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Shelf life trial on cod *(Gadus morhua L.)* and haddock *(Melanogrammus aeglefinus L.)* stored on ice around 0°C.

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ABSTRACT

Chemical and microbiological methods were evaluated during a storage trial of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stored aerobically on ice at around 0°C. Also, sensory methods were used to determine the shelf life of these two temperate marine species. The main emphasis was on the comparison of methods used for total viable bacterial counts (TVC), isolation and identification of the spoilage micro flora that had developed during aerobic storage and understanding the behaviour of these micro organisms.

Sensory evaluation using the Quality Index Method (QIM) on whole fish and the modified Torry scale on cooked fillets were used to determine the shelf life of these two fish species. These two methods have revealed that the shelf lives of the fish stored on ice around 0°C were in the range of 11 to 14 days for haddock and cod, respectively. The chemical indices, TMA and TVB-N, were not found to be proper indicators of shelf life as low production or formation occurred during sensory storage life under iced conditions. The TMA content for cod was 6.15 mg N/100 g and 1.24 mg N/100 g for haddock. Similarly, the TVB-N content for cod (26.7 mg N/100 g) increased to a higher level during ice storage than for haddock (18.7 mg N/100 g.).

Microbiological evaluation of the skin and flesh of the two fish species were carried out using various media (LH, 1% NaCl; IA 1% NaCl, and CFC). In general, total viable counts (TVC) were higher on spread-plated Long and Hammer´s (LH) medium for both cod and haddock. Spread-plating of iron agar (IAS) showed higher total counts and predominantly higher counts of H_2S-producers than poured iron agar (IAP) with an overlay. At the end of the trial, 40% of the skin micro flora of haddock comprised *Pseudomonas* group I-II, 20% *Shewanella putrefaciens*, 15% *Aeromonas*-like spp. and 10% for *Pseudomonas* group III-IV spp.While on the flesh, 32% of the micro flora comprised *Vibrio/ Photobacterium.* The rest of the flesh micro flora was difficult to cultivate and was therefore lost.

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1 INTRODUCTION

1.1 Shelf life

In general, food can be classified into three main categories: perishable, semi-perishable and non-perishable products. Fish is classified as a highly perishable food commodity and its shelf life depends on the initial quality as well as the storage conditions under which it is kept. The type of fishing method employed to catch fish and the subsequent handling practices thereafter greatly influence the shelf life of fish species. Poor bleeding, gutting and inadequate cleaning or washing will also affects the quality of fish during storage. It is well established that spoilage of marine fish whether temperate or tropical is primarily caused by bacteria, or lipid oxidation for fatty fish, and that autolytic changes play only a minor role in spoilage (Liston, 1980; Hobbs & Hodgkiss, 1982; Gram *et al*., 1990). Bacteria decompose several fish constituents; mainly non-protein nitrogen compounds and this can lead to the development of off-odours associated with spoilage.

The rate of fish spoilage depends on several factors, the most important of these are temperature, processing and atmospheric conditions during storage. The bacterial flora on newly caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1977). Several authors (Disney *et al*. 1974; Shewan, 1977; Lisac, 1977; Poulter *et al*., 1981; and Gram *et al*., 1990) have reported on the extended shelf life of iced tropical fish species compared with iced fish from cold or temperate waters. However, Lima dos Santos (1978) noted that although this was true for some tropical fish species, it was not universally the case. Factors such as species, fat content, size, shape, season and feeding ground may also play a part in shelf life stability.

1.2 Rigor Mortis

The first noticeable change in the flesh structure of fish after catch is *rigor mortis*, i.e. the contraction and stiffing of muscles shortly after death. Normally when fish is alive, the heart pumps blood to the gills where it is aerated. This oxygenated blood is circulated to the rest of the body. However when the fish is dead the heart stops functioning and the process of glycol sis takes place in the fish. This process of energy transformation operates under anaerobic conditions. It is very inefficient and the end products are lactic acid, pyruvate and adenosine triphosphate (ATP). When all the energy is used up in the fish muscle, the fish becomes stiff and is said to be in *rigor mortis*. The onset and duration of *rigor mortis* is dependent on temperature, the catching method used and the glycogen reserves of the fish. Amlacher (1961) suggested that the *rigor mortis* process determines the subsequent shelf life on ice since autotypic and bacterial decomposition could not start until after rigor resolved.

1.3 Nucleotides

Apart from events related to *rigor mortis*, the turnover of nucleotides is thought to be the main process influencing the quality of fish. Japanese researchers have for many years studied the autolytic changes occurring during storage of fresh fish with special focus on the breakdown of ATP (Saito *et al*., 1959).

ATP is enzymatic ally dephosphorylated and delaminated to inosine monophosphate (IMP) which through cleavage of phosphate is converted to inosine (HxR). Finally, inosine is split into hypoxanthine (Hx) and ribose (R) as shown in Figure1.

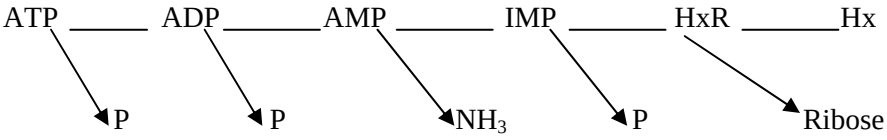

Figure 1: Dephosphorylation and deamination of ATP by enzymes.

In general, the first three reactions in Figure 1 proceed fast, especially at elevated temperatures, where the turnover occurs within hours. This pattern is followed in all bony fish investigated, but the turnover rate at the different steps varies considerably: Especially the last reactions differ since some fish accumulate inosine whereas high concentrations of hypoxanthine are found early during the storage period in other species (Dingle and Hines, 1971). This was confirmed in a study where quality changes during iced storage of 98 fish species were investigated and the species classified as either HxR or Hx accumulating (Ehira and Uchima, 1973). Also Knochel (1985) reported such differences and found cod to be an inosine accumulating species whereas hypoxanthine accumulated in plaice.

As a measurement of freshness, Japanese researchers (Saito *et al*., 1959; Ehira, 1976) have introduced the K-value, which is particularly useful in the initial storage period. The K-value is expressed as the percentage (%) of the ratio of Hx and HxR to the total amount of nucleotides:

$$K = \frac{Hx + HxR}{ATP + ADP + AMP + IMP + HxR + Hx} \times 100$$

1.4 Spoilage at temperatures around 0°C

The spoilage pattern of fresh temperate marine fish usually follows a series of easily recognisable symptom which were translated into a scoring system based on the early work by Castell and Anderson (1948) and Shewan *et al.* (1953). The odours developing were used when rating the fish on a 0 to 10 hedonic scale and when describing the spoilage in four stages as shown in Figure 2.

The characteristic odours and flavours in phase 1 are specific to the fish species and resemble seaweed; during phase 2 these are lost and the odours and flavours of the fish are often referred to as "neutral". In the third phase, there are slight off-odours that may be described as "sweet", "fruity" and later "sour" and can be described as the first signs of spoilage. In the fourth and last stage, the odours become stronger and more offensive and include ammonia, hydrogensulphide (H_2S), methylmercaptan, dimethylsulphide, trimethylamine (TMA) and insole.

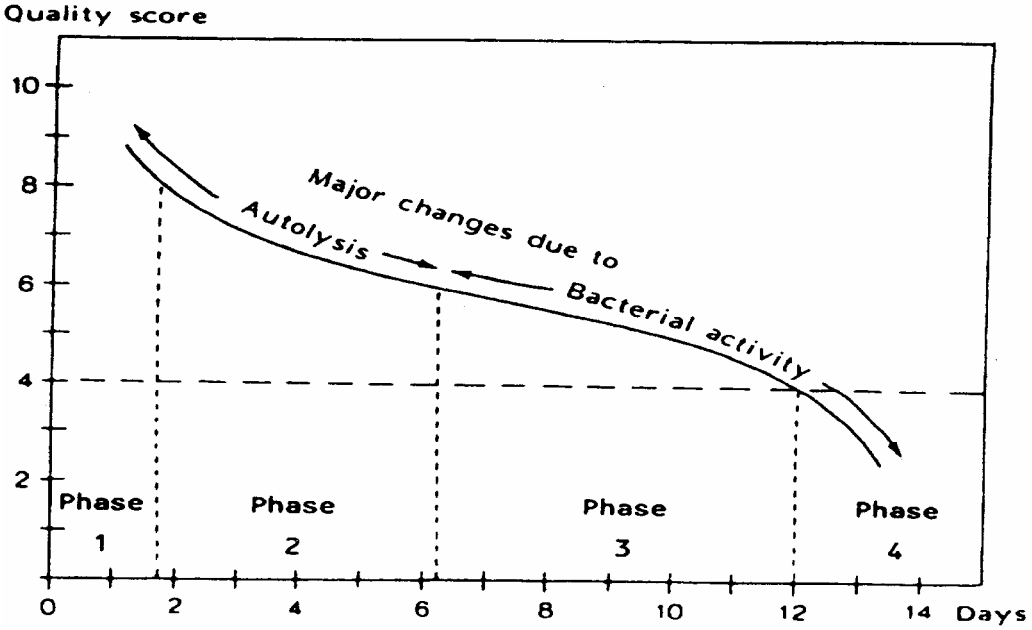

Figure 2: Changes in the eating quality of iced (0°C) cod (Huss, 1976)

