SHELF-LIFE EXTENSION OF FRESH FISH — A REVIEW
PART I — SPOILAGE OF FISH

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INTRODUCTION

In 1982 the world fish catch was 75 million tons live weight. About 70% (52 million tons) was used directly for human food (30% of which was consumed fresh). The catch provided 14% of the world's need for animal protein, 4-5% of the total protein requirements, but less than 1% of the nutritional energy needs (Whittle 1984). Fish is a good source of minerals such as calcium, phosphorous and iron, trace elements like iodine (in marine species), as well as vitamins. The high content of polyunsaturated fatty acids contributes significantly to the essential fatty acid requirements and, in addition, probably helps lower cholesterol levels. Thus, fish is important in the diet of the developing world. Within developing countries there is a recognizable trend for the poor to spend proportionally more of their household expenditure for animal protein on fish rather than on other meat products (James 1984).

By the year 2000 when the anticipated world population will be 6.1 billion people of which 90% will be living in developing countries, about 104 million tons of fish will be needed world-wide to meet the demand. There are three major ways to possibly meet this demand:

(1) By making better use of what is already available, by reducing postharvest losses, and by increasing the use of underutilized species (such as the shrimp by-catch and the pelagic species),

(2) By promoting aquaculture to grow larger quantities of preferred species, and

(3) By diversifying the fishing efforts to focus on the less conventional resources such as krill, mesopelagics, and oceanic cephalopods.

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Of the 15 million tons of fish marketed fresh, 10% is conservatively estimated to end up spoiled. In Mexico alone, for example, the Minister of Commerce admitted early last year that ‘at least’ half of the country’s catch is lost to spoilage each year due to inadequate methods of preservation and distribution (Fishing News International 1984). A study of the Lake Chad fishery also indicated that fish losses were possibly as high as 50% (National Academy Science 1978). Many other countries around the world have equally large losses.

Fresh fish losses are mainly a result of spoilage, caused principally by lack of chilling, coupled with poor storage, distribution, and marketing facilities. If reduction in losses are to be achieved, money will have to be invested in improving the fresh fish distribution system. Given modern technology and sufficient resources, it is possible to conserve food commodities for extended periods of time without loss. A potentially economically and technologically viable method for reducing fresh fish losses is the use of chemical preservatives and modified atmospheres (MA) in addition to ice or refrigeration. Both preservatives and MA can reduce bacterial growth, extend the allowable transit time, provide a higher quality end product and reduce economic losses.

Increasing fresh fish stability will not only permit the retail marketing of an acceptable product over a longer period of time, but will also permit market expansion by allowing high quality fish to be sent to distant inland markets where good fresh fish were previously unknown. In the developed countries MA systems are beginning to be found to be efficient and cost effective.

This review briefly summarizes the ‘extension of shelf-life of fish’ literature with modified atmospheres (MA) and chemical preservatives. This review, which will appear in three separate parts, consists of:

1. Spoilage of Fish,
2. Preservation of Fish, and
3. Fish Quality and Methods of Assessment.

PART I — SPOILAGE OF FISH

Seafoods, especially fish, are one of the most perishable foods. The muscle tissue of fish spoils faster than mammalian muscle. The higher water content, the high free amino acid content, and the lower content of connective tissue as compared to other flesh foods leads to the more rapid spoilage of fish. The higher ultimate pH and the colder water temperature water for at least the temperate water fish also facilitate the decreased lag time, and the more rapid growth and reproduction of bacteria, even with refrigeration.

Indigenous enzymatic reactions and biochemical changes along with microbially-induced activity are involved in the degradation of fish tissue.
Enzymatic and Biochemical Changes

At the time of death, a series of complex changes begin to occur in fish which are caused by both natural fish processes and by the bacteria found on the fish (Regenstein 1983).

Rigor mortis (death stiffening) of fish takes place when the adenosine triphosphate (ATP) level falls below a certain critical level. The colder the fish the longer this takes.

