The high levels of moisture, rich nutrients, including free amino acids, other nitrogenous compounds and digestible proteins renders seafood easily perishable, often spoiling in a short period of time even under refrigeration. Fish and shellfish carry a variety of microorganisms from both aquatic and terrestrial sources. In addition to spoilage microorganisms, seafood may contain various potential pathogens which are public health hazards. It is often difficult to maintain the quality of seafood and seafood products because of the considerable distance between consumers and harvesting areas, which provides opportunities for microbial growth and recontamination. This paper covers the quantitative and qualitative aspects of microorganisms found in fish and the factors affecting seafood quality. Emphasis will be placed on spoilage bacteria, which causes degradation and organisms that presents risk to the public health.

Fish spoilage

Most fish are caught in the wild in nets, or with lines with baited hooks, and hence it is difficult to control the initial quality of the raw material with any degree of repeatability. Fish farming has to some extent reduced the variation of fresh fish quality. The stress and mechanical damage caused during capture, the structure and composition of fish, rate of post-mortem change, minimal pH drop, and storage temperature prior to landing all act to induce rapid spoilage in fish. Like red meat, fish spoils through the combined effects of chemical reactions, via the continuing activities of endogenous enzymes and through bacterial growth. Since fish are poikilothermal, their metabolism and the commensal bacterial flora of their skin, gills and intestines are adapted to lower temperatures than those of mammals. Hence, chilling has less effect on slowing spoilage when compared with chilled red meat and poultry, especially for fish caught in temperate and polar regions, whereas chilled fish caught in tropical waters tend to spoil more slowly, since the initial micro flora is not adapted to growing in chilled conditions. The initial stages of spoilage in fish characterised by the loss of characteristic odour and taste are mainly due to autolytic degradation, while the final stages of fish quality deterioration characterised by softening or toughening of flesh texture and
production of volatile unpleasant-smelling odours and flavours are mainly due to microbial activity. Generally, the rates at which autolytic and microbial spoilage occur are dependent on the degree of microbial contamination and flora, storage temperature and packaging.

**Microbial flora in fresh fish**

The wide range of fish species, the vastly different environments from which they are harvested and the variety of microbiological sampling techniques used has resulted in widely ranging reports of numbers of organisms and flora on fish. The bacterial flora of cold water fish are dominated by the psychrotrophic gram-negative genera (Shewan, 1977). Organisms involved in the genera, Acinetobacter, Flavobacterium, Moraxella, Shewanella and Pseudomonas; members of the Vibrionaceae (Vibrio and Photobacterium) and Aeromonadaceae (Aeromonas spp.) are also common aquatic bacteria, while gram-positive organisms such as Bacillus, Micrococcus, Clostridium, Lactobacillus and coryneforms can also be found in varying proportions (Huss, 1995). Shewan (1977) concluded that the gram-positive Bacillus, and Micrococcus dominate on fish from tropical waters; however, other workers have found that the micro flora on tropical species is very similar to that on temperate species but with a higher load of gram-positive and enteric bacteria (Huss, 1995). Shewan (1977) emphasized that the significance of these organisms in terms of spoilage of fish varies considerably on their growth. Many of the organisms present on spoilt fish are thought to play no active role in spoilage (Huss, 1995). Alur et al. (1989) showed that despite prolific growth of Micrococcus colpogenes (10^7-10^8 cells/ml) in mackerel homogenate there were no signs of spoilage, thus indicating that total bacterial count does not serve as a reliable index of freshness for processed seafood (Alur et al., 1971). It thus, it is a common mistake to think the microorganisms present in the highest numbers are the cause of spoilage and deterioration in fish. A clear distinction should always be made between the terms spoilage flora and spoilage bacteria. Spoilage flora refers to the bacteria present on the fish when considered spoiled, whereas spoilage bacteria are the bacteria responsible for the production of off-odours and off-flavours in the spoilt fish. Examples of compounds produced by microbial metabolism during spoilage are given in Table I. Some of these spoilage compounds are also produced by the action of autolytic enzymes naturally present in the fish.

**Bacterial deterioration of fish**

The fish’s regulatory mechanisms, which prevent invasion of the tissues by bacteria, cease to function after death. Bacteria then invade the fish body through the skin, enter the body cavity and belly via intestines, and penetrate the gill tissue and kidney by way of the vascular system. The low molecular weight compounds and soluble proteins yielded from the fish body during autolysis after rigor mortis provide rich nutrients for bacterial growth.