1.5 Spoilage microorganisms

The microbial flora of spoiling fish consists mainly of gram-negative organisms of the genera *Pseudomonas, Alteromonas, Shewanella, Moraxella, Acinetobacter, Vibrio, Flavobacterium* and *Cytophaga*. The spoilage micro flora of aerobically iced stored fish comprises mainly of *Pseudomonas* spp. and *Shewanella 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*., 1982; Lima dos Santos, 1978; Gram *et al*., 1990. Shamshad *et al*., 1990). At 25°C (ambient temperature), the micro flora is predominantly mesophilic Vibrionaceae (Gorczyca & Pek Poh Len, 1985; Gram *et al*., 1990) and if the fish is caught or harvested from polluted waters mesophilic Enterobacteriaceae become dominant (Gram, 1992).

The meaning of **spoilage flora** and **spoilage bacteria** has been described by Huss (1996). The former is the bacteria present on the fish when it spoils whilst the latter is the bacteria that produce off-odours and off-flavours attributed to spoilage. Most of the bacteria normally present on the fish initially, are not responsible for spoilage (Figure 3). Identification of spoilage bacteria is a tedious task. To evaluate spoilage bacteria (sometimes known as specific spoilage organisms) requires in-depth sensory, microbiological and chemical analyses. The micro flora available at the point of sensory rejection is isolated and the bacteria screened in a sterile fish substrate for their spoilage ability, i.e. the potential to produce off-odours and off-flavours (Castell and Andersson, 1948; Herbert *et al*., 1971; Gram *et al*., 1987). The growth kinetics as well as quantitative and qualitative production of off-odours by the isolated bacterial strains is finally studied.

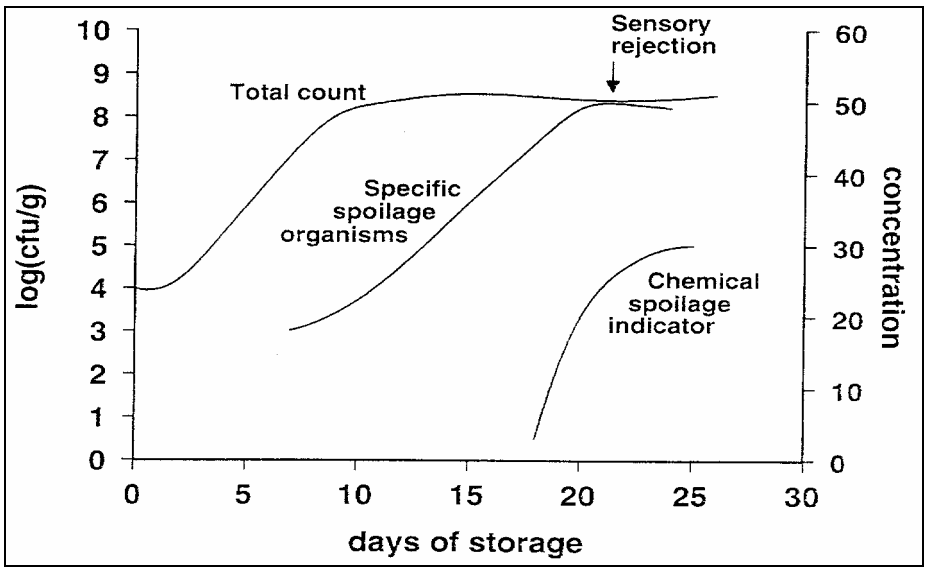

Figure 3: Model of changes in total viable count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during cold storage of fish (modified from Huss *et al*. 1996)

1.6 Isolation and cultivation of bacteria

During the past twenty years many refinements and changes in the techniques of estimating bacterial populations and detecting bacterial growth have occurred. Most of the early estimations were carried out using the pour plate technique, with 1.0 ml amounts of serial dilutions of flesh or skin homogenates, crudely prepared by grinding or shaking with sand (Hobbs and Hodgkiss, 1982). It is now recognised that exposure to the temperature is sufficient to kill a proportion of the psychrophilic organisms found on the fish. The type of dilute, culture media, temperature and time of incubation markedly affect the numbers and types of organisms isolated. If it is accepted that counts and micro flora analyses are intended to determine those organisms which are actively growing in a particular environment then the media and incubation conditions should approximate those in the sample under test (Hobbs and Hodgkiss, 1982). The use of automatic plate pouring machines, application of spiral-plate makers and electric colony counters can reduce the tedium of the detection and enumeration of bacteria.

1.7 Studies on cod and haddock

Shelf life studies on two fish species were undertaken during the winter of 1998 in Iceland. The aim of the trials was to determine the shelf life of whole, gutted cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) under storage in iced (around 0 to 1°C). Sensory shelf life of cod and haddock were determined by using the Quality Index Method (QIM) evaluating the whole fish and the Modified Torry scale for cooked fillets. Microbiological and chemical analyses were also conducted on the two fish species throughout the storage trials. Different microbiological media were used to evaluate total viable counts (TVC) and presumptive identification was done on the spoilage micro flora isolated from haddock on the last sampling day.

2 MATERIALS AND METHODS

Cod used in this experiment was caught on Sunday 29 November 1998 with a long line along the Southern-West coast of Iceland. Immediately after catch, the ungutted fish were bled, washed in running seawater, iced in plastic tubs and brought ashore the same day. On arrival the fish samples were stored in a chilled room over night and brought to the Icelandic Fisheries Laboratories (IFL) around 10.00 a.m. The fish were immediately gutted, iced and stored at chilled temperatures between 0 to 1°C.

Haddock were caught on the 7 December 1998 using a hook and line. The fish were bled alive, gutted, washed in running seawater and put on ice in clean plastic tubs. On arrival ashore the fish were transported to the IFL in a refrigerated and insulated truck on the 9 December 1998. Some of the fish were still in *rigor mortis*. The samples were transferred into cleaned plastic boxes, covered with layers of ice and stored in a chilled storage room at 0 to 1°C. Samples were removed from the chilled room as and when necessary for chemical, microbiological and sensory analyses.

2.1 Microbiological examination

TVC from the two fish species were evaluated from both the skin and the flesh throughout storage. Two fish were analysed at each sampling day. The cotton swab technique was used to take samples from the skin prior to sensory evaluation of the whole fish. Samples were taken by using sterile instruments and aseptically swabbing a 50 cm^2 area of the skin with a hydrophobic cotton swab. After taking samples, each cotton swab was broken into a small plastic bottle. Ten ml of cooled maximum recovery diluents (MRD, Oxoid) were added to each plastic bottle with the swab and mixed thoroughly (60 seconds) until a homogenate solution was achieved. Flesh analyses were performed by aseptically removing the skin on one side of the fish, cutting fillet sections and mincing them. Twenty-five grams of minced flesh samples were weighed in a stomacher bag using sterile equipment, 225 grammas of MRD were added and homogenized using a stomacher for one minute. Tenfold serial dilution was made as required using cooled MRD. Plating of the aliquots was performed on microbiological media. Two bacterial isolation methods were used for Iron Agar medium (1% NaCl; Gram *et al*. 1987): either applied by spread-plating (IAS) avoiding any heat shock or pour-plating of iron agar with an overlay (IAP). Selective counts of H_2S-producing bacteria were done by counting black colonies forming on the iron agar. Another microbiological medium, Long and Hammer's (LH) (Van Spree kens, 1974) was used to evaluate TVC by the spread-plating method. CFC medium (*Pseudomonas* agar base supplemented with CFC supplement, Oxoid) was also spread-plated and used to evaluate the development of *Pseudomonas* spp. during storage. Counts of fluorescent (group I) and non-fluorescent (group II) *Pseudomonas* were estimated by illuminating the CFC plates under a ultra-violet (UV)

light and the respective colonies counted (Shewan, 1963). The plates were aerobically incubated at 15°C for four to five days with the exception of the CFC medium, which was incubated at 22°C for two to three days. After the required incubation, total viable bacteria were counted on the plates using the Darkfield Quebec Colony Counter. The detection limit of bacteria for pour-plating method was 10 CFU/g for flesh samples and 20 CFU/cm^2 for skin samples. For spread plating, the detection limit was 20 CFU/cm^2 for both skin and flesh samples. Some of the LH plates were kept on the last sampling day for isolation and identification of spoilage micro flora of haddock. Bacterial isolates were streaked on iron agar and incubated at 15°C for 3 days before identification tests were performed as described in Table 1.

