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Microbiological spoilage of fish and fish products

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Abstract

Spoilage of fresh and lightly preserved fish products is caused by microbial action. This paper reviews the current knowledge in terms of the microbiology of fish and fish products with particular emphasis on identification of specific spoilage bacteria and the qualitative and quantitative biochemical indicators of spoilage. *Shewanella putrefaciens* and *Pseudomonas* spp. are the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish. Modified atmosphere stored marine fish from temperate waters are spoiled by the CO_2 resistant *Photobacterium phosphoreum* whereas Gram-positive bacteria are likely spoilers of CO_2 packed fish from fresh or tropical waters. Fish products with high salt contents may spoil due to growth of halophilic bacteria (salted fish) or growth of anaerobic bacteria and yeasts (barrel salted fish). Whilst the spoilage of fresh and highly salted fish is well understood, much less is known about spoilage of lightly preserved fish products. It is concluded that the spoilage is probably caused by lactic acid bacteria, certain psychrotrophic *Enterobacteriaceae* and/or *Photobacterium phosphoreum*. However, more work is needed in this area.

Keywords: Fish; Fish products; Spoilage association

1. Introduction

Microbiological spoilage of foods may take diverse forms, but all of them are a consequence of microbial growth and/or activity, which manifests itself as changes in the sensory characteristics as shown in Table 1.

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Raw foods are initially contaminated with a wide variety of microorganisms, but only a selection of these contaminants is able to colonize the food and grow to high numbers. The term 'spoilage association' has been coined for such a specific microbial community. The precise mechanism by which one group of bacteria predominates over another, closely related group is not always fully understood. It is well known that only minor changes in processing and packaging of fish products are causing a dramatic change in the development and composition of the spoilage association and a complete different type of spoilage. However, even for the same type of product, spoilage may develop differently, depending on geographical origin and other unknown factors interacting with the microbial development. The present paper will review the recent findings and try to explain the sensory profile at the time of spoilage in terms of the microbiological activity in the product.

2. Fish as substrate for bacterial growth

All food commodities have their own distinctive microbiology. Important factors contributing to the microbiological complexity of seafood are:

- specific as well as non-specific contamination of the live animal from the environment and of products during processing;
- growth conditions for microorganisms due to specific intrinsic and extrinsic factors (temperature, a_w, pH, Eh, microbial interactions etc.).

The wide range of environmental habitats (freshwater to saltwater, tropical waters to arctic waters, pelagic swimmers to bottom dwellers and degree of pollution) and the variety of processing practices (iced fish products to (sterile) canned products) are all important factors in determining the initial contamination of fish and fish products. The part of the microflora which will ultimately grow on the products will be determined by the intrinsic and extrinsic parameters. There are several important specific intrinsic factors in fish which greatly influence the microbiology and spoilage:

- the poikilotherm nature of the fish and its aquatic environment;
- a high *post mortem* pH in the flesh (usually > 6.0);
- the presence of large amounts of non-protein-nitrogen (NPN);

Table 1
Microbiological spoilage of foods

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odour and -flavour
Production of extracellular polysaccharide material	Slime formation
Growth per se of moulds, bacteria, yeasts	Large visible pigmented or non-pigmented colonies
CO_2—from carbohydrate or aminoacids	Production of gas
Production of diffusible pigments	Discolouration

– the presence of trimethylamine oxide (TMAO) as part of the NPN fraction.

Bacteria estabilsh themselves on the outer and inner surfaces of the live fish (gills, skin, gastro-intestinal tract). The poikilotherm nature of fish allows bacteria with a broad temperature range to grow. Thus, the microflora of temperate water fish is dominated by psychrotrophic Gram-negative, rod-shaped bacteria belonging to the genera *Pseudomonos*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrionaceae* and *Aeroemonadaceae*, but Gram-positive organisms such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus* and *Corynebacterium* can also be found in varying proportions. The flora on tropical fish often carries a slightly higher load of Gram-positive and enteric bacteria, but is otherwise similar to the flora on temperate-water fish (Liston, 1980).

An important intrinsic factor relatcd to fish flesh is the very high post-mortem pH (> 6.0). Most fish contain only very little carbohydrate ($< 0.5\%$) in the muscle tissue and only small amounts of lactic acid are produced post mortem. This has important consequences for the microbiology of fish as amongst other factors it allows the pH sensitive spoilage bacteria *Shewanella putrefaciens* to grow.

The non-protein-nitrogen (NPN) fraction of the fish flesh consists of low-molecular-weight water-soluble nitrogen containing compounds such as free amino acids and nucleotides and is a readily available bacterial growth substrate. The decomposition of the sulphur containing amino acids cysteine and methionine is particularly important in spoilage, as it causes off-odours and -flavours due to formation of hydrogen sulphides and methylmercaptane respectively (Herbert and Shewan, 1975, 1976).

Trimethylamineoxide (TMAO) is part of the NPN fraction and its presence in all marine (Hebard et al., 1982) and some fresh water fish (Gram et al., 1989; Anthoni et al., 1990) species is well established. TMAO is known to cause a high (positive) redox potential (Eh) in the fish flesh (Huss and Larsen, 1979, 1980), however, the significance of this is not clear. The spoilage of fresh fish is certainly influenced by the presence of TMAO, particularly under conditions where oxygen is excluded. A number of well defined spoilage bacteria (*Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*) are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odours and -flavours due to formation of trimethylamine (TMA) (Gram et al., 1987, 1990; Dalgaard et al., 1993). In sugar-salted herring, the presence of TMAO and the high Eh was established as the protective mechanism against the most common type of spoilage (sweet-sour, rotten putrid) as the organism causing this type spoilage is a strict anaerobe requiring a low Eh for growth (Knøchel and Huss, 1984a,b).

3. Principles of bacterial spoilage

In its simplest form, food spoilage is a result of microbiological growth per se and becomes evident as visible growth (moulds, pigmented or non-pigmented, slimy bacterial colonies). In such cases, of course, there is a direct relationship between the total numbers of microorganisms and degree of spoilage.