Various proteases and other hydrolytic enzymes secreted by psychrophilic and psychrotrophic bacteria can act on the fish muscle even at low temperatures (Venugopal, 1990). The factors that influence the microbial contamination and growth include fish species and size, method of catch, on-board handling, fishing vessel sanitation, processing, and storage condition (Chen and Chai, 1982; Ward and Baj, 1988). Fish are subjected to rapid microbial contamination and growth if handling and storage are inadequate. It is estimated that about 10 per cent of the total world catch is lost due to bacterial spoilage (James, 1986). Various micro-organisms involved in spoilage are listed in Table II in descending order of spoilage activity. Some organisms cause spoilage in different degrees depending on the total microbial flora, fish

**Table I** Bacterial spoilage compounds

<table>
<thead>
<tr>
<th>Specific spoilage bacteria</th>
<th>Spoilage compounds</th>
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<tbody>
<tr>
<td><em>Shewanella putrefaciens</em></td>
<td>TMA, H2S, CH3SH, (CH3)2S, HX</td>
</tr>
<tr>
<td><em>Photobacterium phosphoreum</em></td>
<td>TMA, HX</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>Ketones, aldehydes, esters, non-H2S sulphides</td>
</tr>
<tr>
<td><em>Vibrioaceae</em></td>
<td>TMA, H2S</td>
</tr>
<tr>
<td><em>Aerobic spoilers</em></td>
<td>NH3, acetic, butyric and propionic acid</td>
</tr>
</tbody>
</table>

Note: TMA = trimethylamine, H2S = hydrogen sulphide, CH3SH = methylmercaptan, (CH3)2S = dimethylsulphide, HX = hypoxanthine, NH3 = ammonia

Source: Church (1998)
quality, handling and packaging methods, and storage temperature.

Refrigerated fresh haddock fillet contain about 105 g⁻¹ of initial bacteria, predominated by *Moraxella*, *Acinetobacter* and *Corynebacterium*. After storage at 1°C for 14 days the bacterial numbers reaches 2.1 · 10⁸ g⁻¹ and the fish enters the spoilage stage. *Pseudomonas* (*Alteromonas*) *putrefaciens* and fluorescent pseudomonads were the organisms responsible for the spoilage of refrigerated haddock (Chai, 1968). These spoilage bacteria account only for about 1 per cent of the initial microbial load but increase to at least 30 per cent of the spoilage flora. This indicates that whenever *Pseudomonas putrefaciens* and fluorescent pseudomonads reaches 30 per cent of the total bacterial count, fish spoilage will result regardless of total bacterial level.

As *Pseudomonas putrefaciens*, fluorescent pseudomonads and other potential spoilage bacteria increase rapidly in the initial stages of spoilage, they produce vast amounts of proteolytic and hydrolytic enzymes (Shewan, 1961). Proteins are degraded by proteases to peptides and amino acids and then further broken down to indole, amines, acids, sulphide compounds, and ammonia (Liston, 1980). Lipases break down lipids to form fatty acids, glycerol and other compounds.

**Trimethylamine production**

Fish have a unique osmoregulatory mechanism to avoid dehydration in marine environments and waterlogging of tissue in fresh water. One important osmoregulant is trimethylamine oxide (TMAO) by reducing it to TMA (trimethylamine). Endogenous enzymes present in fish can also reduce TMAO to DMA (dimethylamine) and FA (formaldehyde). While TMAO is non-odorous, TMA is a component in the odour of stale fish. The levels of TMA in fish has for sometime been used as an indicator of microbial deterioration of fish. Fish with less than 1.5mg TMA-N 100 g⁻¹ is considered of good quality while 10-15mg TMA-N 100g⁻¹ is considered the limit of acceptability (Huss, 1988). However, these limits cannot be applied universally as an indicator of freshness of fish. Ababouch et al. (1996) have shown that while less than 1mg TMA-N 100 g⁻¹ is considered first grade, 5-10mg TMA-N 100g⁻¹ is the limit of acceptability in sardine. The reduction of TMAO to TMA is pH dependent and will therefore vary depending on the overall condition of the fish. However, the presence of TMA in fish is universally used as an indicator of microbial deterioration.

**Production of other volatiles**

Many other volatile compounds are produced as a result of bacterial catabolism of fish constituents and are sometimes used as indicators of microbial degradation of fish. Volatile sulphide compounds and H₂S gas is produced from sulphur-containing amino acids (Gram et al., 1990). Ammonia is produced as a result of bacterial degradation of other non-protein nitrogen compounds such as urea which is in significantly large amounts in elasmobranches. Bacterial catabolism of amino acids in fish muscle also result in the formation of ammonia and other volatile bases. Limits of acceptability for total volatile base nitrogen (TVB-N) in some fish were 30-35mg 100 g⁻¹ (Huss, 1988); however, Ababouch et al. (1996) found that 25-35mg TVB-N 100g⁻¹ was the limit of acceptability in sardine, while El Marrakchi et al. (1990) found the limit of acceptability in another fish sample to be 25-30mg TVB-N 100 g⁻¹. These findings suggest that the development of TVB-N in fish may vary widely and is dependent on a number of intrinsic and extrinsic factors.