Table 1. Microbiological identification tests performed on selected isolates

Test	Methodology	Reference
Gram type	Gram staining 3% KOH	Hucker *et al.* 1992 Gregersen, 1978
Form and size	1000 x magnification	
Catalase	3% H_2O_2	
Oxidase	Dry slide (Difco)	
Motility	Hanging drop: isolates grown overnight at 15°C in Luminescence broth	Krieg and Holt (1984) (eds) Bergey´s Manual Vol. I
Oxidation/fermentation of glucose	O/F medium	
TMAO reduction	TMAO medium	Hugh and Leifson, 1953 modified with ½ strength artificial seawater (Macleod, 1968)
H_2S production	TMAO medium	
Sensitivity to 0/129 (Vibriostatic agent)	150 µg 0/129 disc (Oxoid) applied on iron agar plate previously swabbed with strain under study. Incubated at 15°C for 3 days.	Gram et al., 1987

2.2 Chemical analyses

Spoilage chemical indicators were evaluated by measuring trimethylamine (TMA) and total volatile basic nitrogen (TVB-N) levels in the two temperate fish species. The determination of TVB-N by steam distillation of TCA extract (Modified method of Malle and Tao, 1987) was used. In this method, one hundred grams of fish muscle were deproteinised and filtered. Steam distillation was carried out using a Struer-type distillatory. Twenty-five ml of the filtrate was put into the distillation flask and 10 ml of 10% NaOH was added to it. An Erlenmeyer flask containing 10 ml of 4% aqueous boric acid solution and 0.04 ml of methyl red and bromocresol green indicator were used for titration of ammonia and placed at the end of the condenser. Distillation was done until the final volume of 90 ml was obtained in the beaker (80 ml of distillate). The boric acid solution turned green when alkalinized by the distilled TVB-N. This solution was titrated using a 0.1 ml graduated burette containing

0.025N H_2SO_4 and complete neutralization was obtained when the colour turned pink on the addition of a further drop of H_2SO_4. The quantity of TVB-N in mg was determined from the volume of H_2SO_4 (n ml) added as follows: TVB-N= (n) (4.2 mg N/100g).

With respect to the determination of TMA content, steam distillation of TCA extract method (Malle and Poumeyrol, 1989) was used. This method is a modification of TVB-N (TCA) assay. Twenty ml of formaldehyde were added to the distillation flask to block the primary and secondary amines. Steam distillation was performed as for the determination of TVB-N in TCA extract. Upon the addition of the required amount of formaldehyde, only the TMA was distilled. The TMA content was calculated from the volume of 0.025 N H_2SO_4 used for titration (n ml) as follows: TMA= (n) (4.2mg N/100g).

The pH was measured in 5 grams of minced flesh moistened with 5 ml of deionised water at room temperature.

2.3 Sensory examination

Staff of the Icelandic Fisheries Laboratories carried out sensory evaluation of whole fish and cooked fillets for both fish species studied. The sensory evaluation panel comprised 10 to 12 trained staff members and a modified Torry scale (Shewan *et al*., 1953) was used to evaluate the quality of cooked fillets. The scale ranges from 10 = very fresh to 3 = very spoiled, with a rejection level at 5.5. The fillets were cooked in a steam oven for approximately 5-6 minutes at 80-90°C.

The quality index method (QIM) (Bremner, 1985) was used in the evaluation of whole cod and haddock. Sensory attributes relating to the gills, eyes, skin and intestinal cavity were evaluated and a quality index score was given for all the attributes assessed. The quality index score was at 0 for very fresh fish and 21 for very spoiled fish with a rejection level at 15. In order words, the lower the rating score the higher the quality of the whole fish and the higher the rating score the poorer the quality of the fish.

3 RESULTS

Bacteriological, chemical and sensory data collected during the shelf life trials are found in Appendix I and only representative figures and tables appear in this section. Appendix II contains sensory assessment schemes drawn up when studying the sensory changes in cod and haddock stored in ice around 0 to 1°C. Appendix III contains the presumptive identification scheme used to identify the bacterial isolates studied.

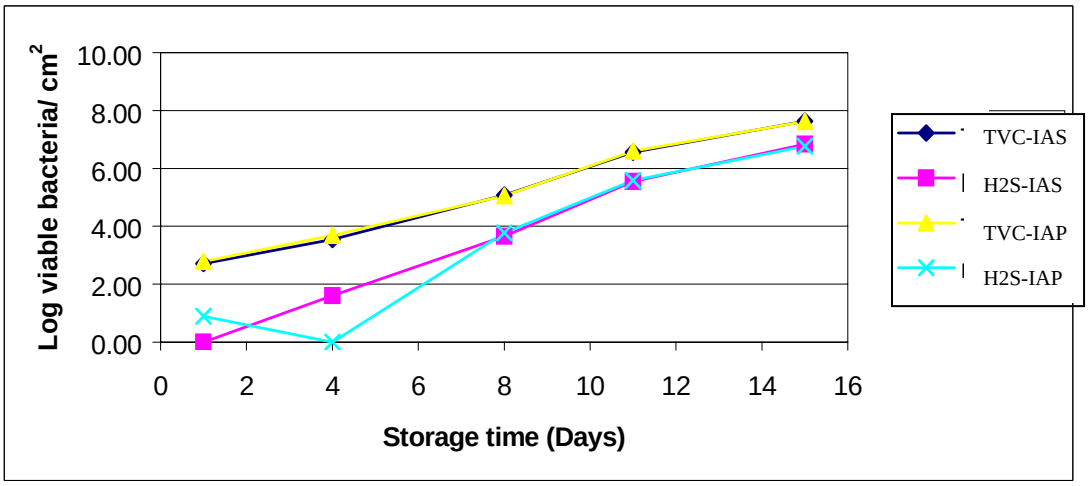

Figure 4: Comparison of two bacterial isolation methods, Iron Agar Poured (IAP) and Iron Agar Spread (IAS) plated, used in evaluating TVC on cod skin.

Two techniques, spread and pour-plating were used with Iron agar medium for the evaluation of total viable bacteria and counts of H_2S-producing organisms. Figure 4 shows the similarity between the two methods when assessing TVC on cod skin. The drop in the number of H_2S-producing organisms as estimated by IAP method during the second sampling day (Day 4) is noticeable and could have been due to experimental error.

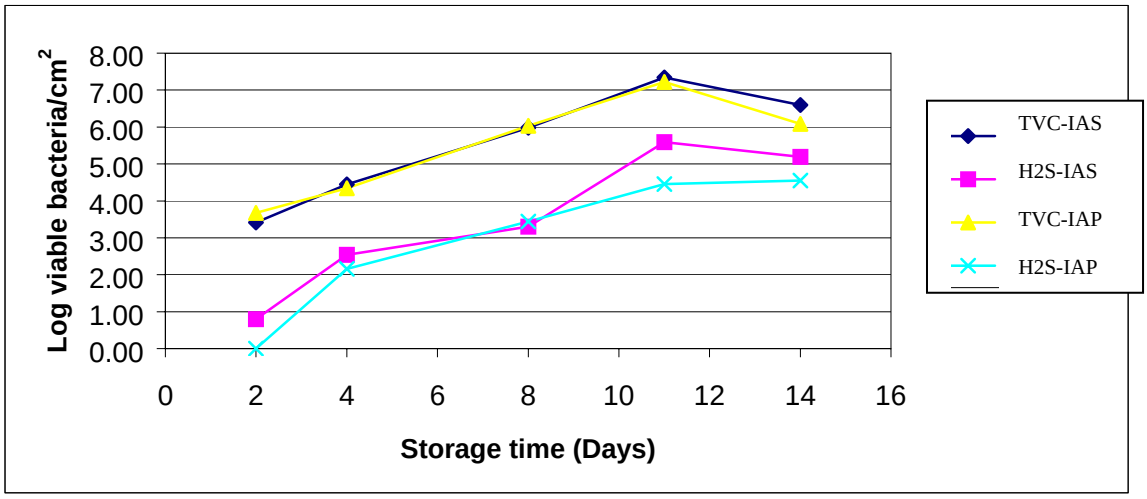

Figure 5. Comparison of two bacterial isolation methods, Iron Agar Poured (IAP) and Iron Agar Spread (IAS) plated, used in evaluating TVC on haddock skin.