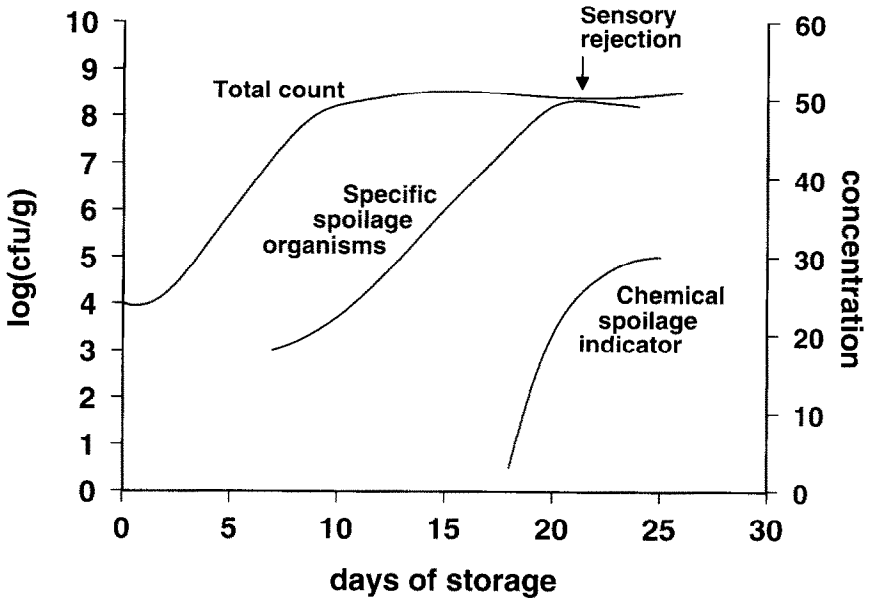

Fig. 1. Model of changes in total count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during chill storage of a fish product (modified from Huss et al., 1996).

More often spoilage is as a result of the production of off-odours and -flavours caused by bacterial metabolism. In this case there is no correlation between total numbers of bacteria and spoilage (Castell et al., 1948; Huss et al., 1974), since only a fraction of the total flora participates in the spoilage.

A clear distinction should be made between the terms 'spoilage association' and 'spoilage organisms' (bacteria) since the first describes merely the bacteria present on the fish when it spoils whereas the latter is the specific group that produces the off-odours and off-flavours associated with spoilage (Fig. 1).

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 (Castell and Anderson, 1948; Herbert et al., 1971; Gram et al., 1987). Spoilage potential can be assessed in substrates such as sterile, raw fish juice (Lerke et al., 1963), heat-sterilized fish juice (Castell and Greenough, 1957; Gram et al., 1987; Dalgaard, 1995a) or on sterile muscle blocks (Herbert et al., 1971). The latter is the most complicated, but is also the one which is most comparable to the product.

Finally, the selected strains are tested to evaluate their spoilage activity, i.e., their growth kinetics and their qualitative and quantitative production of off-odours in the product of concern (Gram, 1989; Dalgaard, 1995a). The latter step is important, as some bacteria may produce the chemical compounds associated with spoilage, but are unable to do so in significant amounts at the normal conditions prevailing in a product 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 of iced fish. The spoilage of chilled, CO_2 packed fish is seen at a much lower level of 10^7 *P. phosphoreum* per gram (Dalgaard et al., 1993). This relatively low cell level is due to the large size (5 μm) of the bacterium resulting in a high amount of TMA per cell (Dalgaard, 1995a) and thus a higher spoilage activity. Calculating the amount of TMA per cell surface or volume, the production of TMA by *P. phosphoreum* is, however, not different from that of *S. putrefaciens*, for example.

Some organisms may not be able to produce off-odours in sterile fish substrates but only when the substrate is digested by other members of the spoilage associateion. Therefore, in some products, spoilage potential and acitivity of a particular strain must be evaluated in competition or symbiosis with the normal spoilage association.

4. Spoilage of fresh fish

Generally the quality deterioration of fresh fish is characterized sensorically by an initial loss of 'fresh fish flavour' (sweet, seaweedy). After a period where the odour and flavour is described as neutral or non-specific, the first indications of off-odours and flavours are detectable. These will progressively become more pronounced and lead to rejection of the fish. The time to spoilage depends mainly on storage temperature and fish species.

The initial quality loss in fish is primarily caused by autolytic changes and is unrelated to microbiological activity. Of particular importance in this respect is the degradation of nucleotides (ATP-related compounds) which is caused by autolytic enzymes. It is now widely accepted that the loss of the intermediate nucelotide, inosine monophosphate (IMP), is responsible for the loss of fresh fish flavour, but apart from this, the autolytic changes are contributing to spoilage mainly by making catabolites available for bacterial growth (see Huss, 1995). The breakdown of IMP and inosine proceeds faster in naturally contaminated fish than in sterile samples and it has been shown repeatedly that several bacteria participate in the degradation (see below).

The off-odours and -flavours developing in fish stored in air depend on the fish species and origin of the fish. The spoilage of marine temperate-water fish is characterized sensorically by development of offensive fishy, rotten, H_2S-off-odours and -flavours. This sensory impression is distinctly different for some tropical fish and freshwater fish, where fruity, sulphydryl off-odours and -flavours are more typical (Lima dos Santos, 1978; Gram et al., 1989).

The spoilage association developing in aerobically stored fish consists typically of Gram-negative psychrotrophic non-fermenting rods. Thus, under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas* sp. and *S. putrefaciens*. This is true for all fish and shellfish whether caught or harvested in temperate (Levin, 1968; Gram et al., 1987) or sub-tropical and tropical waters (Lannelongue et al., 1982a; Gram et al., 1990; Lima dos Santos, 1978; Shamshad et al., 1990). At ambient temperature (25°C), the microflora is dominated by mesophilic *Vibrionaceae* (Gorczyca and Pek Poh Len, 1985; Gram et al., 1990) and, particularly if the fish are caught in polluted waters, mesophilic *Enterobacteriaceae* (Gram, 1992).