Volatile acids such as acetic, propionic, butyric and other short chain fatty acids are also produced as a result of microbial degradation of fish. These compounds contribute to the odour of spoil fish. Ethanol, propanol, isopropanol and phenethyl alcohol are

<table>
<thead>
<tr>
<th>Spoilage activity</th>
<th>Micro-organisms</th>
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<tbody>
<tr>
<td>High</td>
<td><em>Pseudomonas (Alteromonas) putrefaciens</em>, <em>Pseudomonas (altremonas) fluorescens</em>, other fluorescent pseudomonads and other pseudomonads</td>
</tr>
<tr>
<td>Moderate</td>
<td><em>Moraxella</em>, <em>Acinetobacter</em> and <em>Alcaligenes</em></td>
</tr>
<tr>
<td>Low, active only in specific conditions</td>
<td><em>Aerobacter</em>, <em>Lactobacillus</em>, <em>Flavobacterium</em>, <em>Micrococcus</em>, <em>Bacillus</em> and <em>Staphylococcus</em></td>
</tr>
</tbody>
</table>

Source: Hui (1992)
produced by a number of fish spoilers (Chen et al., 1974).

**Histamine production**

Scrombroid fish (tuna, albacore) contain characteristically high levels of free histidine in their muscle tissue (Taylor et al., 1989), which can be converted to histamine by the action of bacterial decarboxylase. Bacteria known to be capable of decarboxylating histidine include Vibrio, Proteus morganii, and Klebsiella pneumoniae, all having minimum growth temperatures between 8-15°C. The formation of histamine in fish is purely from microbial origin and therefore could be used as an indicator of bacterial activity in fish. The presence of histamine in fish is insignificant in its contribution to the sensory acceptability of fish; however, its presence could appreciably compromise its safety when consumed. The guideline established by the US Food & Drug Administration (FDA, 1996), for histamine in fish is 5 mg/100g (50ppm). Fish with levels above this limit are prohibited from being sold for human consumption. Since histamine is only produced at temperatures above 8°C, adequate temperature control is necessary to reduce or prevent its formation. During storage for extended periods at ambient temperature the level of histamine may begin to reduce by bacteria having histaminase activity (Taylor, 1986); however, the fish would have been completely spoiled by the time the histamine levels have reduced to a legally acceptable level. Other biogenic amines formed as a result of bacterial activity on free amino acids in fish muscle are cadaverine and tyramine.

**Pathogenic flora of fresh fish**

Many pathogenic bacteria make up part of the natural micro flora of fresh fish. Some aquatic pathogens found in fish include Vibrio para-hemolyticus, V. vulnificus, Listeria monocytogenes, Aeromonas hydrophila, Yersinia enterocolitica, Clostridium perfringens and Clostridium botulinum. The activity of these pathogens in fish is dependent on the storage, handling and processing of the raw material. Since fish is stored at low temperatures (<2°C), psychrotropic strains of Clostridium botulinum and other psychrotrophic pathogens such as Aeronomas, Listeria and Yersinia, are of greater importance because they can render food unsafe for human consumption. When considering the safety of fish, non-psychrotrophic, psychrotrophic strains of C. botulinum is of particular importance. These strains can grow and produce toxins without producing any overt signs of spoilage, which may also be absent as a result of an inhibition of the normal spoilage flora, e.g. in bulk stored, modified atmosphere packaged (MAP) fish or conditions in which an anaerobic environment is created. Strains of type E and non-proteolytic types B and F are a major concern in MAP as they are able to grow at temperatures as low as 3.3°C, albeit slowly, and, as they do not putrefy proteins they may not show obvious signs of spoilage (Church, 1998).

During storage, handling and processing fish may become contaminated with other pathogens such as Salmonella typhimurium and Escherichia coli. These and other pathogens may cause degradation leading to intoxication and spoilage of fish and fish products stored at elevated temperatures. This is very common when fish is stored in the home refrigerator where the temperature is not not monitored or controlled (Parry, 1993).

**Conclusion**

The preservation of fish quality is of great economic importance to the seafood industry. For a very long time fishermen and seafood processors have employed several methods to preserve the quality and extend the shelf life of fish. Some of the most commonly used methods are low temperature storage, dehydration, canning, modified atmosphere packaging, irradiation, the use of chemical and biological preservatives, and combinations of two or more of these methods. All these have undoubtedly contributed to the improvement of quality and safety of seafood. Autolytic deterioration of fish dominates the earlier changes in fresh fish quality; however, microbial degradation which causes more undesirable changes in fish dominates the latter stages of spoilage. In addition to causing quality deterioration, microbial contaminants can render the food unsafe for consumption. The handling, processing and storage of seafood combined with poor sanitation directly affects the spoilage flora and the ability of these bacteria to cause spoilage; therefore regardless of the initial quality if seafood is improperly handled rapid deterioration will be inevitable. Good manufacturing practices and the implementation of HACCP are there-
fore necessary to ensure safe, good quality seafood.

References


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