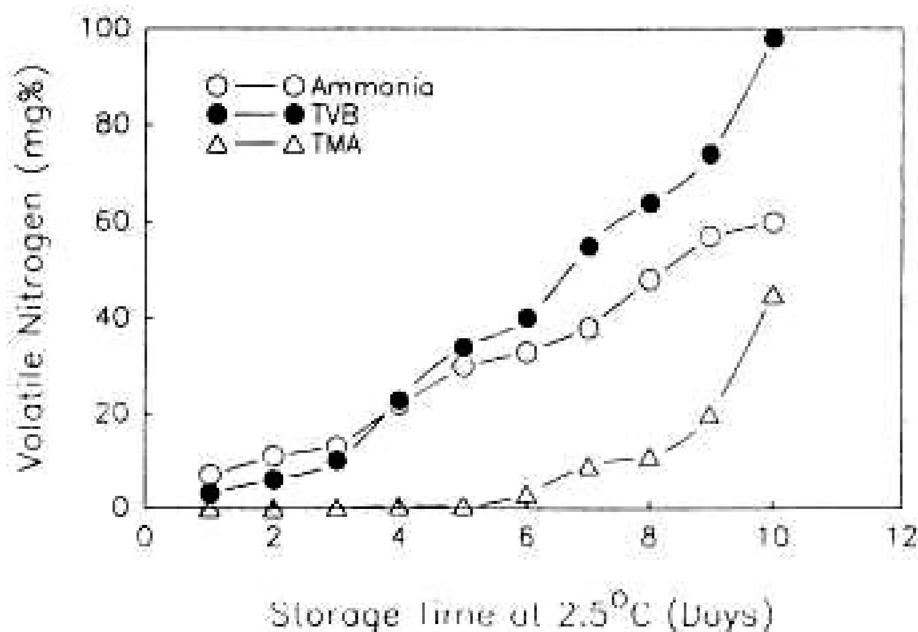


Figure 8.7 Effect of storage time on production of ammonia. TVB and TMA in

short finned squid *(Illex illecebrosus),* adapted from Gill (1990)

Trimethylainine (TMA)

Trimethylamine is a pungent volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. Although TMA is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good. This phenomenon is now thought to be due to the presence of small numbers of "specific spoilage" bacteria which do not always represent a large proportion of the total bacterial flora, but which are capable of producing large amounts of spoilage -related compounds such as TMA. One of these specific spoilage organisms, *Photobactetium phosphoreum,* generates approximately 10 - 100 fold the amount of TMA than that produced from the more commonly-known specific spoiler, *Shewanella putrefaciens* (Dalgaard, 1995) (in press).

As mentioned above, TMA is not a particularly good indicator of edibility of herring quality but is useful as a rapid means of objectively measuring the eating quality of many marine demersal fish. The correlations between TMA level or more preferably, TMA index (where TMA index = log (1 + TMA value)) and eating quality have been excellent in some cases (Hoogland, 1958; Wong and Gill, 1987). Figure 8.8 illustrates the relationship between odour score and TMA level for iced cod. The linear coefficient of determination was statistically significant at the P £ 0.05 level.

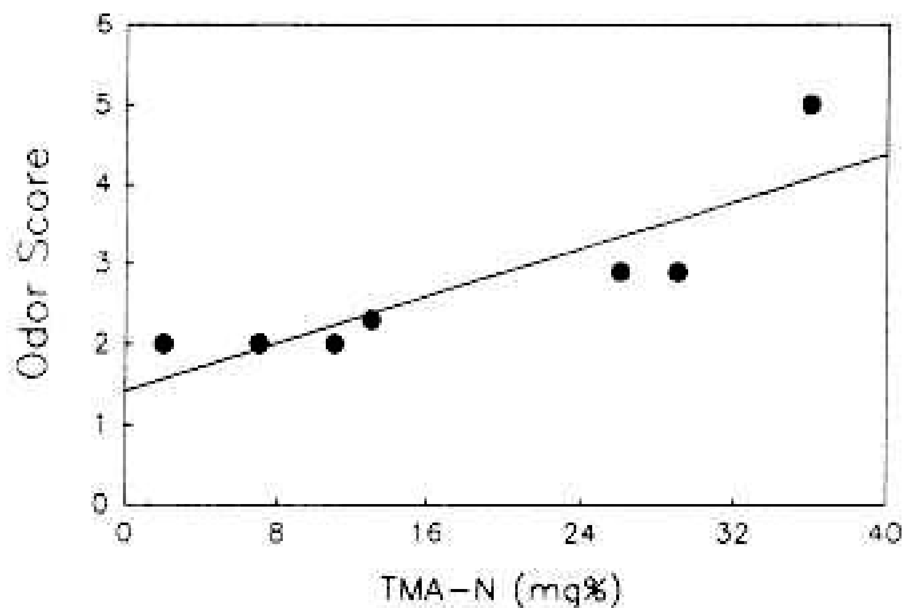


Figure 8.8 Relationship between odour score and TMA levels for iced cod. The

straight line was fitted by linear regression analysis (P£ 0.05) and all data points were averages of data obtained for three individual cod, adapted from Wong and Gill (1987)