Shewanella putrefaciens is the specific spoilage bacteria of marine temperate-water fish stored aerobically in ice and the number of *S. putrefaciens* is inversely linearly related to remaining shelf-life of iced cod (Jørgensen et al., 1988). *S. putrefaciens* strains isolated from fish products have similar spoilage potential (Jørgensen and Huss, 1989; Dalgaard, 1995a), however, the group is phenotypically heterogenous (Stenström and Molin, 1990) and on-going work at our laboratory show that there may be a clonal selection during a storage trial (Fonnesbech et al., in preparation).

Pseudomonas sp. 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, 1992). *S. putrefaciens* has been isolated from tropical freshwaters, but does not appear to be important in the spoilage of iced freshwater fish from tropical waters (Lima dos Santos, 1978; Gram et al., 1990). This may be due to occurrence of very low numbers and the inabililty of the organism to compete with high numbers of antagonistic pseudomonads (Gram, 1993; Gram and Melchiorsen, 1996).

At ambient temperature, motile aeromonads are the specific spoilers of aerobically stored freshwater fish (Gorczyca 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.

In vacuum-packed iced stored fish from temperate marine waters an increased development of TMA is seen (see Fig. 2) while the shelf-life is unaffected compared to aerobically stored fish (Table 2). The number of *Pseudomonas* is reduced, but *S. putrefaciens* which is capable of anaerobic respiration using TMAO grows to levels of 10^6– 10^8 cfu/g (Gram et al., 1987; Jørgensen et al., 1988; Dalgaard et al., 1993). Numbers below 10^8 cfu/g are unlikely to be important in spoilage and consequently other organisms must be involved. Jørgensen et al. (1988) observed that vacuum-packed cod contained some very large, almost yeast-like cells and suggested that these were involved in the spoilage. It was recently shown that these cells are heat sensitive *Photobacterium phosphoreum* (Dalgaard et al., 1993). *P. phosphoreum* is a marine vibrio which has escaped microbiologist as it does not grow when pour plating and incubation at high temperatures are used (van Spreekens, 1974; Dalgaard et al., 1993). It is easily isolated from intestines of various fish (van Spreekens, 1974; Dalgaard, 1995a). The organism produces 10–100 fold more

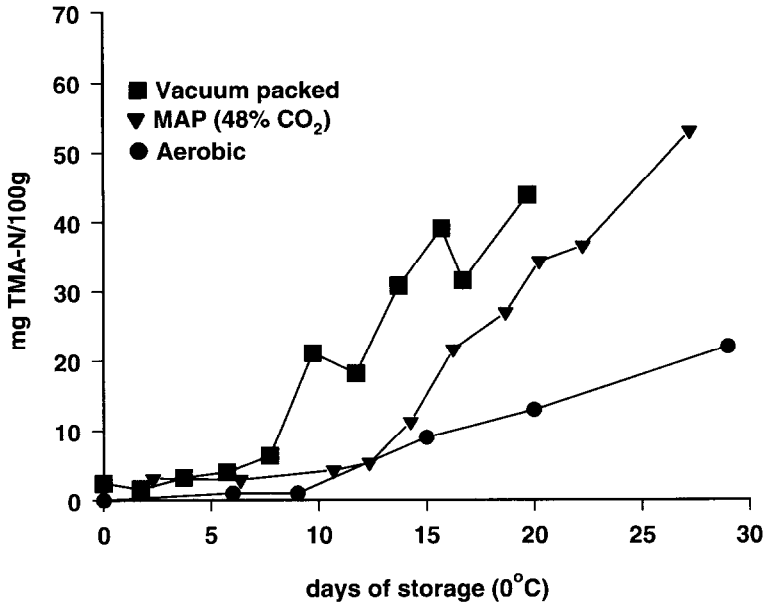

Fig. 2. Development of trimethylamine (TMA) in cod stored at 0°C in air (•), vacuum-packed (■) and modified atmosphere (48% CO_2) (▼) (modified from Dalgaard et al., 1993).

TMA per cell than *S. putrefaciens* (Dalgaard, 1995b) but does not cause off-odours as foul as *S. putrefaciens* (van Spreekens, 1977) probably because it does not produce volatile sulfides (van Spreekens, 1977; Dalgaard et al., 1993, Dalgaard, 1995a). The spoilage of vacuum-packed fish from temperate marine waters is caused by these two bacteria and differences in initial numbers of *S. putrefaciens*

Table 2
Effect of packaging on the shelf life of chilled fish and meat products (Dalgaard, 1995b)

Type of product	Storage temperature (°C)	Shelf life (weeks)		
		Air	VP	MAP
Meat				
Beef, pork, poultry	1.0–4.4	1–3	1–12	3–21
Lean fish				
Cod, pollock, rockfish, trevally	0.0–4.0	1–2	1–2	1–3
Fatty fish				
Herring, trout, salmon	0.0–4.0	1–2	1–2	1–3
Shellfish				
Crabs, scampi, scallops	0.0–4.0	1/2–2	—	1/2–3
Warmwater fish				
Sheepshead, tilapia, swordfish	2.0–4.0	1/2–2	—	2–4

VP, vacuum-packed; MAP, modified atmosphere packed (High CO_2 concentrations (25–100%)).

and *P. phosphoreum* probably decides which of the two becomes most important. Whilst abundant data exists on marine fish, little is known about the spoilage bacteriology of vacuum-packed freshwater fish. It is unlikely that *P. phosphoreum* plays a major role in the spoilage of freshwater fish as it is sodium-requiring and Hussain et al. (1976) found that 4 weeks of iced storage of vacuum-packed trout selected for Gram-positive bacteria.