Similarly to cod, TVC of haddock skin (Figure 5) obtained by both IAS and IAP methods gave similar results except for lower H_2S counts obtained by the IAP method. The IAS method registered higher counts of H_2S-producing organisms than the IAP method by almost 0.5 to 1 log of viable bacteria. This difference may have been due to the different treatment between IAS and IAP methods. The IAP method entailed pouring warm medium and this may have killed or injured some of the bacteria due to the heat shock.

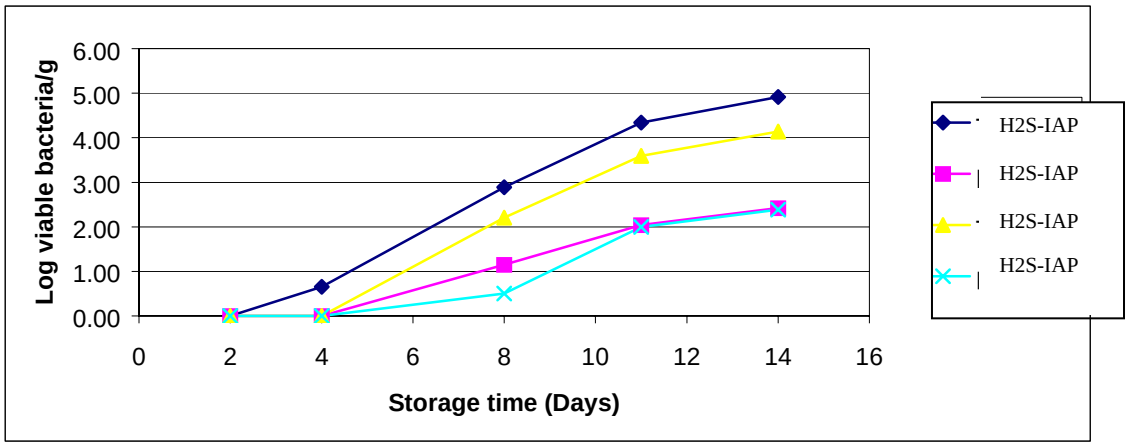

Figure 6. Comparison of two bacterial isolation methods, Iron Agar Poured (IAP) and Iron Agar Spread (IAS) plated, used in evaluating TVC on haddock flesh.

The comparison of bacterial isolation methods between IAS and IAP for haddock flesh as shown in Figure 6 revealed that the IAS method gave higher TVC counts than IAP by a factor of 1 log. This difference is quite important and could have been related to the heat sensitiveness of the flesh spoilage micro flora. On the other hand, H_2S-producer counts obtained by both methods did not differ much.

For the cod flesh, TVC was higher on IAS than IAP and at the end of the sampling trial; the H_2S-producing organism counts in the IAS method were greater than the IAP method by 0.5 log difference (data not shown).

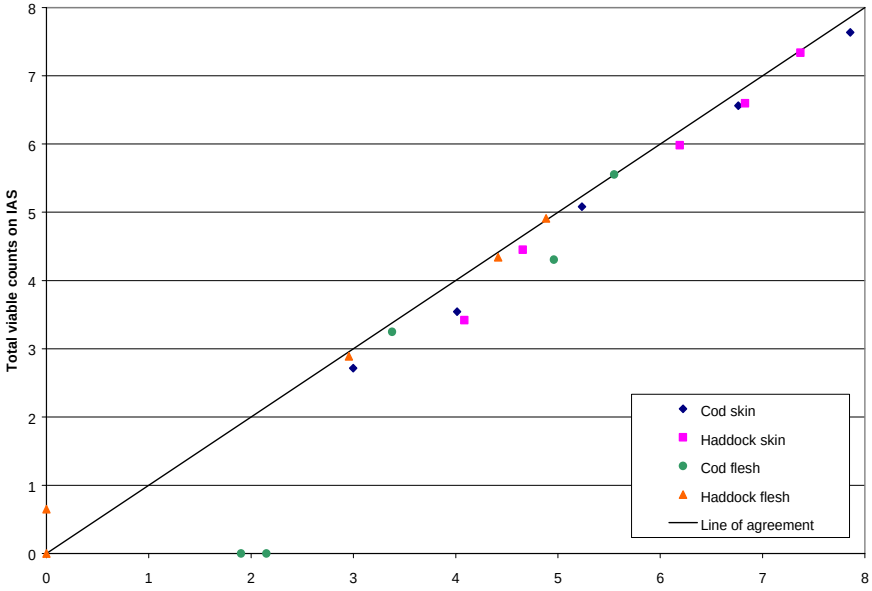

Figure 7. Comparison of TVC as estimated on LH and IA (spread-plated) for skin and flesh of cod and haddock.

A further comparison of the media used to estimate TVC (Figure 7) during the storage trials shows that LH medium generally gave higher TVC than those found on IA spread-plated medium, especially for the skin TVC of both fish species.

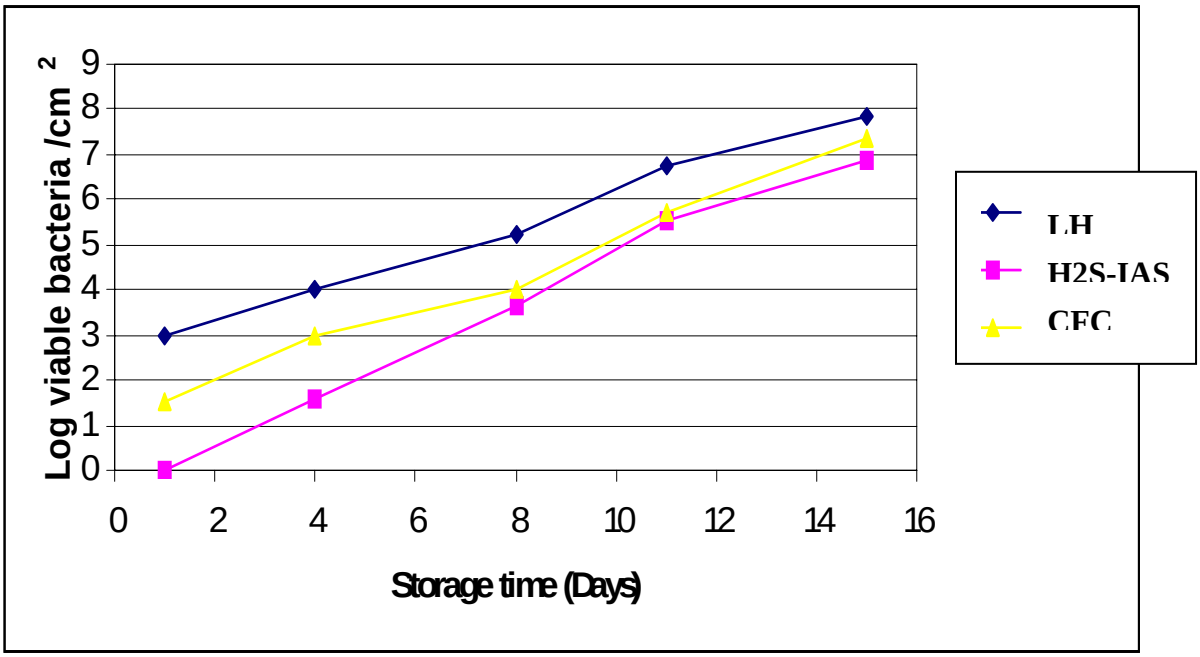

Figure 8. Plot of TVC (LH) and selective counts of *Pseudomonas* (CFC) and H_2S-producing bacteria (H_2S-IAS) as estimated for cod skin.

Figures 8 to 11 summarise the microbiological data collected during the storage trials of both fish species. Based on TVC (LH) and selective counts of *Pseudomonas* (CFC) and H_2S-producing bacteria, it is possible to study the development and estimate the importance of these bacterial groups as fish spoils. Figure 8 indicates that initially the skin micro flora of cod contained a low level of *Pseudomonas* spp. (about 1.5 log less than TVC) while H_2S-producing bacteria were below detection level. However, as storage progressed the level of these bacterial groups increased slightly faster than the overall micro flora (TVC) with a difference in counts of 1 log (for H_2S-producers) or less (for *Pseudomonas* spp.) at the last sampling day.