As described by Hobbs (1982), when a fish dies, some of the enzymes in the muscle continue to perform their functions, including those that maintain the muscle in a state of readiness to contract. The energy for contraction comes from the glycogen stored in the muscle. Adenosine triphosphate (ATP) is split rapidly to adenosine diphosphate (ADP) and a phosphorous group (P) by the Mg-activated actomyosin ATPase. The free energy derived from the splitting of ATP in living tissue is used for contraction. The development of power occurs through the sliding of the actin and myosin filaments past one another. Postmortem the ATP is eventually not restored by other chemical reactions. In the absence of ATP, permanent actomyosin crosslinks are formed. This action results in the stiffening of fish associated with rigor mortis that remains until enzymatic activity, probably proteolysis, releases the tension elsewhere. The release of tension is referred to as the resolution of rigor (Bendall 1969). The fish's backbone prevents the muscle from shortening in the whole fish; but if a fillet is removed before rigor mortis is fully established, the muscle tissue will shorten, perhaps by as much as 40% of its original length (Hobbs 1982). The muscle passing into rigor mortis becomes rigid and inextensible and any forcible attempt to stretch it is then accompanied by fracture, usually in the I-band of the sarcomere (Bendall 1969).

The lack of oxygen causes glycogen to be metabolized anaerobically (glycolysis) to lactic acid. If present in sufficient quantity, lactic acid lowers the pH of the muscle tissue, suppressing the development of microorganisms. Depending upon fish species, the pH immediately after rigor mortis has resolved is usually between 6.2 to 6.5. If it goes below normal for a particular species, the fish will generally lose water easily and this leads to "chalky" fish.

Glycolysis occurs until the glycogen reserve is exhausted. Autolytic breakdown of protein and some other autolytic changes start taking place (Amlacher 1962). Further, the activity of the endoenzymes of fish muscle (proteases, cathepsins, peptidases, etc.) play an important role in the degradation of peptides and proteins by establishing an optimal medium for growth and reproduction of these spoilage microorganisms. If, in addition, fish tissue is mechanically damaged; enzymes stored in the lysosomes will also be released.

Since the exoenzymes in the digestive tract are strong proteases, gutting of fish is highly recommended as soon as fish are caught in order to avoid invasion of
enzymes through the abdominal cavity to the tissues (although a poor gutting job can sometimes be more harmful than not gutting at all). Some fish species, e.g., herring, mackerel, and capelin caught during periods of heavy feeding, are very susceptible to autolytic tissue degradation. The usual explanation has been that fish with high amounts of stomach and gut content also have high activities of digestive enzymes. These leak out postmortem by the bursting of the belly walls and degrade the surrounding tissues (Gildberg and Raa 1980). "Belly Burst" and its effects can be reduced by keeping the fish cool and processing quickly, especially by gutting the fish soon after catching (Hobbs 1982). Love and Lavety (1972) and Gildberg (1978) pointed out that the state of the connective tissue has an important role in the phenomena since heavy feeding fish have a weaker connective tissue than starving fish. The connective tissue is relatively weak if the postmortem tissue pH is low and observations indicate that postmortem tissue pH is lowest when fish are caught during heavy feeding periods (Gildberg 1978).

Microbial Changes

Even though autolytic activity and natural chemical changes start the degradation process in fish, the spoilage of fish held on ice is mainly a bacteriological phenomenon. The chemical changes that take place are mainly due to bacterial enzymes (Liston 1982; Ronsivalli and Charm 1975).

The composition of the microflora of many different species of cold water fishes at spoilage were reported to be dominated by Gram-negative bacteria usually identified as Achromobacter, Flavobacterium, Pseudomonas, or less frequently Vibrio or enterobacterial genera. However, there are a few reports of large numbers of Gram-positive bacteria being the dominant microflora of warm water species (Liston 1980). A close relationship between the initial bacterial microflora of the newly-caught fish and the microflora of the environment has been demonstrated (Shewan 1971).