CO_2-packing of marine fish from temperate waters inhibits the development of the respiratory organisms like *Pseudomonas* and *S. putrefaciens* and their numbers rarely exceed 10^5–10^6 cfu/g. Similar changes resulting in dramatic extensions of shelf-life are seen for meat products (Dainty and Mackey, 1992). However, a number of studies have shown that compared to meat products only limited or no extension of shelf-life is obtained by packing fish in CO_2 atmosphere (Cann et al., 1983, 1984; Farber, 1991; Dalgaard et al., 1993) (see Table 2). The development of TMA is, as shown in Fig. 2, similar to or delayed only a few days compared to vacuum-packed storage, but increased compared to aerobic storage (Cann et al., 1983; Dalgaard et al., 1993). The presence and subsequent growth of CO_2 resistant TMA producing *P. phosphoreum* to levels of 10^7–10^8 cfu/g explains these observations. By comparing Table 2 and Fig. 2 it can further be deduced that it is not development and level of TMA per se which causes sensorical rejection of fish, but rather the presence of TMA in combination with other, yet unidentified compounds.

Carbon dioxide and vacuum-packing of fish caught in freshwater or warmer waters where these particular heat sensitive, sodium-requiring *P. phosphoreum* are probably not as common, logically result in decrease in TMA production. The microflora becomes dominated by various Gram-positive organisms, mainly lactic acid bacteria (Banks et al., 1980; Lannelongue et al., 1982a,b; Oberlender et al., 1983; Pedersen and Snabe, 1995). However, as TMA can be detected later in the storage (Reddy et al., 1995; Oberlender et al., 1983), TMAO-reducing organisms must be present at some level. The specific spoilage bacteria of fresh and packed fish are summarized in Table 3.

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). These include TMA, volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight compounds. The substrates for the production of volatiles are TMAO, sulphur-containing amino acids, 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 (Herbert and Shewan, 1975, 1976; Ringø et al., 1984) (see Table 4).

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 L-cysteine (Gram et al., 1987; Stenström and Molin, 1990). In contrast, 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 detected in spoiling CO_2 packed cod (Dalgaard et al.,

Table 3
Specific spoilage bacteria of fresh and packed fish stored chilled (<4°C) or in ice (Gram and Huss, 1996)

Atmosphere	Specific spoilage organisms of fresh, chilled fish			
	Temperate waters		Tropical waters	
	Marine	Fresh	Marine	Fresh
aerobic	*S. putrefaciens*[a] *Pseudomonas* spp.	*Pseudomonas* spp.[d]	*S. putrefaciens*[f]/ *Pseudomonas* spp.[f]	*Pseudomonas* spp.[i]
vacuum	*S. putrefaciens*[b]/ *P. phosphoreum*[b]	Gram-positive bacteria[e] Lactic acid bacteria[d]	Lactic acid bacteria[g]/ others?	Lactic acid bacteria?
CO_2	*P. phosphoreum*[c]	Lactic acid bacteria[d]	Lactic acid bacteria[h]/ TMAO reducing bacteria	Lactic acid bacteria?/ TMAO reducing bacteria[j]

/ = and/or; ? = not known.
[a] Levin (1968), Herbert et al. (1971), Jørgensen and Huss (1989).
[b] Dalgaard et al. (1993), Jørgensen et al. (1988).
[c] Dalgaard et al. (1993), Dalgaard (1995a).
[d] Assumed to be the most likely spoilage bacteria as typical marine bacteria are not present.
[e] Hussain et al. (1976).
[f] Gillespie and MacRae (1975), Gram (1992).
[g] Pedersen and Snabe (1995).
[h] Oberlender et al. (1983), Banks et al. (1980), Lannelongue et al. (1982a).
[i] Lima dos Santos (1978), Gram et al. (1990).
[j] Reddy et al. (1995).

1993). Methylmercaptan (CH_3SH) and dimethylsulphide ($(CH_3)_2S$) are both formed from methionine (Herbert and Shewan, 1975). Taurine, which is also sulphur-containing, occurs as free amino-acid in very high concentrations in fish muscle and disappears from the fish flesh during storage but this is because of leakage rather than because of bacterial attack (Shewan and Jones, 1957).

The development of TMA is in many fish species paralleled by a production of hypoxanthine. Hypoxanthine, which may cause a bitter off-flavour in the fish, can 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 Jørgensen et al. (1988) and Dalgaard et al. (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod. Several of the spoilage bacteria produce hypoxanthine from inosine or inosine mono-phosphate, including *Pseudomonas* sp. (Surette et al., 1988) *S. putrefaciens* (van Spreekens, 1977; Jørgensen and Huss, 1989; Gram, 1989) and *P. phosphoreum* (van Spreekens, 1977).

Table 4
Substrate and typical spoilage compounds produced by bacteria during storage of fresh and packed fish (Gram and Huss, 1996)

	Production (+) of spoilage compounds						Product examples
Substrate:	TMAO	Cysteine	Methionine	Other amino acids	IMP, inosine	Carbohydrates, lactate	
Compounds:	TMA	H_2S	CH_3SH, $(CH_3)_2S$	Ketones, esters, aldehydes, NH_3	Hypoxanthine	Acids	
Spoilage bacteria							
S. putrefaciens	+	+	+	?	+	+	Iced fish
Pseudomonas sp.	–	–	+	+	+	?	Iced fish
P. phosphoreum	+	–	–	?	+	?	CO_2 packed fish
Vibrionaceae	+	+	?	?	?	?	Ambient stored fresh fish
Enterobacteriacea	+	(+)	?	+	+	+	Lightly preserved fish
Lactic acid bacteria	–	(+)	?	+	?	+	Lightly preserved fish
Yeast	–	–	–	+	?	+	Sugar-salted fish
Anaerobic rods	–		?	+	?	?	Sous-vide fish

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* sp. is characterized by absence of these compounds (Gram et al., 1989, 1990). Fruity, rotten, sulphydryl odours and flavours are typical of the *Pseudomonas* spoilage of iced fish. *Pseudomonas* sp. produce a number of volatile aldehydes, ketones, esters and sulphides (Edwards et al., 1987; Miller et al., 1973a,b). However, it is not known which specific compounds are responsible for the typical off-odours. The fruity off-odours produced by *Pseudomonas fragi* originate from monoamino-monocarboxylic amino-acids.

Lerke et al. (1967) separated fish juice into a protein and a non-protein fraction and found that 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.