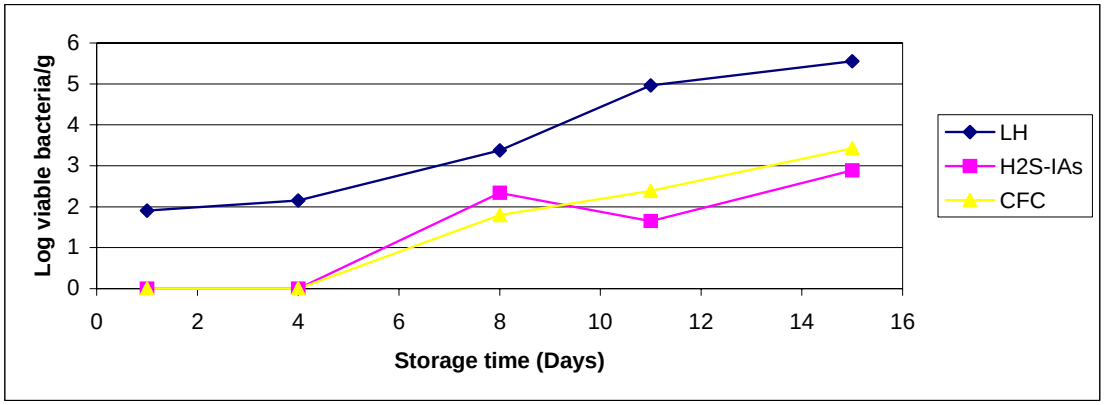

Figure 9. Plot of TVC (LH) and selective counts of *Pseudomonas* (CFC) and H_2S- producing bacteria (H_2S-IAS) as estimated for cod flesh.

Initially no H_2S-producers and *Pseudomonas* could be detected on cod flesh while TVC was over 2 logs during the first four days of storage life (Figure 9). TVC gradually increased and by the end of the storage trial it had became greater than H_2S-producers and *Pseudomonas* counts by almost 3 logs. On the other hand, *Pseudomonas* counts were higher than H_2S-producer counts.

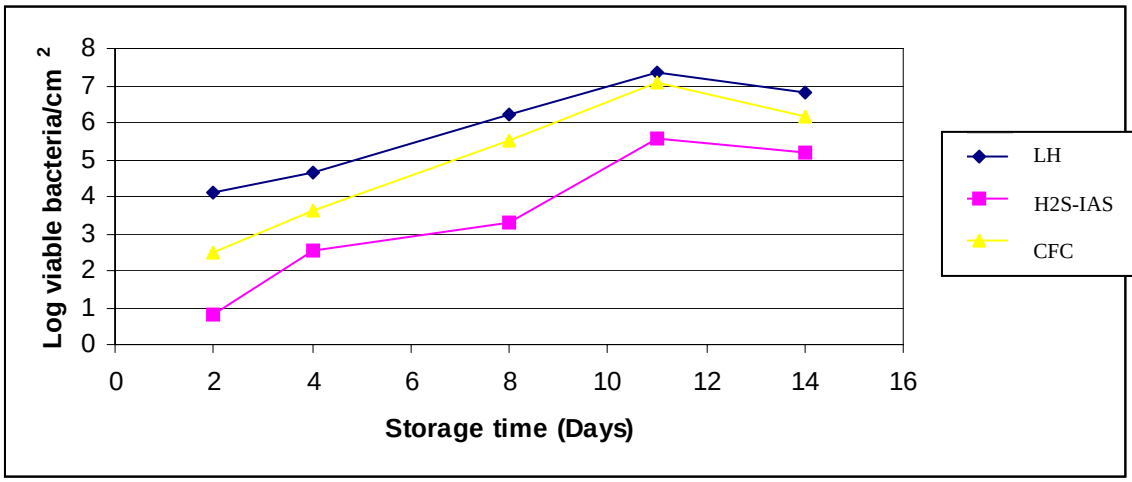

Figure 10. Plot of TVC (LH) and selective counts of *Pseudomonas* (CFC) and H_2S-producing bacteria (H_2S-IAS) as estimated for haddock skin.

The results are similar to these obtained for cod skin, TVC for haddock skin (Figure 10) was higher than the selective counts for H_2S-producers and pseudomonas by a difference of 3 and 1.5 logs respectively, at the beginning of the trial. A decrease in TVC and selective counts was observed on the last sampling day probably due to biological variation of fish samples. However, as storage time increased this difference in counts decreased leading to a higher proportion of *Pseudomonas* spp. and H_2S-producers at the end of the storage period. Still, the level of *Pseudomonas* was higher than that of H_2S-producers throughout storage.

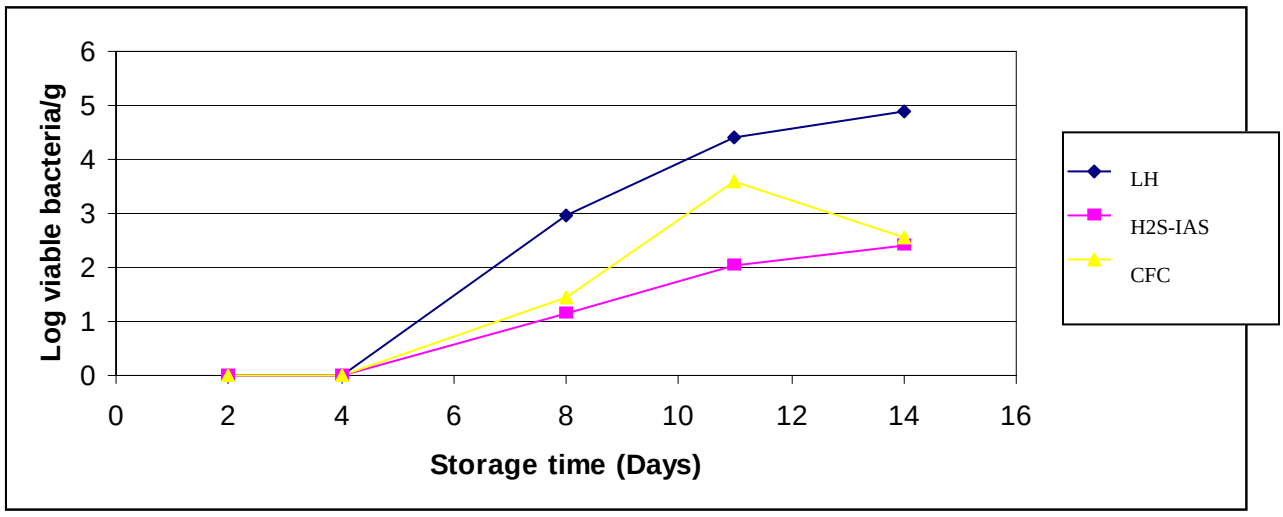

Figure 11. Plot of TVC (LH) and selective counts of *Pseudomonas* (CFC) and H2S-producing bacteria (H_2S-IAS) as estimated for haddock flesh.

During the storage trial of haddock, no bacteria could be detected during the first 2 days (Figure 11). On the third sampling day (day 8), TVC was higher than the selective counts by almost 2 logs. This trend was continuously seen throughout the storage life. The selective counts for *pseudomonas* were higher than H_2S-producers and by the end of the shelf life trial, both counts were almost the same and reached levels of 2.5 logs, which represented less than 1% of the spoilage micro flora.