It is usually believed or stated that bacteria penetrate the gill tissue and proceed along the vascular system, particularly along the caudal vein, through the kidney and thence, after some days into the flesh; or through the intestines into the body cavity and belly walls; or through the skin into the flesh; but the truth is we have very little direct proof for any of the above statements (Shewan 1971).

The use of ice for chilling is still one of the most important methods of preserving fish, but ice merely slows down the microbial activity since fish can support a population of psychrotrophic (cold-loving) bacteria (Liston 1982). These bacteria are predominantly at the surface of the fish but secrete enzymes into the tissues, bringing about a complex series of chemical changes (Shewan 1977). The microflora of fresh fish initially use many of the low molecular weight substances in the tissue (carbohydrates, free amino acids, small peptides and lactic acid) as a source of energy for further growth (Gill and Newton 1977). The Pseudomonas-Achromobacter group rapidly metabolize most amino acids,
dipeptides and tripeptides found in the nonprotein nitrogen (NPN) fraction of muscle. Oxidative deamination of amino acids seems to be the primary pathway leading to ammonia and volatile fatty acids accumulation (Liston 1982). Proteolysis does not seem to be significant in the early stages of spoilage because the high concentration of NPN (free amino acids) apparently inhibits the proteinases (Chung 1968). Proteolysis become more important in the later stages of spoilage when most of the free amino acids have been depleted.

Other breakdown compounds are produced during fish spoilage. Sulphur compounds (H₂S, (CH₃)₂S and CH₃SH) are produced mainly from the sulphur amino acids: H₂S from cysteine and (CH₃)₂S + CH₃SH from methionine (Herbert and Shewan 1976).

Trimethylamine (TMA) is produced by the reduction of trimethylamine oxide (TMAO), possibly by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria. Dimethylamine (DMA) and formaldehyde (FA) can be produced by fish enzymes, but this reaction is slow enough that it is generally not important in fresh fish spoilage. The FA leads to crosslinking of the proteins that is described as a "cottony" and "spongy" texture. This reaction occurs in frozen storage and may also occur if fresh fish are stored for long periods of time, such as when the fish are treated with chemicals and/or MA.

TMA is associated with the odor of fish spoilage and is clearly a part of the spoilage pattern of many fish species. When TMA reacts with fat in the muscle of fish, the characteristic fishy odor of low quality fish is produced. Odors appear at TMA levels of 2.9 to 4.3 mM (4-6 mg N/100 mL) of muscle extract. At a level of 7.2 mM (10 mg N/100 mL) of the muscle extract there are definite off-odors. Beatty and Gibbons (1937) found that the TMA level in cod muscle press juice rose rapidly from the almost negligible level in fresh fish and these levels correlated with odors. Subsequently, a number of experiments have shown positive correlations of TMA levels and organoleptic scores (cited by Hebard et al. 1982).

TMAO is found in a large number of marine fish and shellfish. Generally the largest amounts are in the elasmobranch fishes. Negligible amounts are found in fresh water fish (Dyer 1952; Groninger 1959; Ruiter 1971 as cited by Hebard et al. 1982). However, burbot (Lota lota), the only freshwater gadoid fish does seem to have TMAO (Samson 1983). TMAO is believed to be involved in the osmotic regulation of marine fish, as well as being part of the body's buffer system (Regenstein et al. 1982). Hebard et al. (1982) indicate that Yamada (1967) suggested that TMAO was a waste product in fish, a detoxified form of TMA derived from choline, betaine, methionine, etc. Ogilvie and Warren (1957) suggested that the presence of TMAO in fish was the results of both exogenous and endogenous processes. Salt water fish have varying amounts of TMAO due to differences in the TMAO present in their food but mostly because they must lower their TMAO excretion to the level that counteracts the osmotic
pressure of sea water. TMAO degradation is due to the bacterial enzyme triamineoxidase (TAO) which apparently activates the substrate (TMAO) so that a bacterial dehydrogenase can reduce it to TMA. Figure 1 illustrates the TMAO degradation reactions. Among the many potential hydrogen donors, the most important is probably lactic acid (one of the final products of glycolysis) and pyruvic acid. Psychrotrophic bacteria, particularly Achromobacter are capable of reducing TMAO to TMA (Laycock and Regier 1970; Lee et al. 1967). However, significant amounts of TMA are not produced until after the bacterial lag phase, which extends from the onset of rigor to its resolution, due to a postulated bacteriostatic effect of rigor mortis. When bacterial counts are sufficiently high, the amount of TMAO reduced to TMA is significant.