Table 4 summarizes what is currently known about the spoilage bacteria on fresh and packed fish and the compounds associated with spoilage.

5. Spoilage of fish products

5.1. Ligthly preserved fish products

This group includes fish products preserved by low levels of salt ($< 6\%$ NaCl (w/w) in the water phase) and, for some products, addition of preservatives (sorbate, benzoate, NO_2 or smoke). Product pH is high (> 5.0) and they are often packaged under vacuum and must be stored and distributed at chill temperatures ($\leq 5°C$). This is a group of high-value delicatessen products (cold-smoked, pickeled ('gravad') or marinated fish, brined shellfish) that are typically consumed as ready-to-eat products with no heat treatment.

The microbial spoilage developing in these products is not well understood. By comparing the sensory changes in naturally contaminated cold-smoked salmon with changes in samples with much reduced bacterial load Truelstrup Hansen et al. (1996) concluded that while autolytic enzymes caused tecture changes (softening) bacterial activity was indeed the cause of spoilage. The off-odours and -flavours developing are variously described as putrid, cabbage-like, sour, bitter, fruity, sweet and the normal shelf-life of this product also varied considerably from about three weeks to eight weeks for vacuum-packed, cold-smoked salmon (4–5% NaCl in water phase, pH 6.3–6.4) stored at 5°C.

Several studies have shown that the microflora developing in this type of products is dominated by lactic acid bacteria (LAB) (Magnusson and Traustadóttir, 1982; Civera et al., 1995; Truelstrup Hansen et al., 1995; Leisner et al., 1994). Often high levels (10^7–10^8 cfu/g) of LAB are present for several weeks before the product becomes sensorically rejectable, which demonstrates that the often used 'total count of bacteria' is completely useless as a spoilage indicator for this type of products, see Fig. 3.

Truelstrup Hansen (1995) reported that *Lactobacillus curvatus* was the most common species occurring, but also *Lb. saké/bavaricus*, *Lb. plantarum*, *Carnobacterium* and *Leuconostoc* sp. were present in smaller numbers. The specific microflora developing seemed to be related to production environment in the smokehouse (an in-house flora).

Until recently, it was believed that the lactic acid bacteria were of little importance for the sensory changes during storage of fish products and Leisner (1992) showed that no or very faint off-odours were produced by lactic acid bacteria compared to the very obnoxious off-odours produced by Gram-negative spoilers. However, Truelstrup Hansen (1995) found that several lactic acid bacteria were able to produce some of the off-odours (sour, cabbagey, sulphurous) associated with spoilage of cold-smoked salmon. Furthermore, a strain of *Lb. saké* produced H_2S during growth on cold-smoked salmon. Preliminary work at our laboratory has shown that elimination of the lactic acid bacteria by e.g. nisin causes extension of the shelf-life of cold-smoked salmon compared to samples with normal levels of LAB (Gram, Nilsson and Paludan-Møller, unpublished data).

Although most studies report dominance of lactic acid bacteria in cold-smoked fish, the chemical changes during storage varies as does the composition of the remaining microbial flora. The TMA content of cold-smoked salmon sometimes increases to 25–30 mg TMA-nitrogen per 100 g (Civera et al., 1995) whereas other

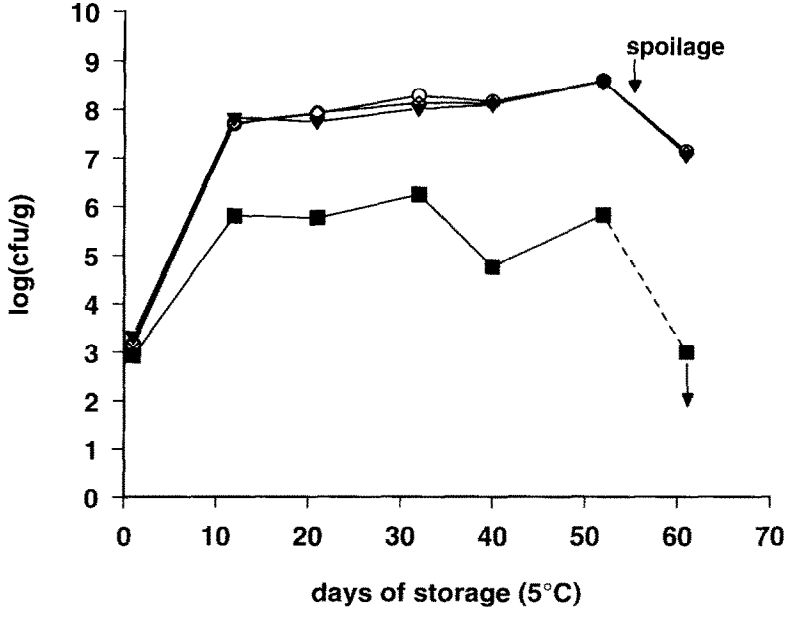

Fig. 3. Changes in total count (○), total psychrotrophic count (▼), lactic acid bacteria (◇) and *Enterobacteriaceae* (■) during storage of vacuum-packed cold-smoked salmon (NaCl 4.6% w/w in water phase) at 5°C (Truelstrup Hansen et al., 1995).

samples in the same study contained only low levels (5 mg TMA-N per 100 g). Similarly, Truelstrup Hansen et al. (1995) concluded that TMA could not be used as an objective quality indicator of cold-smoked salmon. Since LAB are known not to reduce TMAO to TMA, other bacteria must at times be participating in the spoilage process.

