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FISHERIES TRAINING PROGRAMME

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Shelf life of herring (*Clupea harengus*) kept at different temperatures

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ABSTRACT

The objective of this project was to learn about the main types of evaluation of quality of fish, and quality changes during different storage condition. Atlantic herring (*Clupea harengues)* was chosen as a similar fish species to mackerel, the important fish species in Cape Verde. Herring was stored in ice and kept for 9 days, at +1, 5ºC and -1, 5ºC. Quality changes during chilled and superchilled storage was evaluated by sensory analyses using Quality Index Method (QIM) and Torry score, by microbiological analyses; total viable counts (TVC), hydrogen sulphide (H_2S) producing bacteria and by chemical analyses of total volatile bases (TVB) and trimethylamine (TMA). The modified QIM scheme for herring had higher correlation (R^2=0,914) than published QIM schemes with correlation of R^2 =0,740. Storage time could be predicted to approximately 8 days in ice. During the chilled and superchilled storage of herring very low number of microorganisms was detected. On day 9 TVC and H_2S – producing bacteria values were log 4.3 and log 4.1 (+1,5^{o}C) and log 5 and log 4.4 (-1,5^{o}C) respectively. Low levels of TVB-N and TMA were found during the storage time. The reason for these unexpected results was probably due to temperature fluctuation. There was no significant difference between the two storage groups.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Cape Verde (CV) is a small archipelago where the fish and fish products have a considerable importance for national economy, employment and foreign exchange. Access to global markets requires not only meeting changing consumer demands but also having the ability to sell at competitive prices. Fish from Cape Verde is largely targeted for overseas markets such as the European Union. International trade in fish and fisheries products has been increasingly brought under the WTO agreements. This has resulted in increased trade liberalization and competition in the global market. In view of the increased demand for foreign exchange and changes in the global fish market, the government has plans to expand exports of fish and fisheries products to generate economic growth. Under the EU regulations, standards and procedures applicable to fish products originating within the EU also apply to fish products entering the EU. All fish and fisheries products imported from third countries must come from processing facilities which are approved by a competent authority in the exporting country (91/493/EEC).

Currently in Cape Verde, there is only one competent laboratory. The CV government has plans to establish the second laboratory in Sao Vicente Island at the National Institute for Fishery Development (INDP). Sao Vicente already has a major fish port, and is increasingly servicing a large number of fishing vessels .The airport is currently being upgraded for international flights, therefore opening the possibility of export of fresh fish. Hence, the government aims to increase exports of fresh fish from S. Vicente islands as a way to increase exports from Cape Verde. The success of any effort in food industry including fisheries depends on one single factor; getting the product to the consumer in an acceptable condition. Consequently, efforts are required to maintain quality and acceptability of the fish and fisheries products to consumers. Most fish processing companies are located on the windward group of Cape Verde islands (as Sao Vicente), the INDP realizes that it would greatly enhance exports if the companies have easy access to a high quality fish laboratory facility. Plans are already underway for the establishment of a second laboratory in Cape Verde. However, there is a need for skilled personnel and technicians to be able to carry out necessary activities. This project focus on testing and evaluating the primary methods to measure freshness of fish at various temperature levels to determine their relative storage time. Herring was chosen as a sample to be studied, because it is fat pelagic fish species like mackerel, the most popular species in Cape Verde.

The main objective of this study is to:

- Gain experience in applying microbiological, chemical and sensory methods in evaluating the quality and shelf life of herring kept at superchilled (-1.5°C) and chilled (+ 1.5°C) temperature.

- Estimate storage time of the product using QIM scheme, Torry scheme and measurement of total viable count (TVC), H2S – producing bacteria, total volatile bases (TVB-N) and trimethylamine (TMA).

2 LITERATURE REVIEW

2.1 Importance of quality

Fresh fish is an extremely perishable food and deteriorates very rapidly at normal temperatures. For all of types of fish and fishery products freshness makes a major contribution to the quality of the products (Olafsdottir *et al*. 1997a). From the moment the fish is caught the deterioration process starts and quality of the fish is affected. Changes occur in composition and structure because of chemical, physical, enzymatic and bacterial influence. Another cause of spoilage may be rancidity, especially in fat fish species as herring, tuna, and mackerel. Spoilage is a natural process once the fish dies, but chilling can slow down this process and prolong the shelf life of fish as food (Graham *et al.* 1992). There are three important ways of preserving the freshness of fish: cooling (as previously explained), hygienic practice and good handling (Graham *et al.* 1992).