The chief advantages of TMA analysis over the enumeration of bacterial numbers are that TMA determinations can be performed far more quickly and often reflect more accurately the degree of spoilage (as judged organoleptically) than do bacterial counts. For example, even high quality fillets cut with a contaminated filleting knife may have high bacterial counts. However, in such a case the bacteria have not had the opportunity to cause spoilage, thus TMA levels are bound to be low. The chief disadvantages of TMA analyses are that they do not reflect the earlier stages of spoilage and are only reliable for certain fish species. A word of caution should be given concerning the preparation of fish samples for amine analysis. TMA and many other amines become volatile at elevated pH. Most analytical methods proposed to date begin with a deproteinization step involving homogenization in perchloric or trichloroacetic acids. Volatilization of amines from stored samples may result in serious analytical errors. Therefore, samples should be neutralized to pH 7 immediately before analysis and should be left in their acidified form in sealed containers if being stored for extended time periods prior to analysis. It is also important to note that appropriate protection for hands and eyes be worn when handling perchloric and/or trichloroacetic acids. In addition, perchloric acid is a fire hazard when brought into contact with organic matter. Spills should be washed with copious quantities of water. Some of the methods of analysis reported to date include colorimetric (Dyer, 1945; Tozawa, 1971), chromatographic (Lundstrom and Racicot, 1983; Gill and Thompson, 1984) and enzymatic analysis (Wong and Gill, 1987; Wong *et al.,* 1988), to name but a few. For a more comprehensive review of the analytical techniques for TMA see the recent review articles: (Gill 1990, 1992).

Dimethylarnine (DMA)

As outlined in section 5.2, certain types of fish contain an enzyme, TMAO dimethylase (TMAO-ase), which converts TMAO into equimolar quantities of DMA and formaldehyde (FA). Thus for fish in the cod (gadoid) family, DMA is produced along with FA in frozen storage with the accompanying FA-induced toughening of the proteins. The amount of protein denaturation is roughly proportional to the amount of FA/DMA produced, but it is most common to monitor the quality of frozen-stored gadoid fish by measuring DMA rather than FA. Much of the FA becomes bound to the tissue and is thus not extractable and cannot be measured quantitatively. The most common method for DMA analysis is a colorimetric determination of the DMA in deproteinized fish extracts. The Dyer and Mounsey (1945) procedure is still in use today although

perhaps more useful is the colorimetric assay proposed by Castell *et al.* (1974) for the simultaneous determination of DMA and TMA, since both are often present in poor quality frozen fish. Unfortunately, many of the colorimetric methods proposed to date lack the specificity where mixtures of different amines are present in samples. The chromatographic methods including gas-liquid chromatography (Lundstrom and Racicot, 1983) and high performance liquid chromatography (Gill and Thompson, 1984) are somewhat more specific, and are not as prone to interferences as the spectrophotometric methods. Also, most of the methods proposed to date for the analysis of amines are destructive and not well suited for analyzing large numbers of samples. Gas chromatographic analysis of headspace volatiles has been proposed as a non-destructive alternative for amine determinations; however, none of the methods proposed to date are without serious practical limitations.

Dimethylamine is produced autolytically during frozen storage. For gadoid fish such as hake, it has been found to be a reliable indicator of FA-induced toughening (Gill et *al.,* 1979). Because it is associated with membranes in the muscle, its production is enhanced with rough handling and with temperature fluctuations in the cold storage facility. Dimethylamine has little or no effect on the flavour or texture of the fish *per se,* but is an indirect indicator of protein denaturation which is often traceable to improper handling before and/or during frozen storage.

BiogenicAmines

Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, presumably so that the organisms may raise the pH of the growth medium through the production of amines.

Histamine, putrescine, cadaverine and tyramine are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. Histamine has received most of the attention since it has been associated with incidents of scombroid poisoning in conjunction with the ingestion of tuna, mackerel, mahi-mahi (dolphinfish from Hawaii). However, the absence of histamine in scombroid fish (tuna, mackerel, etc.) does not ensure the wholesomeness of the product since spoilage at chill storage temperatures does not always result in the production of histamine. Mietz and Karmas (1977) proposed a chemical quality index based on biogenic amines which reflected the quality loss in canned tuna where:

$$\text{Quality Index} = \frac{\text{ppm histamine} + \text{ppm putrescine} + \text{ppm cadaverine}}{1 + \text{pm spermidine} + \text{ppm spermine}}$$

They found that as the quality index ratio increased, the sensory scores on the canned' product decreased. Later, Farn and Sims (1987) followed the production of histamine, cadaverine and putrescine in skipjack and yellowfin tuna at 20°C and found that cadaverine and histamine increased exponentially after an initial lag period of about 36 hours. However, putrescine increased slowly after an initial lag period of 48 hours. Levels of cadaverine and histamine increased to maximum levels of 5-6μg/g tuna but the authors reported that the absence of such amines in raw or cooked product did not necessarily mean that the products were not spoiled.

It is interesting to note that most of the biogenic amines are stable to thermal processing, so their presence in finished canned products is a good indication that the raw material was spoiled prior to heating.

Some of the methods for biogenic amine analysis include high pressure liquid chromatography (Mietz and Karmas, 1977), gas chromatography (Staruszkiewicz andBond, 1981), spectrofluorometric (Vidal-Carou *et al.,* 1990) and a newly-developed rapid enzymatic method for histamine using a microplate reader (Etienne and Bregeon, 1992).

Nucleotide Catabolites

A discussion of the analysis of nucleotide catabolites has been presented in section 5.2 -Autolytic Changes, although all of the catabolic changes are not due to autolysis alone. Most of the enzymes involved in the breakdown of adenosine triphosphate (ATP) to inosine monophosphate (IMP) are believed in most cases to be autolytic whereas the conversion of IMP to inosine (Ino) and then hypoxanthine (Rx) are believed mainly to be due to spoilage bacteria although Hx has been shown to accumulate slowly in sterile fish tissue. Since the levels of each of the catabolic intermediates rise and fall within the tissue as spoilage progresses, quality assessment must never be based upon levels of a single catabolite since the analyst has no way of knowing whether a single compound is increasing or decreasing. For example, if the IMP content of a fish sample were determined to be 5 μmoles/g tissue, the sample might well have been taken from a very fresh fish or a fish on the verge of spoilage, depending on whether or not AMP were present.