Spoiled lightly preserved fish products may also contain low (10^3 cfu/g) or high (10^6–10^7 cfu/g) levels of *Enterobacteriaceae*, *Brochotrix thermosphacta*, yeasts and *Photobacterium phosphoreum* (Cann et al., 1984; Civera et al., 1995; Leisner et al., 1994, Truelstrup Hansen et al., 1996). Truelstrup Hansen (1995) concluded that one of three different microfloras were present when cold-smoked salmon was sensorically rejected: (a) dominated by lactic acid bacteria (10^7–10^9 cfu/g); (b) dominated by lactic acid bacteria and *Enterobacteriaceae* (10^7–10^8 cfu/g); and (c) dominated by *Photobacterium phosphoreum* (10^6–10^7 cfu/g) with occasional high levels of lactic acid bacteria. The *Enterobacteriaceae* growing in lightly preserved fish products have been identified as psychrotrophic *Hafnia alvei*, *Serratia liquefaciens* or *Enterobacter* sp. All strains produce spoilage off-odours and reduce TMAO but the ability to produce H_2S from protein substrates varies (Truelstrup Hansen, 1995) and little is known about their spoilage activity in lightly preserved fish products.

5.2. Salt curing and fermentation

There are essentially two types of products where the preservative action of salt is the predominant process—dry salted and wet salted or pickeled fish products.

Dry-salting is used only for non-fatty fish. There are two types of spoilage of this product. One is growth of the extremely halophilic bacteria which causes a condition known as 'pink'. These pink halophilic bacteria (*Halococcus*, *Halobacterium*) are strongly proteolytic and produce off-odours and -flavours in the product. The other type of spoilage is moulding by a highly osmophilic type of fungus known as 'dun' (*Sporendonema* and *Oospora*) (Huss and Valdimarsson, 1990).

Wet salting or barrel-salting is used for fatty fish species such as herring and anchovy. The fish are mixed with salt and kept in a closed container. The waterphase salt in barrel-salted herring is typically 15–20%. Three types of spoilage are known for this product (Knøchel and Huss, 1984a,b). The most common type is characterized by the presence of sour, sour/sweet and putrid off-odours and -flavours. This type of spoilage is caused by growth of a Gram-negative, halophilic, obligate anerobic rod (up to 10^6–10^7 cfu/g). The growth of this organism is not possible until the general microflora has reduced all the TMAO causing the Eh to drop to negative values. This may take more than one year at chill storage (2–4°C) as the general flora consisted of low levels (10^3–10^5 cfu/g) of mainly Gram-negative, halophilic rods able to reduce TMAO.

The second type of spoilage is characterized by the development of fruity off-odours and is caused by growth to levels of 10^5 cfu/g of osmotolerant yeast species. Finally the term 'ropiness' or 'ropy brine' is used to describe the phenomen that the brine becomes highly viscous or slimy. This third type of spoilage is caused

by a Gram-negative, halophilic, aerobic non-motile rodshaped (*Moraxella*-like) bacteria (Magnusson and Møller, 1985).

The authors are not aware of any reports of microbiological spoilage of high-salt fermented fish products such as fish sauce and paste. This type of products is, despite their name, not really fermented products as very high salt levels (20–30%) are used and the liquefying action believed to be caused by autolytic enzymes.

5.3. Heat treated products

Many types of seafood products receive a heat treatment as part of their processing. Particularly the products receiving only a mild heat treatment and distributed at chill temperatures (Refrigerated processed foods with extended durability; REPFEDS) are likely to spoil due to microbial action. Unusual clostridia have been found as poilers of pasteurized crab meat (Segner, 1992) and Ben Embarek (1994) found that sous vide packed cod stored at 5°C spoiled due to growth of a Gram-positive sporeforming bacteria producing extremely obnoxious and putrid off-odours.

6. Conclusions

A thorough understanding of the spoilage process and knowledge of the specific spoilage organisms are necessary for design of an optimal, product specific quality assurrance programme or if microbiological data are to be utilized to predict the shelf-life of a product at specific conditions (temperature, packaging). For a number of fish products (heavily preserved as iced or salt cured products) the situation is reasonably well described and understood. One or only a few organisms invariably appear as the spoilage organisms in the same type of product under the same geographical conditions.

However, the situation in e.g. lightly preserved fish products is much more complicated and the assumption mentioned appears not to hold. Much more work is needed to understand the significance of such factors as the role of bacterial interactions, possible clonal selection of specially active groups within one species and possible changes of bacterial metabolism due to intrisic and extrinsic conditions.

References

Anthoni, U., Børresen, T., Christophersen, C., Gram, L. and Nielsen, P.H. (1990) Is trimethylamine oxide a reliable indicator for the marine origin of fishes. Comp. Biochem. Physiol. 97B, 569–571.

Banks, H., Nickelson, R. and Finne, G. (1980) Shelf-life studies on carbon dioxide packaged finfish from the Gulf of Mexico. J. Food Sci. 45, 157–162.

Barile, L.E., Estrada, M.H., Milla, A.D., Reilly, A. and Villadsen, A. (1985) Spoilage patterns of mackerel (*Rastrelliger faughni* Matsui) 2. Mesophilic versus psychrophilic fish spoilage of tropical fish. ASEAN Food J. 1, 121–126.

Ben Embarek, P.K. (1994) Microbial Safety and Spoilage of sous vide Fish Products. Ph.D. Thesis. Technological Laboratory, Lyngby, and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Cann, D.C., Smith, G.L. and Houston, N.C. (1983) Further Studies on Marine Fish Stored Under Modified Atmosphere Packaging. Torry Research Station, Aberdeen, Scotland.

Cann, D.C., Houston, N.C., Taylor, L.Y., Smith, G.L., Thomson, A.B. and Craig, A. (1984) Studies of Salmonids Packed and Stored Under a Modified Atmosphere. Torry Research Station, Aberdeen, Scotland.

Castell, C.H. and Anderson, G.W. (1948) Bacteria associated with the spoilage of cod fillets. J. Fish. Res. Board Can. 7, 370–377.

Castell, C.H. and Greenough, M.F. (1957) The action of *Pseudomonas* on fish muscle. 1. Organisms responsible for odours produced during incipient spoilage of chilled fish muscle. J. Fish. Res. Board Can. 14, 617–625.

Castell, C.H., Anderson, G.W. and Pivnick, H. (1948) Relation of bacterial counts to quality of cod fillets. J. Fish. Res. Board Can. 7, 378–388.