2.2 Spoilage and shelf life of fish

Spoilage begins as soon as the fish dies. The stress and mechanical damage caused during capture, the structure and composition of the fish, pH and storage temperature prior to landing all influence the spoilage rate of the fish (Church 1998). Fish, like red meat, spoils because of the combined effects of enzymatic activity and bacterial growth (Church 1998). These factors all influence the sensory quality of fish during storage time. The factors contributing to the spoilage are degradation of protein, development of oxidative rancidity and microbiology activities. Lipid oxidation represents a major loss of quality during refrigerated and frozen storage of fish (Colby, 1993). The enzymes remain active after the fish dies, and cause self-digestion, especially in small fatty fish. In fatty fish, such as herring (Figure 1), the most important changes occur in the lipid fraction where oxidative processes are purely of chemical nature. These changes may give rise to serious quality problems such as rancid flavours and odours as well as discoloration. Two types of rancidity are found, auto-oxidation and lipid autolysis. Auto oxidation is a reaction involving oxygen and unsaturated lipid which is accelerated by heat and light (especially UV). Lipid autolysis is an enzymatic hydrolysis with free fatty acid and glycerol as major products (Huss 1988). Bacteria are present on the gills, in the surface slime, and in the intestines of live, healthy fish, but are precluded from invading the sterile flesh of the fish by the animal's normal defences (Bonnell 1994). The release of the fatty acids and breakdown of sulphur-containing ammonium acids to methyl mercantan, dimethlsulphide and hydrogen sulphide due to bacterial action contributes to the characteristic smell of spoiled fish (Gram and Huss 1996).

The most important fish spoilage bacteria are characterized by the ability to produce H_2S and reduce trimethylamine oxide (TMAO), and these abilities have been used in the development of indicative agar media and specific chemical and physical assays (Huss e*t al.* 1992). The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colours (discoloration), odours (off odour), texture and flavour (off flavour).

A wide range of terms are used in discussing shelf life, such as quality, acceptability, preference, keeping time, storage time, storage life and potential shelf life; these terms are not interpreted uniformly. The simplest definition of the end of shelf life, or storage life, is the point at which the product is considered spoilt (Graham 1992). The shelf life of food is defined as maximum length of time a given product is fit for human consumption. For fish, it is the time from when the fish is caught until it no longer is fit to eat (Huss 1995). Estimated storage time in ice is the number of days that the fish has been stored in ice. From these results a

prediction can be calculated for the remaining shelf life. Various factors can affect the remaining shelf life. It depends on the handling of fresh fish, rapid cooling after the catch and uninterrupted cold storage. An estimated shelf life for most species is from 13-18 days in ice but may be shorter for herring (eight days)and for shrimp (six days), but longer for salmon (20 days) (Martinsdottir *et al.* 2001)

2.3 Herring

Atlantic herring (*Clupea harengus*) (Figure 1) is a pelagic fish, 23-36 cm long, weighs approximately 100-400 g. The weight for a given length can vary considerably from season to season and from year to year (Stroud 2001). Herring is a highly perishable fish, therefore rapid cooling and careful handling are very important to keep it fresh for human consumption. Principal spoilage factor is deterioration, which can be enzymatic or bacterial and one major quality defect is belly bursting which occurs mainly in feeding herring due to high enzymatic activity (Nielsen and Hulding 2004). Herring is susceptible to lipid oxidation due to its high fat content and high level of polyunsaturated fatty acids. Lipid oxidation results in formation of secondary volatile oxidation compounds (aldehydes, ketones, alcohols etc.), that will give rise to rancid off-flavour formation (Jacobsen *et al*. 2003).