Thus, the analysis of the complete nucleotide catabolite profile is nearly always recommended. A complete analysis of nucleotide catabolites may be completed on a fish extract in 12-25 minutes using a high pressure liquid chromatographic (HPLC) system equipped with a single pump and spectrophotometric detector (wavelength 254 nm). Perhaps the simplest HPLC technique published to date is that proposed by Ryder (1985).

Several other approaches have been proposed for the analysis of individual or combination of nucleotide catabolites but none are more reliable than the HPLC approach. A word of caution is perhaps in order with regard to the quantitative analysis of nucleotide catabolites. Most methods proposed to date involve deproteinization of the fish samples by extraction with perchloric or trichloracetic acids. It is important that the acid extracts are neutralized with alkali (most often potassium hydroxide) as soon as possible after extraction to prevent nucleotide degradation in the extracts. Neutralized extracts appear to be quite stable even if held frozen for several weeks. One advantage of using perchloric acid is that the perchlorate ion is insoluble in the presence of potassium. Thus, neutralizing with KOH is a convenient method of sample "clean-up" before HPLC analysis and this procedure helps to extend the life of the HPLC column. Also, it should be noted that nucleotide determination on canned fish does not necessarily reflect the levels in the raw material. *Gill et al.* (1987) found recoveries of 50%, 75%, 64% and 92% for AMP, IMP, Ino and Hx standards which were spiked into canned tuna prior to thermal processing.

Several unusual but innovative approaches utilizing enzymatic assays have been proposed over the years and are presented in Table 8.3. All of the approaches to date rely on destructive sampling (tissue homogenization). It should be noted that regardless of the approach, enzymes denature with time and thus test kits, enzyme-coated strips, electrodes or sensors have a limited shelf life whereas the HPLC techniques do not.

Table 8.3 Fish Freshness Testing Using Enzyme Technology

Analyte (s)	**Principle**	**Advantages**	**Disadvantages**	**Reference**
Hx	enzymes (xanthine oxidase, X0) immobilized on a test strip	rapid simple to use outside the lab	semi -quantitative only capable of measuring Hx (later stages of spoilage)	Jahns *et al.* (1976)

Hx, Ino	test strip, with immobilized enzymes	rapid simple to use outside the lab	semi -quantitative poor reproducibility limited to Hx and Ino (later stages spoilage)	Ehira *et al.* (1986)
IMP, Ino, Hx	enzyme-coated oxygen electrode	rapid accurate	more complicated and time consuming than test strip technology	Karube *et al.* (1984)
K-index	coupled enzyme assay "KV-101 Freshness Meter"	rapid results comparable to HPLC	must purchase enzymes and reagents cost ?	commercially available from Orienta Electric, Niiza Saitama 352, Japan
K-index	enzyme-coated oxygen electrode "Microfresh"	rapid results comparable to HPLC	cost ?	commercially available from Pegasus Instruments, Agincourt, ON, Canada

The factors which have been shown to affect the nucleotide breakdown pattern include species, temperature of storage and physical disruption of the tissue. In addition, since nucleotide breakdown reflects the combined action of autolytic enzymes and bacterial action, the types of spoilage bacteria would no doubt affect the nucleotide patterns. The selection of which nucleotide or combination of nucleotide catabolites to be measured should be made carefully. For example, in certain cases one or two of the catabolites change rapidly with time of chilled storage, whereas the remaining components may change very little. The technical literature should be consulted for guidance on this matter. An excellent overview on the biological and technological factors affecting the nucleotide catabolites as quality indicators was presented by Frazer Hiltz *et al.* (1972).

Ethanol

Ethanol has been used for many years as an objective indicator for seafood quality although it is not nearly as common as the analysis of TMA. Since

ethanol can be derived from carbohydrates via anaerobic fermentation (glycolysis) and/or deamination and decarboxylation of amino-acids such as alanine, it is a common metabolite of a variety of bacteria. It has been used to objectively measure the quality of a variety of fish including canned tuna (Iida *et al.,* 1981 a, 1981b; Lerke and Huck, 1977), canned salmon (Crosgrove, 1978*;* Hollingworth and Throm, 1982)*,* raw tuna (Human and Khayat, 1981), redfish, pollock, flounder and cod (Kelleher and Zall, 1983).

To date, the simplest and perhaps most reliable means of measuring ethanol in fish tissue is the use of the commercial enzyme test kits available from Boehringer Mannheim (German) or Diagnostic Chemicals (Charlottetown, P.E. I., Canada). One advantage of using ethanol as a spoilage indicator is that it is heat-stable (although volatile) and may be used to assess the quality of canned fish products.

Measurements of oxidative rancidity

The highly unsaturated fatty acids found in fish lipids (section 4.2) are very susceptible to oxidation (section5.4).The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential to oxidize iodide to iodine or to oxidize iron(II) to iron(III). The concentration of the hydroperoxides may be determined by titrimetricor by spectrophotometric methods, giving the peroxide value (PV) as milliequivalents (mEq) peroxide per 1 kg of fat extracted from the fish. A method for PV- determination by iodometry has been described by Lea (1952)*,* and for determination by spectrophotometry of iron (III)thiocyanate by Stine *et al.* (1954).The methods for PV-determination are empirically based, and comparisons between PVs are only possible for results obtained using identical methods. For instance, the thiocyanate-method may give values 1.5 - 2 times higher than the iodine titration method (Barthel and Grosch, 1974).