\[
\text{AH}_2 + (\text{CH}_3)_3\text{NO} \rightarrow A + (\text{CH}_3)_3\text{N} + \text{H}_2\text{O}
\]

\[
\text{CH}_3\text{CHOHCOOH} + \text{TMAO} \rightarrow \text{CH}_3\text{COCOOH} + \text{TMA} + \text{H}_2\text{O}
\]

Lactic Acid \hspace{1cm} \text{Pyruvic Acid}

\[
\text{CH}_3\text{COCOOH} + \text{TMAO} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + \text{TMA} + \text{H}_2\text{O}
\]

**FIG. 1. TMAO DEGRADATION REACTIONS**
*(Regenstein et al. 1982)*

**Pathogenic Bacteria**

Usually there are only two species of pathogenic bacteria which seem to occur naturally on fish. These are *Clostridium botulinum* type E and *Vibrio parahemolyticus*.

The following description of *Clostridium botulinum* was taken from Eklund’s general review (1982).

*Cl. botulinum* is a spore forming anaerobic bacteria that exists in either the spore or vegetative state. The spores are widespread in nature and frequently contaminate food products. With appropriate environmental conditions the spores will germinate and develop into vegetative cells which then grow and produce their lethal toxin. During the later stages of their growth cycle, the vegetative cells again form spores. The spores are very resistant to heat, drying, salting, freezing and other physical and chemical treatments and can remain dormant for many years in the soil and in places such as food processing plants.
When these spores contaminate foods, they are very difficult to destroy. Bottom sediments of marshes, lakes, and coastal ocean waters contain *Cl. botulinum*. The primary type in marine environments in northern areas appears to be type E; however, other types are occasionally found. Different strains of *Cl. botulinum* are divided into types A through G on the basis of the antigenic specificity of the neurotoxins that they produce. Types A, B, and some F are proteolytic. Type G is weakly proteolytic and Types C, D, E and some F are nonproteolytic.

Types A, B, E and F have caused the majority of human botulism outbreaks. These botulinum types can be further divided into two groups based upon their biochemical and physiological characteristics. Group 1 consists of the proteolytic organisms of types A, B and F and group 2 consists of nonproteolytic organisms of types A, B, F and E.

Group 1 species are more heat resistant. Some spores withstand boiling water for 6 to 8 h. The minimum temperature at which they will grow is 10°C (50°F). They attack complex proteins, and their growth is often accompanied by off-odors.

Types B, E and F of group 2 are more sensitive to heat than group 1 types, being rapidly killed in buffer solutions at 212°F. These three types of *Cl. botulinum* have the important characteristic of growing and producing toxins at temperatures as low as 38°F (3.3°C). They do not attack complex proteins and their growth in food cannot be detected by off-odors and off-flavors.

*Cl. botulinum* toxins are the most potent poison known. Under ideal conditions the growth of *Cl. botulinum* is accompanied by the release of this potent neurotoxin into the food. When the food is eaten, the toxin enters the circulatory system through the small intestines. The toxin causes paralysis by acting on the nervous system. If sufficient toxin is present in the blood, the diaphragm and chest muscles are paralyzed and death may occur because of asphyxiation. Usually symptoms develop between 8 and 72 h after eating the toxic food (Eklund 1982).