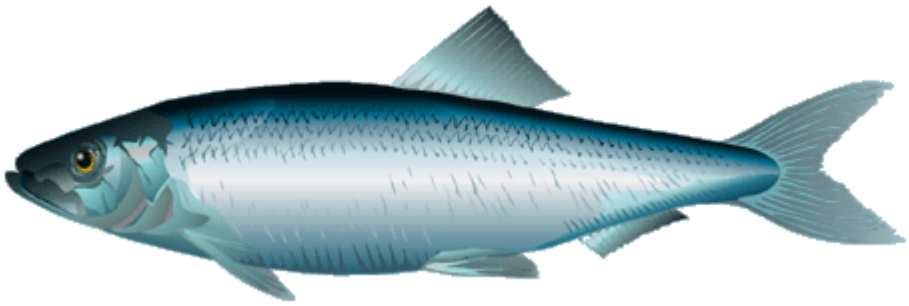

Figure 1: Atlantic Herring *(Clupea harengus*) used in this project

In most cases, small- and medium-size fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is partly that a large number of small fish are caught at the same time and partly because of problems with discoloration and the acceleration of rancidity (Huss 1995). It is often chilled or frozen whole soon after capture. It is traditionally chilled on board with ice. Storage time depends on the fat content of the fish and the amount of food in the gut. Shelf life in ice for fat (summer herring) and low fat (winter herring) fish is 2-6 days and 7-12 days, respectively (Huss 1995).

2.3.1 Chemical composition

The chemical composition of herring depends of the season and the spawning time. The fat content of herring may be less than 1% (right after spawning), or more than 20% (before spawning season). Table1 below shows the water, fat and protein content of herring.

Table 1: Chemical composition of herring (Stroud G.D. 2001).

	Water %	**Fat %**	**Protein %**
Whole herring	60-81	1-24	17-21
Herring flesh	57-79	0.8-24.9	14-17

2.4 Methods to evaluate fish freshness

Freshness is the most important attribute when assessing the quality of fish or fishery products. Most of the methods that have been used to estimate the quality of fresh fish measure or evaluate parameters that change, disappear or are formed during deterioration of fish. These methods may be divided into several groups such as sensory, microbiological and chemical methods.

2.4.1 Sensory evaluation

"Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing" (Huss 1995 p.130). Sensory evaluation is an important method for the assessment of freshness and quality and is commonly used in the fish sector and fish inspection services (Luten and Martinsdottir 1997). Sensory evaluation is used as tool for: 1) grading according to product standards, 2) studying specific properties of the fish species in connection with evaluation of quality, shelf life, storage condition and product development (Olafsdottir *et al*.1997b). In sensory evaluation a panel performs the sensory analysis. To get good results with sensory evaluation, panellist must be trained and have clear and descriptive guidelines. The Quality Index Method (QIM) is a promising method to measure the freshness of whole fish stored in ice, and is both rapid and reliable (Martinsdottir *et al*. 2001). To evaluate sensory attributes of cooked fish, the Torry scheme is often used (Martinsdottir *et al.* 2001) and (Huss 1995).

Quality Index Method (QIM)

Quality Index Method (QIM) is a seafood freshness quality control system it is promising method in assessing the freshness of many fish species in a rapid and reliable way (QIM Eurofish 2004). The method originally developed by the Tasmanian Food Research unit (Bremner *et al.* 1985) is now used by the Lyngby Laboratory (Jonsdottir, 1992) for fresh and frozen cod, herring and saithe (Huss 1995). QIM is based on well-defined characteristic changes of appearance attributes that occur in raw fish such as eyes, skin, gills and changes that occur in odour and texture with storage time. A score from 0 to 3 demerit (index) points is given for each quality parameter according to the specific parameter descriptions. The scores are summarized to give an overall sensory score referred to as the Quality Index (QI). QIM gives scores of zero for very fresh fish and an increasingly larger total result as the fish deteriorates. QIM-schemes for several fish species including herring have been published (Martinsdottir *et al*. 2001).

Torry Scheme

For sensory evaluation of fish fillets, it is common to cook the fillets and evaluate their odour and flavour. The Torry scale is the most-used scale for evaluate the freshness of cooked fish (Martinsdottir 1997).The Torry-scale is a descriptive 10-point scale developed at the Torry Research Station. This scale has been developed for lean, medium fat and fat fish species. Scores are given from 10 (very fresh in taste and odour) to 3 (spoiled). It is considered unnecessary to have descriptions below 3, as the fish is then no longer fit for human consumption (Martinsdottir *et al.* 2001). The maximum storage time of fish can be determined by sensory evaluation of cooked samples. The average score of 5.5 has been used for most fish species as the limit for consumption (Martinsdottir *et. al*. 2001). Then the members of the sensory panel detect evident spoilage characteristics, such as sour taste and hints of "off" flavour (Martinsdottir *et al*. 2001).