For several reasons, interpretation of the PV as an index of quality is not straightforward. First, the hydroperoxides are odour- and flavour-less, thus the PV is not related to the actual sensory quality of the product analyzed. However, the peroxide value may indicate a potential for a later formation of sensorial-objectionable compounds. Second, lipid hydroperoxides break down with time, and a low PV at a certain point during the storage of a product can indicate both an early phase of autoxidation and a late stage of a severely oxidized product, where most hydroperoxides have been broken down (Kanner and Rosenthal, 1992),e.g., in dried, salted fish (Smith *et al.,* 1990).

In later stages of oxidation *secondary oxidation products will* usually be present and thus be indicative of a history of autoxidation. These products (section *5.4)*

comprise aldehydes, ketones, short chain fatty acid and others, many of which have very unpleasant odours and flavours, and which in combination yield the fishy and rancid character associated with oxidized fish lipid. Some of the aldehydic secondary oxidation products react with thiobarbituric acid, forming a reddish coloured product that can be determined spectrophotometrically. Using this principle, a measure of thiobarbituric acid-reactive substances (TBA-RS) can be obtained. Several method variations exist; one method for fish lipids is described by Ke and Woyewoda *(1979),* and for fish by Vyncke *(1975).* The results are expressed in terms of the standard (di-)aldehyde used, malonaldehyde, and reported as micromoles malonaldehyde present in 1 g of fat. *(A note of caution:* Sometimes the TBA-results may be expressed as mg malonaldehyde in 1 g of fat, or as amount of malonaldehyde (μmol or μg) in relation to amount of tissue analyzed.) Several reports (e.g., by Hoyland and Taylor *(1991)* and by Raharjo *et al. (1993))* speak of some correlation between TBA-RS and sensory assessments, but other authors fail to find a correlation (e.g., Boyd *et al., 1993).* Thus, caution is necessary in interpretation of TBA-RS values into measures of sensory quality.

Provided that the PV has not been lowered through extended storage or high temperature exposure, the PV (by iodometric titration) should not be above 10-20 meq/kg fish fat (Connell, *1975).*

Examples of guidelines for TBA-RS-values: foods with TBA-RS above 1-2 μmol MDA-equiv per g fat (Connell, *1975)* or above 10,μmol MDA-equiv per 1 kg fish (Ke *et al.,* 1976) will probably have rancid flavours.

Modern instrumental methods allow analysis of better defined oxidation products (specific hydroperoxides, actual content of malonaldehyde), but for general quality estimation, methods that determine a broader range of oxidation products (such as PV and TBA-RS) are to be preferred, although these methods have their limitations as discussed above. Headspace analysis of the volatile oxidation products gives results correlating well with sensory evaluation (e.g., in catfish (Freeman and Hearnsberger, *1993)),* but the method requires access to gas chromatographic equipment.

8.3 Physical methods

Electrical Properties

It has long been known that the electrical properties of skin and tissue change after death, and this has been expected to provide a means of measuring *post mortem* changes or degree of spoilage. However, many difficulties have been encountered in developing an instrument: for example, species variation;

variation within a batch of fish; different instrument readings when fish are damaged, frozen, filleted, bled or not bled; and a poor correlation between instrument reading and sensory analysis. Most of these problems, it is claimed, are overcome by the GR Torrymeter (Jason and Richards, 1975). However, the instrument is not able to measure quality or freshness of a single fish, although it may find application in grading batches of fish, as shown in Figure 8.9.

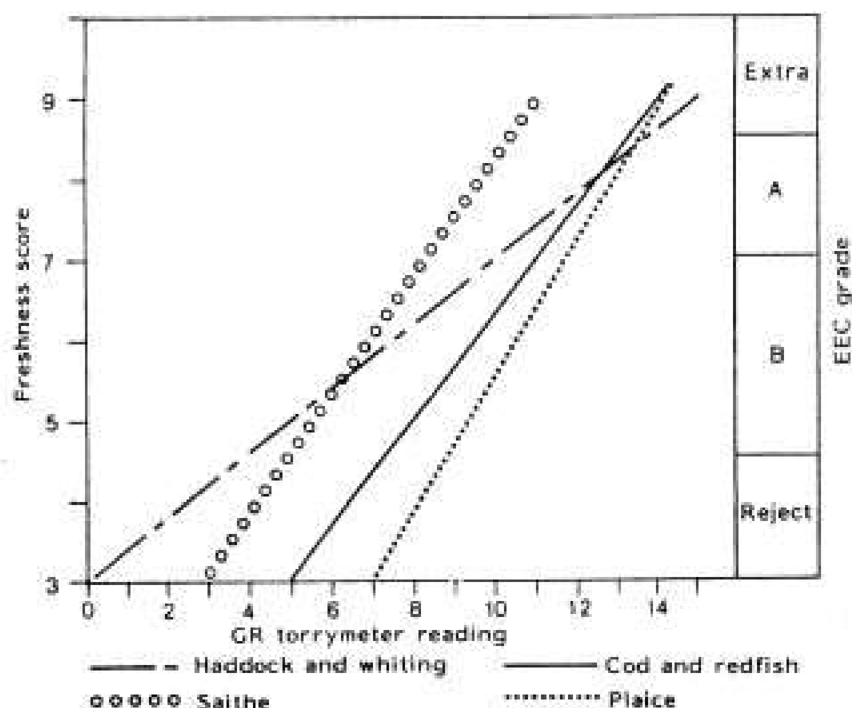


Figure 8.9 Relationship between GR Torrymeter readings of various species of fish and freshness, adapted from Cheyne (1975)

Until recently, no instruments have been capable of on-line determination of quality although this type of mechanized quality evaluation would be highly desirable on the processing floor. The RT Freshness Grader development began in 1982 and, by 1990, a production model capable of sorting 70 fish per minute over 4 channels was made available. The developer was Rafagnataekni Electronics (Reykjavik, Iceland) based the sensing unit on the GR Torrymeter.

pH and Eh

Knowledge about the pH of fish meat may give valuable information about its condition. Measurements are carried out with a pH-meter by placing the electrodes (glass-calomel) either directly into the flesh or into a suspension of fish flesh in distilled water. Measurements of Eh are not carried out routinely, but it is likely that a freshness test can be based on this principle.