Civera, T., Parisi, E., Amerio, G.P. and Giaccone, V. (1995) Shelf-life of vacuum-packed smoked salmon: microbiological and chemical changes during storage. Arch. Lebensmittelhyg. 46, 13–17.

Dainty, R.H. and Mackey, B.M. (1992) The relationship between the phenotypic properties of bacteria from chilled-stored meat and spoilage processes. Soc. Appl. Bacteriol. Symp. Suppl. 21, 103S–114S.

Dalgaard, P. (1995a) Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int. J. Food Microbiol. 26, 319–333.

Dalgaard, P. (1995b) The effect of anaerobic conditions and carbon dioxide. In: H.H. Huss (editor), Quality and Quality Changes in Fresh Fish. FAO Fischeries Technical Paper No. 348. FAO, Rome, Italy.

Dalgaard, P., Gram, L. and H.H., Huss, (1993) Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. Int. J. Food Microbiol. 19, 283–294.

Edwards, R.A., Dainty, R.H. and Hibbard, C.M. (1987) Volatile compounds produced by meat pseudomonads and related reference strains during growth on beef stored in air at chill temperatures. J. Appl. Bacteriol. 62, 403–412.

Farber, J.M. (1991) Microbiological aspects of modified-atmosphere packaging technology—a review. J. Food Prot. 54, 58–70.

Gillespie, N.C. and MacRae, I.C. (1975) The bacterial flora on some Queensland fish and its ability to cause spoilage. J. Appl. Bacteriol. 39, 91–100.

Gorczyca, E. and Pek Poh Len (1985) Mesophilic spoilage of bay trout (*Arripis trutta*), bream (*Acanthropagrus butchri*) and mullet (*Aldrichetta forsteri*). In: A. Reilly (editor), Spoilage of Tropical Fish and Product Development, FAO Fish. Rep. 317 Suppl. FAO, Rome, Italy, pp. 123–132.

Gram, L. (1989) Identification, Characterization and Inhibition of Bacteria Isolated from Tropical Fish. Ph.D. Thesis. Technological Laboratory, Lyngby, and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Gram, L. (1992) Spoilage of three Senegalese fish species stored in ice at ambient temperature. In: E.H. Bligh (editor), Seafood Science and Technology. Fishing News Books, Blackwell, Oxford, pp. 225–233.

Gram, L. (1993) Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. Appl. Environ. Microbiol. 59, 2197–2203.

Gram, L. and Huss, H.H. (1996) Fresh and processed fish and shellfish. Chapter 23. In: B.M. Lund, A.C. Baird-Parker and G.W. Gould (editors), Microbiology of Food. Chapman and Hall, London.

Gram, L. and Melchiorsen, J. (1996) Interaction of two fish spoilage bacteria, *Shewanella putrefaciens* and *Pseudomonas* sp. in fish model systems. J. Appl. Bacteriol. 80, 589–595.

Gram, L., Trolle, G. and Huss, H.H. (1987) Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4, 65–72.

Gram, L., Oundo, J. and Bon, J. (1989) Storage life of Nile perch (*Lates niloticus*) dependent on storage temperature and initial bacterial load. Trop. Sci. 29, 221–236.

Gram, L., Wedell-Neergaard, C. and Huss, H.H. (1990) The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lates niloticus*). Int. J. Food Microbiol. 10, 303–316.

Hebard, C.E., Flick, G.J.J. and Martin, R.E. (1982) Occurrence and significance of trimethylamine oxide and its derivates in fish and shellfish. In: R.E. Martin, G.J.J. Flick and C.E. Hebard (editors), Chemistry and Biochemistry of Marine Food Products. AVI Publishing, Westport, Connecticut, pp. 149–304.

Herbert, R.A. and Shewan, J.M. (1975) Precursors of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). J. Sci. Food Agric. 26, 1195–1202.

Herbert, R.A. and Shewan, J.M. (1976) Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). J. Sci. Food Agric. 27, 89–94.

Herbert, R.A., Hendrie, M.S., Gibson, D.M. and Shewan, J.M. (1971) Bacteria active in the spoilage of certain seafoods. J. Appl. Bacteriol. 34, 41–50.

Huss, H.H. (editor) (1995) Quality and Quality Changes in Fresh Fish. FAO Fish. Tech. Pap. 348, FAO Rome, Italy.

Huss, H.H. and Larsen, A. (1979) The post-mortem changes in the oxidation-reduction potential (Eh) of fish muscle and internal organs. In: K. Sobolenska-Ceronik, E. Ceronik and S. Zaleski (editors), Food as Ecological Environment for Pathogenic and Index Microorganisms. Ars Polona, Warsaw, Poland, pp. 265–279.

Huss, H.H. and Larsen, A. (1980) Changes in the oxidation-reduction potential (Eh) of smoked and salted fish during storage. Lebensm.-Wiss. Technol. 13, 40–43.

Huss, H.H. and Valdimarsson, G. (1990) Microbiology of salted fish. Fish Tech. News (FAO) 10, 1.

Huss, H.H., Dalsgaard, D., Hansen, L., Ladefoged, H., Pedersen, A. and Zittan, L. (1974) The influence of hygiene in catch handling on the storage life of iced cod and plaice. J. Food Technol. 9, 213–221.

Huss, H.H., Dalgaard, P. and Gram, L. (1996) Microbiology of fish and fish products. Where are we - and where should we go? Proceedings "Seafood from producer to consumer, integrated approach to quality". Elsevier Science. To be pulished.

Hussain, A.M., Ehlerman, D. and Diehl, J.-F. (1976) Effect of radurization on microbial flora of vacuum-packaged trout (*Salmo gairdneri*). Arch. Lebensmittelhyg. 27, 223–225.

Jørgensen, B.R. and Huss, H.H. (1989) Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. Int. J. Food Microbiol. 9, 51–62.

Jørgensen, B.R., Gibson, D.M. and Huss, H.H. (1988) Microbiological quality and shelf life prediction of chilled fish. Int. J. Food Microbiol. 6, 295–307.

Knøchel, S. and Huss, H.H. (1984a) Ripening and spoilage of sugar salted herring with and without nitrate. I. Microbiological and related chemical changes. J. Food Technol. 19, 203–213.