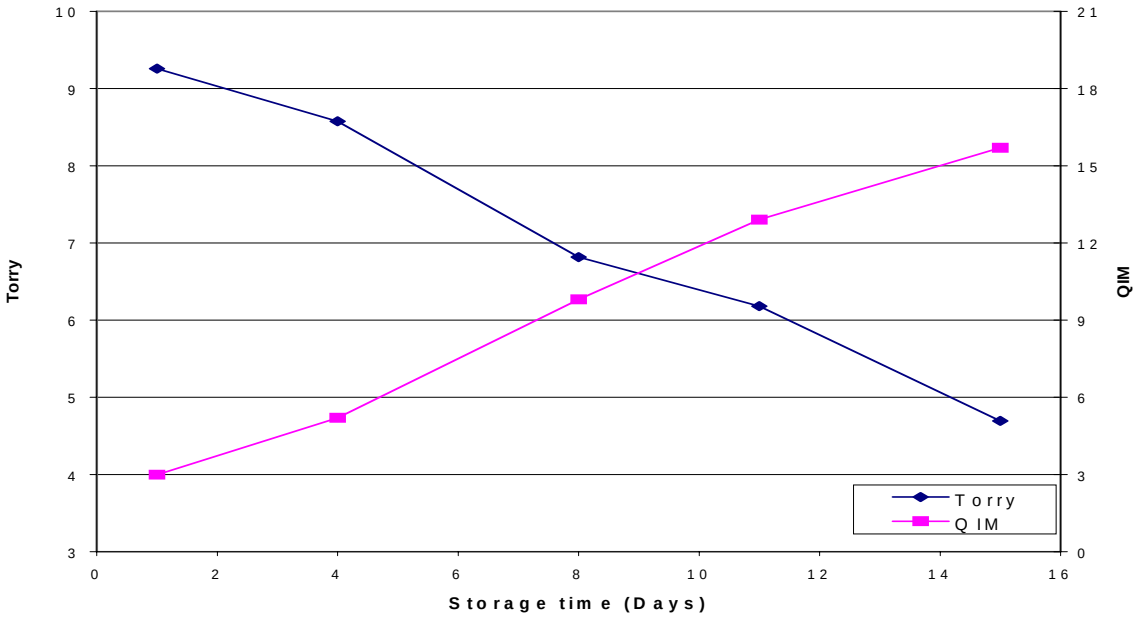

Figure 12. Sensory assessment for whole cod (QIM) and cooked fillets (Torry) stored on ice at around 0°C

The sensory assessment results in Figure 12 for whole cod showed that it had reached the rejection point at day 14 of storage time on ice and the cooked fillets passed the rejection limit on day 13.

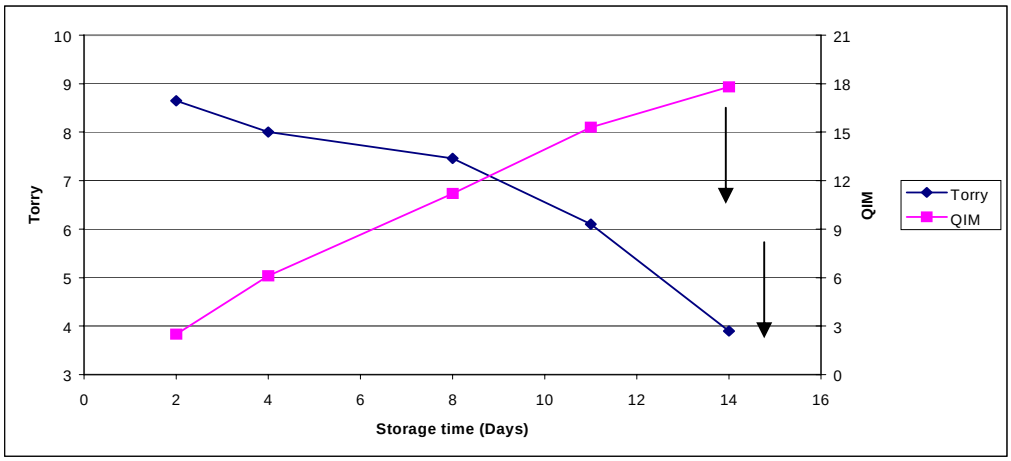

Figure 13. Sensory assessment for whole haddock (QIM) and cooked fillets modified Torry scale stored on ice at around 0°C.

Sensory evaluation results in Figure 13 for whole haddock and cooked fillets indicated that the fish was spoiled at day 11 based on QIM while for the cooked fillets the results showed that the fish was spoiled at day 12.

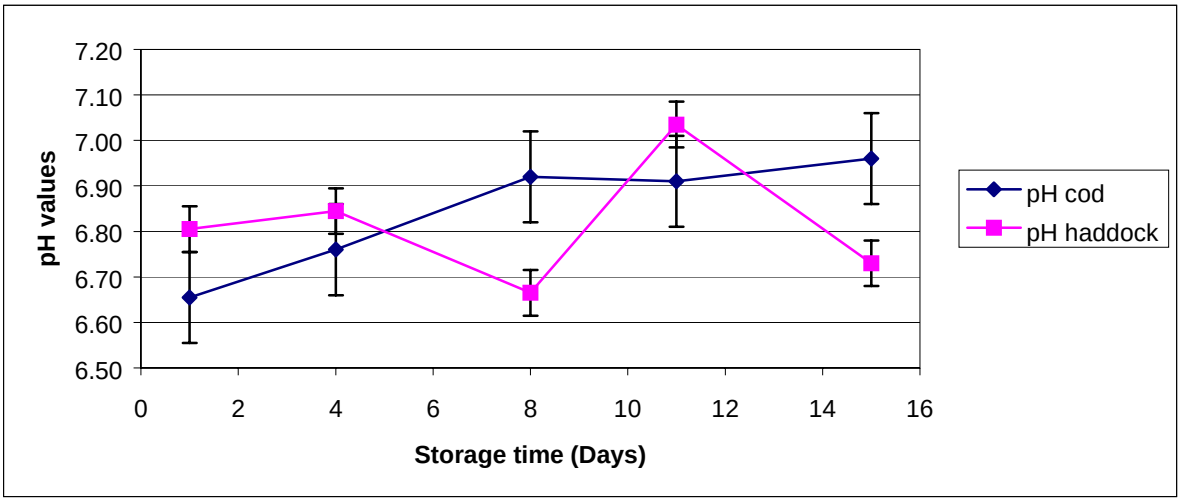

Figure 14. PH values for cod and haddock stored on ice at around 0°C.

The pH values in Figure 14 for cod were low initially (day 1 pH was 6.66) following *rigor mortis* but increased steadily over the storage period to approach pH 7.0. For haddock, fluctuation in pH was observed during storage life and probably due to biological variation.

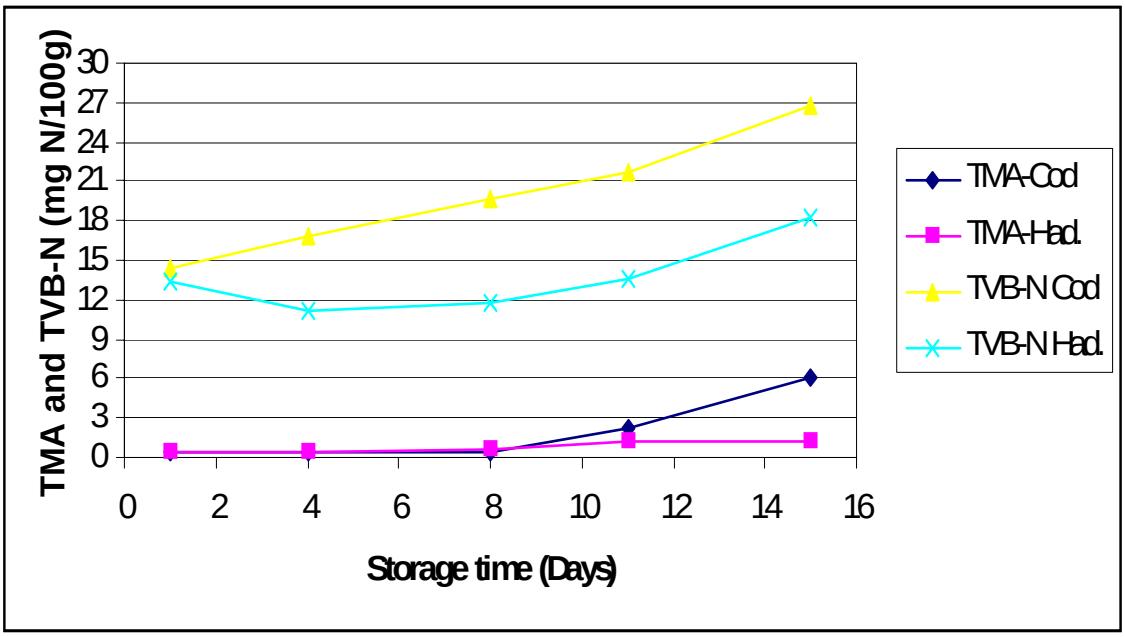

Figure 15. TMA and TVB-N content for cod and haddock stored on ice at around 0°C.

Other chemical indices measured were TMA and TVB-N content. TVB-N is mainly a composition of TMA and ammonia. The TMA and TVB-N values for cod and haddock in Figure 15 did not increase much during storage. In cod, there was an increase in TVB-N of approximately 13 mg N/100 g that was partly due to the formation of ammonia during early storage and to an increase in TMA (6 mg N/100g) during late storage. In haddock, there was no increase in TMA content but TVB-N increased and was therefore due to the production of ammonia.

Table 2. Composition of flesh and skin micro flora of haddock isolated on the last sampling day on Long and Hammer's medium after 4 days of isolation at 15°C.