Measuring Texture

Texture is an extremely important property of fish muscle, whether raw or cooked. Fish muscle may become tough as a result of frozen storage or soft and mushy as a result of autolytic degradation. Texture may be monitored organoleptically but there has for many years been a need for the development of a reliable objective rheological test which would accurately reflect the subjective evaluation of a well-trained panel of judges. Gill *et al.* (1979) developed a method for evaluating the formaldehyde-induced toughening of frozen fish muscle. The method utilized an Instron Model TM equipped with a Kramer shear cell with 4 cutting blades. This method correlated well with data obtained from a trained texture panel. A method for measuring hardness/ softness of fish flesh, designated as compressive deformability, has been reported by Johnson *et al.* (1980). An accuratelycut fish sample is compressed by a plunger, and the stress-strain curve recorded. A modulus of deformability is calculated from the recorded graph. The results from such measurements may, however, be difficult to interpret.

Another method, measuring the shear force of fish flesh, has been investigated by Dunajski (1980). From this work, it has been concluded that a thin-bladed shear force cell of the Kramer type can be applied.

These are but a few of the examples cited in the literature and until recently all involved expensive equipment and destructive sampling. Therefore, Botta (1991) developed a rapid non-destructive method for the measurement of cod fillet texture. It is a small, portable penetrometer which measures both firmness and resilience. Each test takes only 2-3 seconds to complete and results appear to correlate well with subjective texture grades.

8.4 Microbiological methods

The aim of microbiological examinations of fish products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality of the fish including temperature abuse and hygiene during handling and processing. Microbiological data will in general not give any information about eating quality and freshness. However, as outlined in sections 5 and 6, the number of specific spoilage bacteria will be related to the remaining shelf life and this can be predicted from such numbers (see Figure 5.8).

Traditional bacteriological examinations are laborious, time-consuming, costly and require skill in execution and interpretation of the results. It is recommended that such analyses be limited in number and extent. Various

rapid microbiological methods have been developed during the last decade and some of these automated procedures may be of use when large numbers of samples are to be analyzed.

Total counts

This parameter is synonymous with Total Aerobic Count (TAC) and Standard Plate Count (SPC). The total count represents, if carried out by traditional methods, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. This figure is seldom a good indicator of the sensoric quality or expected shelf life of the product (Huss *et al.,* 1974). In ice-stored Nile perch, the total count was 109 cfu/g for days before the fish was rejected (Gram *et al., 1989)* and in lightly preserved fish products high counts prevail for long time before rejection. If a count is made after systematic sampling and a thorough knowledge of the handling of the fish before sampling, temperature conditions, packaging etc., it may give a comparative measure of the overall degree of bacterial contamination and the hygiene applied. However, it should also be noted that there is no correlation between the total count and presence of any bacteria of public health significance. A summary of different methods used for fish and fish products is given in Table 8.4.

Common plate count agars (PCA) are still the substrates most widely used for determination of total counts. However, when examining several types of seafood a more nutrient rich agar (Iron Agar, Lyngby, Oxoid) gives significantly higher counts than PCA (Gram, 1990). Furthermore, the iron agar yields also the number of hydrogen sulphide producing bacteria, which in some fish products are the specific spoilage bacteria. Incubation temperature at and above 30°C are inappropriate when examining seafood products held at chill temperatures. Pour plating and a 3-4 day incubation at 25°C is relevant when examining products where psychrotrophs are the most important organisms, whereas products where the psychrophilic *Photobacterium phosphoreum* occurs should be examined by surface plating and incubation at maximum 15°C.

Several attempts have been made to ease the procedures for determination of the content of bacteria (Fung *et al., 1987).* Both *Redigel* (RCR Scientific) and *Petrifilm*™ SM (3M Company) are dried agars with a gelling agent to which the sample is added directly. The main advantage of Redigel and Petrifilm compared to conventional plate counts in addition to the costs, is the ease of handling. However, all agar-based methods share a common drawback in the lengthy incubation required.

Microscopic examination of foods is a rapid way of estimating bacterial levels. By phase contrast microscopy the level of bacteria in a sample can be determined within one log-unit. One cell per field of vision equals approximately 5 -10^5cfu/ml at 1000 X magnification. The staining of cells with acridine orange and detection by fluorescence microscopy has earned widespread acceptance as the direct epifluorescence filter technique (DEFT). Whilst microscopic methods are very rapid, the low sensitivity must be considered their major drawback.

Bacterial numbers have been estimated in foods by measuring the amount of bacterial adenosine triphosphate (ATP) (Sharpe *et al., 1970)* or by measuring the amount of endotoxin (Gram-negative bacteria) by the *Limulus* amoebocytes lysate (LAL) test (Gram, *1992).* The former is very rapid but difficulties exist in separating bacterial and somatic ATP.