Knøchel, S. and Huss, H.H. (1984b) Ripening and spoilage of sugar salted herring with and without nitrate. II. Effect of nitrate. J. Food Technol. 19, 215–224.

Lannelongue, M., Hanna, M.O., Finne, G., Nickelson, R. and Vanderzant, C. (1982a) Storage characteristics of finfish fillets (*Archosargus probatocephalus*) packaged in modified gas atmospheres containing carbon dioxide. J. Food Prot. 45, 440–444.

Lannelongue, M., Finne, G., Hanna, M.O., Nickelson II, R. and Vanderzant, C. (1982b) Microbiological and chemical changes during storage of swordfish (*Xiphias gladius*) steaks in retail packages containing CO_2-enriched atmospheres. J. Food Prot. 45, 1197–1203.

Leisner, J. (1992) Characterization of Lactic Acid Bacteria Isolated from Lightly Preserved Fish Products and Their Ability to Metabolise Various Carbohydrates and Amino Acids. Ph.D. Thesis. Technological Laboratory, Lyngby, and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Leisner, J.J., Millan, J.C., Huss, H.H. and Larsen, L.M. (1994) Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. J. Appl. Bacteriol. 76, 417–423.

Lerke, P., Adams, R. and Farber, L. (1963) Bacteriology of spoilage of fish muscle. I. Sterile press juice as a suitable experimental growth medium. Appl. Microbiol. 11, 458–462.

Lerke, P.A., Farber, L. and Adams, R. (1967) Bacteriology and spoilage of fish muscle. 4. Role of protein. Appl. Microbiol. 15, 770–776.

Levin, R.E. (1968) Detection and incidence of specific species of spoilage bacteria on fish. I. Methodology. Appl. Microbiol. 16, 1734–1737.

Lima dos Santos, C.A.M. (1978) Bacteriological Spoilage of Iced Amazonian Freshwater Catfish (*Brachyplatistoma vaillanti valenciennes*). M.Sc. Thesis. Loughborough University of Technology, England.

Liston, J. (1980) Microbiology in fishery science. In: J.J. Connell (editor), Advances in Fishery Science and Technology. Fishing News Books, Farnham, England, pp. 138–157.

Magnusson, H. and Møller, A (1985) Ropiness in the brine of sugar-salted herring. Int. J. Food Microbiol. 1, 152–261.

Magnússon, H. and Traustadóttir, K. (1982) The microbial flora of vacuum packed smoked herring fillets. J. Food Technol. 17, 695–702.

Miller III, A., Scanlan, R.A., Lee, J.S. and Libbey, L.M. (1973a) Identification of volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas fragi*. Appl. Microbiol. 25, 952–955.

Miller III, A., Scanlan, R.A., Lee, J.S. and Libbey, L.M. (1973b) Identification of volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrefaciens*, *Pseudomonas fluorescens* and an *Achromobacter* species. Appl. Microbiol. 26, 18–21.

Oberlender, V., Hanna, M.O., Miget, R., Vanderzant, C. and Finne, G. (1983) Storage characteristics of fresh swordfish steaks stored in carbon dioxide-enriched controlled (flow-through) atmospheres. J. Food Prot. 46, 434–440.

Pedersen, L. and Snabe, L. (1995) Isolation of Bacteriocin-producing Lactic Acid Bacteria from Chilled, Vacuum-packaged Temperate and Tropical Fish Products. M.Sc. Thesis. Danish Institute for Fisheries Research, Lyngby, and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Reddy, N.R., Villanueva, M. and Kautter, D.A. (1995) Shelf life of modified-atmosphere-packaged fresh Tilapia fillets stored under refrigeration and temperature-abuse conditions. J. Food Prot. 58, 908–914.

Ringø, E., Stenberg, E., and Strøm, A.R. (1984) Amino-acid and lactate catabolism in trimethylamine oxide respiration of *Alteromonas putrefaciens*. Appl. Environm. Microbiol. 47, 1084–1089.

Segner, W.P. (1992) Spoilage of pasteurized crabmeat by a nontoxigenic psychrotrophic anaerobic sporeformer. J. Food Prot. 55, 176–181.

Shamshad, S.I., Kher-un-Nisa, Riaz, M., Zuberi, R. and Qadri, R.B. (1990) Shelf life of shrimp (*Penaeus merguiensis*) stored at different temperatures. J. Food Sci. 55, 1201–1205,1242.

Shewan, J.M. (1962) The bacteriology of fresh and spoiling fish and some related chemical changes. In: J. Hawthorn and J. Muil Leitch (editors), Recent Advances in Food Science, 1, pp. 167–193.

Shewan, J.M. and Jones, N.R. (1957) Chemical changes occurring in cod muscle during chill storage and their possible use as objective indices of quality. J. Sci. Food Agric. 8, 491–498.

Stenström, I.-M. and Molin, G. (1990) Classification of the spoilage flora of fish, with special reference to *Shewanella putrefaciens*. J. Appl. Bacteriol. 68, 601–618.

Surette, M.E., Gill, T.A. and MacLean, S. (1988) Biochemical basis of post-mortem nucleotide catabolism in cod (*Gadus morhua*) and its relationship to spoilage. J. Agric. Food Chem. 36, 1435–1439.

Truelstrup Hansen, L. (1995) Quality of Chilled, Vacuum-packed Cold-smoked Salmon. Ph.D. Thesis. Danish Institute for Fisheries Research and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Truelstrup Hansen, L., Gill, T. and Huss, H.H. (1995) Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. Food Res. Int. 28, 123–130.

Truelstrup Hansen, L., Gill, T., Drewes Røntved, S. and Huss, H.H. (1996) Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Res. Int. (submitted).

van Spreekens, K.J.A. (1974) The suitability of a modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Antonie Leeuwenhoek 25, 213–219.

van Spreekens, K.J.A. (1977) Characterization of some fish and shrimp spoiling bacteria. Antonie Leeuwenhoek 43, 283–303.