Other potentially pathogenic bacteria occasionally associated with seafood products are: *Vibrio parahemolyticus*, *Clostridium perfringens*, Staphilococcus, Erysipletrix, Edwardsiella, Salmonella, *Escherichia coli*, Shigella, Franciscella, *Vibrio cholerae* and other Vibrios. All of these organisms probably represent contamination from terrestrial sources (Shewan 1971) through contaminated waters. In fish of the Scombridae family (tuna, mackerel, etc.), *Proteus morgani*, and possibly other organisms, if given the opportunity to grow, can rapidly synthesize histidine carboxylase and cause histamine to accumulate to levels that can be toxic for man. Histamine is a potent capillary dilator. Its physiological effects are basically hypotension and hemoconcentration. The major symptoms are headache, nausea, cramps, diarrhea, vomiting, thirst and a burning sensation in the throat.
Although it is clear that scombroid poisoning is caused by histamine, Ferencik (1970) suggested that other biologically active substances may also be involved as the effect of a particular histamine level in fish is generally greater than that of the equivalent histamine level in a purer system (as cited by Eitenmiller et al. 1982). Arnold and Brown (1978) also suggest the possibility that other amines present may act as synergists although this had not been clearly established (cited by Ritchie and Mackie 1979; Eitenmiller et al. 1982).

Chemical Changes and Other Deteriorative Processes

Fish fats contain a high proportion of unsaturated fatty acids which are subject to attack by atmospheric oxygen leading to deteriorative changes especially in fatty fish (Hobbs 1982). Fat oxidation is an important deteriorative reaction causing flavor, color, and possibly textural changes associated with rancidity (Hobbs 1982). Lipid hydrolysis is a common postmortem feature in fish and fish products. The major products are free fatty acids (FFA) and glycerol (Hardy 1980). Hydrolysis is caused by the lipolytic activity of fish tissues. These are species dependent, but hydrolysis can also be promoted by bacterial lipases during fish spoilage. The consequences of lipolysis on fish product acceptability are not always clear. Attention has concentrated instead on whether the acids produced interact with the proteins and thus affect the textural qualities of the fish (Sikorski 1980).

Although oxidative color changes are more significant in frozen fish, the oxidation of myoglobin (purple red) to metmyoglobin (brown) has been reported as a major cause of discoloration of both fresh and frozen fish (Benedict et al. 1975). Fat and myoglobin oxidation can be avoided or at least retarded by lowering the oxygen level by vacuum packaging or by modified atmospheres packaging (MAP) with different concentrations of nitrogen and carbon dioxide in the absence of oxygen.

High concentration of CO₂ can promote oxidation of myoglobin to metmyoglobin. This can be offset by the addition of 1% carbon monoxide (CO) (Gee and Brown 1978). The mechanism of this reaction will be discussed in Part II under modified atmospheres. Even though lipolysis and oxidation of fats are mostly due to endoenzymes of fish tissue and the presence of oxygen, respectively, lipolytic and oxidative rancidity can be promoted by bacterial activity if fatty fish are mishandled and abused particularly by activating microbial lipases.

Autolytic activities are responsible for the degradation of nucleotides. The principal pathway of ATP decomposition in fish is ATP to ADP to AMP to IMP to I to HX (hypoxanthine) to xanthine to uric acid.

Generally the reactions involved in converting ATP to IMP took place in the early stage after death or before the ultimate pH is reached. The IMP to inosine step is often rate-limiting. IMP accumulates in fish meat in the early stage after
death and acts as a flavor enhancer in the fish (Ikeda 1980). Inosine and hypoxanthine concentrations increase with time (decrease in freshness). This series of reactions is the basis of the K value test for freshness discussed in Part III.

The disappearance of nicotinamide-adenine-dinucleotide (NAD) in fish muscle affects the rates of various oxidation-reduction reactions in which NAD participates; for example: browning, the ultimate postmortem pH, and the maintenance of color (Ikeda 1980).

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