Table 8.4 Methods for determination of the content of bacteria in seafood

Method	Temperature, °C	Incubation	Sensitivity, cfu/g
Plate count or Iron agar	15-25	3-5 days	10
"Redigel"/"Petrifilm™ SM"	15-25	3-5 days	10
Microcolony-DEFT	15-30	3-4 hours	10^4-10^5
DEFT	-	30 min.	10^4-10^5
ATP	-	1 hour	10^4-10^5
Limulus lysate test	-	2-3 hours	10^3-10^4
Microcalorimetry/Dye reduction Conductance/Capacitance	15-25	4-40 hours	10

Several methods (microcalorimetry, dye reduction, conductance and capacitance) used for rapid estimation of bacterial numbers are based on the withdrawal of a sample, incubation at high temperature (20-25°C) and detection of a given signal. In microcalorimetry the heat generated by the sample is compared to a sterile control, whereas in conductance and capacitance

measurements of the change in electrical properties of the sample, as compared to a sterile control, is registered. The time taken before a significant change occurs in the measured parameter (heat, conductance, etc.) is called the Detection Time (DT). The DT is inversely related to the initial number of bacteria, i.e., early reaction indicates a high bacterial count in the sample. However, although the signal obtained is reversely proportional to the bacterial count done by agar methods, it is only a small fraction of the microflora that give rise to the signal and care must be taken in selection of incubation temperature and substrate.

Spoilage bacteria

The total number of bacteria on fish rarely indicates sensoric quality or expected storage characteristics (Huss *et al.,* 1974). However, it is recognized that certain bacteria are the main cause of spoilage (see section 5.3). Different peptone-rich substrates containing ferric citrate have been used for detection of H_2S-producing bacteria such as *Shewanella putrefaciens,* which can be seen as black colonies due to precipitation of FeS (Levin, 1968; Gram *et al.,* 1987). Ambient spoilage is often caused by members of *Vibrionaceae* that also will form black colonies on an iron agar to which an organic sulphur source is added (e.g., Iron Agar, Lyngby). No selective or indicative medium exists for the *Pseudomonas spp.* that spoil some tropical and freshwater fish or for *Photobacterium phosphoreum* that spoil packed fresh fish. At the Technological Laboratory, Lyngby, a conductance- based method for specific detection of P. *phosphoreum is* currently being developed (Dalgaard, personal communication). The presence or absence of pathogenic bacteria is often evaluated by methods based on immuno- or molecular biology techniques. Such techniques may also be developed for specific spoilage bacteria, and the Technological Laboratory has been currently investigating the use of antibodies specific for S. *putrefaciens* (Fonnesbech *et al.,* 1993).Also, a gene-probe which is specific for S. *putrefaciens* has been developed but has not been tried on fish products (DiChristina and DeLong, *1993).*

Spoilage reactions

Several spoilage reactions can be used for evaluation of the bacteriological status of fish products. As described above, agars on which H_2S producing organisms are counted have been developed. During spoilage of white lean fish, one of the major spoilage reactions is the bacteriological reduction of trimethylamine oxide to trimethylamine (Liston, 1980; Hobbs and Hodgkiss, 1982).When TMAO is reduced to TMA several physical changes occur: the redox-potential decreases, the pH increases and the electrical conductance increases. The measurement of such changes in a TMAO containing substrate

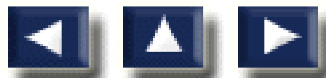
inoculated with the sample can be used to evaluate the level of organisms with spoilage potential; thus the more rapid the change occurs the higher the level of spoilage organisms.

Several authors have inoculated a known amount of sample in a TMAO-containing substrate and recorded the time taken until a significant change in conductivity occurs (Gibson *et al.,* 1984;Gram, *1985;* Jorgensen *et al.,* 1988). This time, the detection time, has been found to be inversely proportional to the number of hydrogen sulphide producing bacteria in fresh aerobically-stored fish, and rapid estimation of their numbers can be given within 8-36 hours.

The changes in redox-potential in a TMAO-containing substrate can be recorded either by electrodes or by observing the colour of a redox-indicator (Huss *et al.,* 1987).As with the conductimetric measurements, the time taken until a significant change is recorded is inversely proportional to the initial amount of bacteria.

Pathogenic bacteria

Several pathogenic bacteria may either be present in the environment or contaminate the fish during handling. A detailed description of these organisms, their importance, and detection methods is given by Huss (1994).



9. ASSURANCE OF FRESH FISH QUALITY

The artisanal fisherman, fishing for a few hours and returning to sell his catch on the beach while the fish is still alive or very fresh, does not need a complicated quality assurance system. His customers know very well the quality of the fish, and most often the fish are caught, sold and consumed within the same day. However, no food production, processing or distribution company can be self-sustained in the medium- or long-term, unless the issues of quality are properly recognized and addressed and an appropriate quality system is put into operation in the processing establishment. The need for effective quality assurance systems is further underlined by the fact that global demand for fish and fishery products is continuously growing while production level is approaching its maximum with limited possibilities for future increase. The need for improved utilization of present harvest including a reduction of fish wasted due to spoilage is therefore a strong incentive to introduce an effective quality assurance system. Further benefits are increasing efficiency, increasing employee satisfaction and lower costs to the processing industry.

Traditionally, fish processors have regarded quality assurance as the responsibility of the regulatory governmental agency, and the means used by these agencies have been the formulation of food laws and regulations, inspection of facilities and processes and final product testing. The processors' own efforts have in many cases been based entirely on final product testing. Such a system is costly, ineffective, provides no guarantee of quality but merely a false sense of security.

At this point, a distinction needs to be drawn between Quality Assurance and Quality Control. Unfortunately, these two terms have been used indiscriminately and the difference between them has become blurred. According to International Standards (ISO 8402), **Quality Assurance (QA)** is "all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality". In other words, QA is a strategic management function which establishes policies, adapts programmes to meet established goals, and provides confidence that these

measures are being effectively applied.

Quality Assurance is the modern term for describing the control, evaluation and audit of a food processing system. The primary function is to provide confidence for both management and the ultimate customer that the company is supplying products with the desired quality which has been specified in trade agreements between the producer and the customer. Only by having a planned QA- programme can a firm continue to succeed in supplying the customer with the desired products.