Genera	**Flora Percentage (%)**	
	Flesh (n=19)	**Skin (n=20)**
Pseudomonas group I-II	-	40
Pseudomonas group III-IV	-	15
Aeromonas-like	-	15
Vibrio/ Photobacterium	32	-
Shewanella putrefaciens	-	20
Lost isolates	68	10

(The composition of the flesh and skin micro flora were also studied on the last sampling day on LH medium after 4 days of incubation at 15°C). The *Pseudomonas* groups are to those described by Shewan *et al*., (1960b) where groups I and II are glucose oxidisers and groups III and IV are not. Groups I and II are generally further differentiated by the ability of group I to fluoresce on certain media. The genera of bacteria found on haddock skin included *Pseudomonas* groups I to IV, *Shewanella putrefaciens*, and Aeromonas-like spp. On the other hand, the flesh micro flora were difficult to cultivate (68% of lost isolates) but some of them were found to belong to the *Vibrio*/*Photobacterium* group. *Pseudomonas* and *Vibrio* spp. are commonly found in soil, fresh water, seawater and ice (Castell & Mapplebeck, 1952).

Table 3. Values at the last sampling day for cod and haddock stored in ice at around 0°C

Fish	Shelf life[a] (days)	TVC (LH) (log /cm^2 or g)	H_2S-producers (log/ cm^2 or g)	*Pseud.* count (log /cm^2 or g)	TMA (mg N/100g)	TVB-N (mg N/100g)
Cod-skin	-	7.86	6.84 (9.5%)[b]	7.32 (28.8%)[b]	-	-
Flesh	13	5.55	3.11 (0.4%)[b]	3.41 (0.7%)[b]	6.15	26.7
Haddock skin	-	6.83	5.19 (2.3%)[b]	6.00 (14.8%)[b]	-	-
Flesh	12	4.88	2.42 (0.4%)[b]	2.35 (0.3%)[b]	1.24	18.3

a) based on cooked fillets
b) percentage of this group of bacteria based on overall TVC.

The TVC values for the skin of both fish were higher than the flesh of the two fish species by a difference of almost 2 logs. This difference is significant. The percentage of H_2S-producers shown in Table 3 on whole cod skin represented 9.5% of the skin microflora while *Pseudomonas* amounted to 28.8% of the spoilage micrflora. On the other hand, H_2S-producers accounted for only 0.4% of the spoilage microflora of cod flesh and *Pseudomonas* spp. for 0.7%. For haddock skin, H_2S-producers represented 2.3% of the microflora and *Pseudomonas* 14.8%, while on the flesh, these organisms formed 0.3% of the microflora and H_2S-producers consisted of 0.4%. The counts for H_2S-producers and *Pseudomonas* spp. were higher on the skin than the flesh by a difference ranging from 2.8 to 3.9 logs.

4 DISCUSSION

Many types of analyses have been developed and are used to measure the loss of fish freshness and to detect spoilage. Chemical, electrochemical, physical, microbiological and sensory methods have been used to evaluate indices of fish spoilage.

Total viable bacteria count (TVC) is the most common method for determination of the bacteriological quality of seafood. This measurement is seldom a good indicator of the sensory quality or expected shelf life of the seafood product (Huss *et al*., 1974), but it is taken as an indicator of the hygiene status of the product.

The initial average bacterial load on the skin of whole cod was log 3.00 per cm^2 and log 4.09 for haddock. These numbers are low and may indicate the initial quality of the fish was good. Long and Hammer´s (LH) medium generally showed higher counts than Iron Agar (IA) used by either spreading or pouring method. H_2S-producers were found in low levels (at or below detection) initially on the skin of the fish studied. Initially, *Pseudomonas* counts were lower on cod skin (log 1.5/cm^2) than haddock skin (log 2.5/cm^2). On the other hand, bacteria were not detected in cod and haddock flesh on IA and CFC media during the first 4 days of storage but were detected from the flesh of cod by LH medium. However, as storage in ice progressed, bacterial growth increased steadily on the skin and flesh of both cod and haddock. These results indicated that microbial invasion of fish occurs from the skin to the flesh. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during ice storage. Ruskol and Bendsen (1992) showed that bacteria could be detected by microscope in the flesh when the number of organisms on the skin surface increased above 10^6 cfu/cm^2.

The methods used to evaluate H_2S-producers on Iron agar (IAS and IAP) showed that higher counts were usually obtained when using the IAS method than the IAP. This can be explained by the fact that the application of the IAP method entailed pouring the warm agar. This may have killed or injured bacteria due to heat shock or made their growth on this medium rather difficult. Similarly, the TVC on IAS was higher on both the skin and flesh of both fish than TVC on IAP.

If *pseudomonas* groups I and II are compared the results (App I) on whole cod and haddock indicated higher counts for non-fluorescent group II *Pseudomonas* than the fluorescent group I *Pseudomonas.*

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 growth around 25°C. Psychrophiles (cold-loving) are bacteria with maximum growth temperature around 20°C and optimum temperature at 15°C (Morita, 1975). The micro flora on temperate water fish is dominated by psychotropic Gram-negative, rod-shaped bacteria belonging to the genera *Pseudomonas, Moraxella, Acinetobacter, Shewanella* and *Flavobacterium.* Members of the Vibrionaceae (*Vibrio* and *Photobacterium*) and Aeromonadaceae (*Aeromonas* spp.) are also common aquatic bacteria and typical of fish flora. In this trial, the bacteria found on the flesh and skin have been presumptively identified to be *Pseudomonas* groups I to IV, *Aeromonas*-like spp., *Vibrio*/ *Photobacterium* spp. and *Shewanella putrefaciens.*

The Quality Index Method (QIM) is a sensory method that was developed by the Tasmanian Food Research Unit (Bremner, 1985) to evaluate the freshness of fish. The method is fast, simple, sensitive and objective, but it relies on human judgement and proper training of panels (Sims *et al*., 1992; Strachan and Nicholson, 1992). Sometimes sensory tests are also

perceived to be inherently subjective (Krzymien and Elias, 1990). The QIM is a non-destructive method evaluating characteristic features relating to skin, gills, stiffness, smell, eyes and belly cavity of the whole raw fish using a score system. The scores for all sensory attributes are added to give an overall sensory score known as the quality index. A linear correlation has been found between the sensory quality expressed as a demerit score and storage life on ice, and this makes it possible to predict the remaining shelf life on ice (Nielsen, 1995).

The decrease in sensory quality of whole cod and haddock stored at around $0^{\circ}C$ for 14-15 days are shown in Figures 12 (a) and (b). As expected with storage time the sensory quality of cod and haddock passed the limit of acceptability. The shelf life that was determined from the graphs in Figures 12 (a) and (b) were 12 and 13 days for haddock and cod respectively, as determined by the cooked sensory assessment but 11 and 14 days by QIM. There is a good agreement between the Quality Index method and the Modified Torry scale. Therefore the good agreement between the two methods makes it possible to predict shelf life of fish using the Modified Torry scale just as it is possible with the Quality Index method.

Overall, the spoilage pattern for cod and haddock was very similar with the first weak off-odours detectable at the gills. The final spoilage was characterised by sour, rotten, sulphide off-odours and a very soft texture.

The chemical indices (TMA, TVB-N and pH of the flesh) measured during the trial did not provide valuable information as spoilage indices (Figures 13 and 14). PH was not expected to be a valuable indicator as it is expected to decrease initially during storage in ice and later to increase as non-protein nitrogen and other volatile amines are produced as a consequent of bacterial activity. According to Huss (1996), in cod, the *post mortem* pH drops following *rigor mortis* from 6.8 to 6.1-6.5. In this trial, the pH of both cod and haddock was below 6.8 initially following *rigor mortis* but increased during storage due to the formation of amines and reached about 7.0. Love (1975) indicated that loss of water in fish has a detrimental effect on the texture of fish muscle and that there is an inverse relationship between muscle toughness and pH.

The TMA content for both cod and haddock did not increase to levels usually seen in spoiling fish (10-15 mg N/100 g of flesh) (Magnusson and Martinsdottir, 1995). The H_2S-producers and *Pseudomonas* spp. are also responsible for causing fish spoilage (Gram, 1989a). The percentage of H_2S-producers and *Pseudomonas* spp. in the flesh of cod and haddock was too low at the time the fish were sensorial rejected. The low amount of TMA observed in this investigation was due to the low counts of H_2S-producers on the flesh reword of both cod and haddock since TMA is mostly a microbial metabolite.