A large part of a quality assurance programme is built around **Quality Control (QC).** QC is "the operational techniques and activities that are used to fulfil requirements for quality" (ISO 8402), i.e., a tactical function which carries out the programmes established by the QA. Thus quality control is quite often equated with "inspection" or measurements within a quality assurance programme. Thus QC means to regulate to some standard, most often associated with the processing line, i.e., specific processes and operations. QC is the tool for the production worker, to help him operate the line in accordance with the predetermined parameters for any given quality level.

In contrast to the principles in traditional quality programmes relying heavily on control of end- products, a preventative strategy based on a thorough study of prevailing conditions is much more likely to provide a better guarantee of quality, and even at a reduced cost. Such a strategy was first introduced by microbiologists more than 20 years ago to increase safety of food products and is named the Hazard Analysis Critical Control Point (HACCP) System. The principles of the HACCP system can very easily be used also in the control of other aspects of quality.

The principles of the HACCP system are now being introduced in food production in many parts of the world. One reason for this development is that a number of national food legislations today are placing full responsibility for food quality on the producer (e.g., EEC Council Directive no. 91/493/EEC) and the use of the HACCP system is required (EEC 1993, 1994).

The Hazard Analysis Critical Control Point (HACCP) system

The main elements of the HACCP system are:

A. Identify potential hazards. Assess the risk (likelihood) of occurrence.
B. Determine the Critical Control Points (CCPs). Determine steps that can be controlled to eliminate or minimize the hazards. A CCP that can completely control a hazard, is designated CCP-1, while a CCP that

minimizes, but not completely controls a hazard is designated a CCP-2.

C. Establish the criteria (tolerances, target level) that must be met to ensure that a CCP is under control.
D. Establish a monitoring system.
E. Establish the corrective action when CCP is not under control.
F. Establish procedures for verification.
G. Establish documentation and record-keeping.

For detailed information on introduction and application of the HACCP system, Huss (1994) should be consulted.

The great advantage of the HACCP system is that it constitutes a scientific and systematic, structural, rational, multi-disciplined, adaptable and cost-effective approach of preventive quality assurance. Properly applied, there is no other system or method which can provide the same degree of safety and assurance of quality, and the daily running cost of a HACCP system is small compared with a large sampling programme.

By using the HACCP concept in food processing it is possible to assure and - as all actions and measurements are recorded - to document assurance of a quality standard as specified in the product specification.

Application of the HACCP system for fresh or frozen fish production

A starting point in design and implementation of any quality programme is to achieve a complete and correct definition and description of the product. Further, it must be ensured that each and every quality attribute is included and is written such that any ambiguity is avoided. Thus the critical limits for defects such as presence of bones, pieces of skin and membranes on skinless fillets, maximum permitted short weights, etc., must be clearly stated. When this task is completed, and the processes within the operation have been considered, it is possible to identify the hazards to be controlled. A list of possible hazards and Critical Control Points in production and processing of fresh and frozen boneless fillets is given in Table 9.1.

In most presentations it is recommended that hazards are limited to safety hazards and decomposition (spoilage). However, in the present presentation commercial quality (defects) have also been included as hazards.

When all hazards, defects and Critical Control Points (CCP) have been identified, an appropriate monitoring and checking system must be established at each CCP. This includes:

a. a detailed description of control measure, frequency of control and nomination of who is responsible
b. establishment of critical limits for each control measure
c. records to be kept for all actions and observations
d. establishment of a corrective action plan.

Table 9.1 Hazards and Critical Control Points (CCP) in production and processing of fresh and frozen boneless fish fillets

Processing flow	**Hazard**	**Preventive Measure**	**Degree of control**
LIVE FISH	Contamination (chemicals, enteric pathogens) biotoxins	Avoid fishing in contaminated areas and areas where biotoxins are prevalent	CCP-2
CATCH			
CATCH HANDLING	Growth of bacteria Gaping in fillets Discolorations	Short handling time Avoid rough handling	CCP-1 CCP-2
CHILLING	Growth of bacteria	Low temperature	CCP-1
LANDING			
ARRIVAL OF RAW MATERIAL AT FACTORY	Substandard quality entering processing	Ensure reliable source (HACCP-plan onboard or list of approved suppliers) Sensory evaluation	CCP-2
CHILLING	Growth of bacteria (deterioration)	Ensure low temperature	CCP-1
PROCESSING: De-icing Washing Filleting Skinning,Trimming Candling	Pieces of skin, bones and membranes left on fillet Visible parasites left on fillet	Proper setting of machinery Instructions of personnel Ensure light intensity on candling table Frequent change of personnel	CCP-2 CCP-2

Weighing Packaging	Short weights/over weights Deterioration during (fresh/frozen) storage	Ensure accuracy of scales Ensure adequate packaging material and method (e.g., vacuum)	CCP-1 CCP-2
All processing steps	Growth of bacteria Contamination (enteric bacteria)	Short processing time Factory hygiene/ sanitation water quality	CCP-1 CCP-2 CCP-1
CHILLING/FREEZING - STORAGE	Deterioration	Ensure correct (low) temperature	CCP-1

A precise and detailed description of all CCPs is not possible as the individual and local situation may vary. However, some general points are considered as follows:

LIVE FISH - before being caught. The hazards are presence of biotoxins and contamination with chemicals and/or enteric pathogens:

a. control measures are monitoring of the environment (fishing areas) for pollution and presence of biotoxins. The government will be responsible for this activity in most countries and regular surveys should be carried out
b. critical limits should be set by national governments
c. results of surveys should be published at regular intervals
d. corrective action is restricted fishing in grossly polluted areas

CATCH HANDLING - hazards are growth of bacteria (causing histamine formation and/or decomposition), discoloration and gaping in fillets:

a. control measures are restricted time for catch handling (time from catch to chilling) and visual check that crew are following prescribed procedures to avoid rough handling. The control should be continuous and the skipper or first mate on deck is responsible
b. time for catch handling is limited to max 3 h
c. a detailed log on each haul, proper marking of boxes or containers for identification of lot, time (day and hour) for catch, catch handling time, deviations - if any - from prescribed procedure
d. corrective actions are check of product (sorting) and rejection of low quality product

CHILLING - the hazard is growth of bacteria:

a. control measures are continuous recording of temperature (automatic) or visual control of icing of the fish. The skipper or chief is responsible
b. the critical limit for fish temperature is 1°C
c. a log on temperature and icing observations must be kept
d. corrective action is checking of fish from period out of control, sorting and rejection of low quality fish. Identification of reason(s) for temperature out of control

ARRIVAL OF RAW MATERIAL AT FACTORY - the hazard is risk of substandard quality entering processing:

a. control measures are check of identity of raw material, sensory assessment (visual) and control of fish temperature of all arriving raw material. Processing manager or person specially designated may be responsible
b. no low quality fish will be accepted (company specification)
c. a log on all daily actions and observations Must be kept
d. rejection of low quality fish. Identify reason for low quality. Change of supplier

CHILLING - the hazard is growth of bacteria (deterioration):

a. control measures are continuous recording (automatic) of temperatures in chill room and check on icing of fish. Accuracy of thermometer must be checked regularly against mercury-thermometers. Responsible person is the processing manager or designated person
b. chill room temperature must be £ 5°C
c. a continuous log on temperatures and observations must be kept
d. if temperatures are out of control, all products must be reinspected, sorted and low quality material rejected

PROCESSING Filleting, skinning/ trimming - the hazards are pieces of skin, bones and membranes left on fillet:

a. control measures are daily check of machinery for correct setting. Instructions of personnel. A sample of x kilo of fillet is taken x times daily for careful visual examination. Frequency of sampling is company policy, on-line electronic control is possible (Pau and Olafsson, 1991). Line manager is responsible for the on-line control, while QC-manager is responsible for collecting and examination of samples (verification)
b. critical limits are specified in product specification by the buyer
c. records on all actions and observations
d. sort and reprocess fillets with defects. Identify reason for processing out

of control

Candling - the hazard is visible parasites left on fillet:

a. control measures are continuous candling of all fillets, packaging personnel is instructed to observe for parasites. The sample taken for control of bones, membranes and skin is also checked for parasites and same person is responsible. The production manager is responsible for the on-line control while the QC manager is responsible for collecting and examination of samples (verification)
b. critical limits may be set by buyer or by company policy. See also Codex Alimentarius and EEC regulations
c. records on all actions and observation
d. fillets with visible parasites are reprocessed or rejected. Adjustment of candling light. Frequent change of personnel

Weighing - the hazards are short weight or over-weight:

a. control measures are frequent (1-2-3 times daily) check of weighing procedures, control weighing of samples and daily check of accuracy of scales. Line operator is responsible
b. critical limits are specified by company policy or buyer
c. daily records of all actions and observation
d. reweighing of products processed when out of control. Identification of reason for deviation

Packaging - the hazard is spoilage in frozen storage if packaging (packaging material, vacuum) is inadequate:

a. the processing manager must ensure daily that packaging is in agreement with product specification

All processing steps - the hazards are 1) growth of bacteria and 2) (gross) contamination by enteric pathogens:

a. control measure for 1) is establishment of short processing time - which must be checked on a daily basis by the line manager. For control of contamination, the personal hygiene must be supervised continuously by production manager, and prescribed procedures must be followed (medical certificate, report on illness, dress, etc.). Microbiological control of water quality must be carried out on a regular basis (daily - weekly - monthly - depending on the source of water) and is the responsibility of the QC-manager. If chlorination of water is applied, the level of free

chlorine must be determined on a daily basis

b. critical limits for water quality are standards for drinking water. Limits for chlorine is 0.5 mg/l. No person with gastro-intestinal disorder must work in direct contact with unwrapped fish
c. records on tests for water quality. Actions and observations on personal hygiene must be recorded
d. corrective action is microbiological testing of products. Rejection of all contaminated products

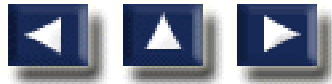
CHILLING /FREEZING - the hazard is deterioration:

a. continuous temperature control (automatic recording) or frequent check of icing. Accuracy of thermometers must be checked regularly against an accurate mercurythermometer. Foreman in charge of stores is responsible
b. critical limits are + 1°C for chilled fish and -18°C for frozen fish
c. log on all temperature readings must be kept
d. corrective action is reinspection of fish exposed to elevated temperature - and rejection of low quality products

In order to be effective, the HACCP system needs to be applied from origin of food (catch) to consumption. In the case of fresh fish, the situation is most often that the fish change owner at the time of landing. Here, the new owner (the processor) must ensure that the fish are supplied from a reliable source (fisherman) who also applies the HACCP principles. If this is possible, the processor has the situation under control and needs only occasionally to verify the quality on arrival to the factory by checking quality (sensory evaluation) and temperature of fish on arrival. In this case it is not a critical situation and this step can be designated a Control Point (CP) only.

The situation is very different if the processor needs to buy fish from a number of unknown fishermen (auction system). This will require constant checking of fish quality on arrival to the factory in order to ensure compliance with all the requirements of the product. In this case, it is therefore a critical Control Point, and since there is still a risk of substandard quality entering the processing line, it is a CCP-2.

Most on-line control (continuous control of temperatures, quality of work, sensory quality of product) should be the responsibility of the processing manager.



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*These references are presented here as submitted by the authors

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