5 CONCLUSION

The two fish species studied in this trial were of the highest quality and microbiological examination of the fish leads to the conclusion that Long and Hammer´s (LH) medium incubated at 15°C generally gave higher TVC for both cod and haddock. On the other hand, Iron agar spread-plated (IAS) was preferred to Iron agar pour-plating with overlay (IAP) since it gave higher TVC and furthermore, it often gave higher counts of H_2S-producing organisms. *Pseudomonas* group I-II were found to be the dominant micro flora (40%) on haddock skin stored in ice at around 0°C while *Shewanella putrefactions* comprised 20%. However, on the flesh of haddock *Vibrio/Photobacterium* (32%) was the only group of bacteria that could be identified as many isolates (68%) were difficult to cultivate.

Chemical indices, mainly TVB-N and TMA, were not found to be good indicators of spoilage as noticeable increases of these amounts were not seen in either temperate fish species studied. There seems to be a good agreement between the Quality Index Method (QIM) and the modified Torry Scale in evaluating the shelf life of fish stored in ice. Either method can be used by the seafood industry but for convenience it is recommended for the industry to use the QIM method.

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APPENDIX I: Bacteriological counts for cod and haddock stored in ice.

Average log counts on cod skin.							Fluorescent	Non-fluorescent
Days	LH	TVC-IAs	H_2S-IAs	TVC-IAp	H2S-IAp	CFC	*Pseudomonas* I	*Pseudomonas* II
1	3.00	2.72	0	2.78	0.89	1.54	0.65	1.45
4	4.01	3.54	1.60	3.69	0.00	2.98	2.65	2.75
8	5.24	5.08	3.65	5.06	3.78	4.03	3.07	3.98
11	6.76	6.56	5.55	6.60	5.59	5.74	4.45	5.72
15	7.86	7.64	6.84	7.62	6.77	7.35	6.15	7.32

Average log counts On cod Flesh.							Fluorescent	Non-fluorescent	
Days	LH	TVC-Ias	H2S-IAs	TVC-IAp	H2S-IAp	CFC	*Pseudomonas* I	*Pseudomonas* II	pH
1	1.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.66
4	2.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.76
8	3.38	3.25	2.34	3.20	1.85	1.80	0.00	1.80	6.92
11	4.96	4.31	N/A	4.46	3.06	2.39	1.15	2.36	6.91
15	5.55	5.55	2.89	4.64	3.11	3.42	1.00	3.41	6.96

NA: not available

Average log counts on haddock skin							Fluorescent	Non-fluorescent
Days	LH	TVC-IAs	H2S-IAs	TVC-IAp	H2S-IAp	CFC	*Pseudomonas* I	*Pseudomonas* II
2	4.09	3.42	0.80	3.67	0.00	2.48	1.75	2.33
4	4.66	4.45	2.54	4.34	2.16	3.60	2.21	3.59
8	6.19	5.98	3.30	6.03	3.44	5.53	3.80	5.47
11	7.37	7.34	5.59	7.22	4.45	7.07	5.45	7.06
14	6.83	6.60	5.19	6.09	4.55	6.18	5.45	6.00

Average log counts on haddock flesh							Fluorescent	Non-fluorescent	
Day	LH	TVC-Ias	H2S-IAs	TVC-Iap	H2S-IAp	CFC	*Pseudomonas* I	*Pseudomnas* II	pH
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.81
4	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	6.85
8	2.96	2.89	1.15	2.21	0.50	1.45	0.00	1.45	6.67
11	4.41	4.34	2.04	3.59	2.00	3.58	2.39	3.54	7.04
14	4.88	4.91	2.42	4.14	2.39	2.57	2.15	2.35	6.73

Results of TMA and TVB-N for cod fillets.

Results of TMA and TVB-N for haddock fillets.

Sample Days	Sample number	TMA (mg N/100g)	TVB-N (mg N/100g)
1	176	<0.5	13.3
1	178	<0.5	15.5
4	179	<0.5	17.8
4	180	<0.5	16.0
8	181	<0.5	20.4
8	182	<0.5	19.0
11	183	2.0	20.4
11	185	2.3	23.0
14	187	2.3	25.3
14	188	10.0	28.0

Sample Days	Sample number	TMA (mg N/100g)	TVB-N (mg N/100g)
2	130	0.42	12.94
2	131	0.37	13.68
4	132	0.40	10.38
4	133	0.52	11.83
8	134	0.48	11.46
8	135	0.57	12.20
11	136	1.03	13.49
11	137	1.26	13.68
14	138	0.94	15.30
14	139	1.54	21.29

Sensory evaluation QIM-cod

Days	1	4	8	11	15
	1.9	4.4	9.2	14.1	14.2
	3.5	5.8	9.5	12.2	16.2
	3.3	4.3	9.7	12.9	15.8
	2.9	4.4	11	12.2	15.9
	3.3	7.1	9.6	13.1	16.5
Average score	**2.99**	**5.2**	**9.8**	**12.9**	**15.7**

Sensory evaluation QIM-haddock

Days	2	4	8	11	14
	2.3	6.3	12.1	15	17.4
	2.5	5.5	12.2	15.9	18
	2.6	5.8	11.0	15.3	17.7
	2.5	6.8	10.7	14.5	17
	2.8	6.0	9.8	15.8	18.9
Average score	**2.5**	**6.1**	**11.2**	**15.3**	**17.8**

Sensory evaluation on cod fillets using the modified Torry scale.

Sample days	Min. score	Max. score	Average score
1	6.0	10.0	**9.26**
4	7.0	10.0	**8.58**
8	4.5	9.5	**6.82**
11	3.5	9.0	**6.18**
15	3.0	8.0	**4.70**

Sensory evaluation on haddock fillets using the modified Torry scale.

Sample days	Min. score	Max. score	Average score
1	6.0	10.0	**8.65**
4	6.5	10.0	**8.00**
8	5.0	9.0	**7.46**
11	3.5	9.0	**6.06**
14	3.0	6.0	**3.86**

APPENDIX II: Sensory assessment schemes for whole cod and haddock (QIM).

Attribute		Description	Score
Appearance	Skin	Bright, iridescent pigmentation	**0**
		Rather dull, becoming discoloured	**1**
		Dull	**2**
	Stiffness	In rigor	**0**
		Firm, elastic	**1**
		Soft,	**2**
		Very soft	**3**
Eyes	Cornea	Clear	**0**
		Opalescent	**1**
		Milky	**2**
	Form	Convex	**0**
		Flat, slightly sunken	**1**
		Sunken, concave	**2**
	Pupil	Black	**0**
		Opaque	**1**
		Grey	**2**
Gills	Colour	Bright	**0**
		Less coloured, some discoloured	**1**
		Discoloured, brown spots	**2**
		Brown, discoloured	**3**
	Smell	Fresh. seaweedy, metallic	**0**
		Neutral, grassy, musty	**1**
		Yeast, bread, beer, sour milk	**2**
		Sulphuric, very sour	**3**
	Mucus	Clear	**0**
		Milky	**1**
		Milky, dark, opaque	**2**
Blood in belly cavity	Colour	Red	**0**
		Dark red	**1**
		Brown	**2**
Quality index			**0-21**

APPENDIX II: Sensory assessment schemes for cooked cod and haddock fillets (modified Torry method).

QUALITY SCORE	ODOUR	**FLAVOUR**
10	Initially weak odour of sweet, boiled milk, starchy followed by strengthening of these odours.	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop.
9	Shellfish, seaweed, boiled meat.	Sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity.
7	Wood shavings, wood sap, vanilla	Neutral.
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours, boiled clothes-like	Slight sourness, trace of off-flavours
4	Lactic acid, sour milk TMA	Slight bitterness, sour, off-flavours, TMA
3	Lower fatty acids (e.g. acetic or butyric acids) composed grass, soapy, turnip, tallow	Strong bitter, rubber, slight sulphide

APPENDIX III: Presumptive identification scheme for Gram-negative bacteria.

Oxidase +, fermentative bacteria:

0/129 sensitivity (150 ug)	-	*Aeromonas* spp.
	+	*Vibrio, Photobacterium* or *Plesiomonas* spp.

Oxidase +, oxidative bacteria:

Motility	+	TMA	-	Fluorescein	+	*Pseudomonas* group I spp.
	+	TMA	-	Fluorescein	-	*Pseudomonas* group II

Oxidase +, non-oxidative bacteria:

Motility	+	TMA	+	*Shewanella puterfaciens* (H2S +)
	+	TMA	-	*Pseudomonas* group III spp.(no change in O/F medium, can be oxidase-)
	+	TMA	-	*Pseudomonas* group IV spp.(alcaline in O/Fmedium, can be oxidase -)