2.4.2 Microbiological methods

Microbiological examination of fish aims at evaluating hygienic quality of fish, including temperature abuse, and the possible presence of pathogenic microorganisms in the fish (Huss 1995).The activity of microorganisms is the main factor limiting the shelf life of raw fish. Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms varies enormously and states a normal range of 10^2-10^7 cfu/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 -10^9 cfu/cm^2. (Huss 1995). When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibres.

An estimation of the total viable counts (TVC) is usually used as an acceptability index in standards, guidelines and specifications (Ólafsdóttir *et al*. 1997a). The total count represents, if carried out by traditional methods, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. This figure is seldom a good indicator of the sensory quality or expected shelf life of the product (Huss *et al*. 1974; Huss 1995). While early studies of seafood microbiology acknowledged that only part of the spoilage microflora participated in the spoilage process (Huss 1974), the recent establishment of the specific spoilage organism (SSO) concept (Dalgaard 1995) has contributed significantly to our understanding of seafood spoilage. *Shewanella purifaciens* has been identified as the main specific spoilage bacteria of marine temperate-water fish stored aerobically in ice and the number of *S. purifaciens* is inversely linearly related to remaining shelf-life of iced cod (Gram and Huss 1996) These species produce H_2S and TMA.

2.4.3 Chemical analysis

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 (Huss 1995). A number of spoilage indicators have been used, including total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and formation of biogenic amine , whereas nucleotide degradation product ratios (such as hypoxanthine, K, Ki values) have been used as freshness indicators (Dalgaard 2000). The K or "freshness" index gives a relative freshness rating based primarily on the autolytic changes which take place during post mortem storage of the muscle. Thus, the higher the K value, the lower the freshness level. Unfortunately, some fish species such as Atlantic cod reach a maximum K value well in advance of the shelf life as determined by trained judges, and K is therefore not considered reliable as a freshness index for all marine finfish (Huss 1994).

Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) is one of the most widely used measurement of seafood quality. It is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB-N analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of cod as well as several other species (Huss 1995). According to Icelandic regulation number 233 from 1999, about "Hygiene and handling on fish during processing and distribution" which is based on EC directives number 91/493, gives us maximum TVB-N (Total volatile bases content) in various fish species. If over this limits the fish is not fit for human consumption (EU. Directive 91/493/EEC).

Table 2: Limits for TVB-N for some fish species

Types of fish	Maximum TVB-N value in mg/100g
Red fish	25
Flat fish	30
Cod and related species	35
Salmon…	35

Trimethylamine (TMA)

Trimethylamine oxide is generally present in seawater fish. TMA is formed in spoiling fish from TMAO by bacterial reduction during iced storage (Magnusson and Martinsdottir 1995). The reduced component, TMA, which is one of the dominant components of spoiling fish, has a typical fishy odour. However, gram-negative bacteria such as *Shewanella putrifaciens* can obtain energy from TMAO by reducing it to TMA. Endogenous enzymes present in fish can also reduce TMAO to DMA (dimethylamine) and FA (formaldehyde). While TMAO is non-odorous, TMA is a component in the odour of stale fish. The levels of TMA in fish has for sometime been used as an indicator of microbial deterioration of fish. Fish with less than 1.5 mg TMA-N/100g is considered of good quality while 10-15 mg TMA-N/100g is considered the limit of acceptability (Huss 1988).

Histamine

Scrombroid fish (tuna, albacore) contain characteristically high levels of free histidine in their muscle tissue (Taylor et al., 1989), which can be converted to histamine by the action of bacterial decarboxylase. Bacteria known to be capable of decarboxylating histidine include *Vibrio*, *Proteus morgani*, and *Klebsiella pneumoniae*, all having minimum growth temperatures

between 8-15°C. The presence of histamine in fish is insignificant in its contribution to the sensory acceptability of fish; however, its presence could appreciably compromise its safety when consumed. For analyzing quantity of histamine in fish, we need to take 9 samples of each lot to fulfil the demands, which are specified in the same regulation as referred to before (Icelandic regulation 1999 number 233; Cape Verde regulation 11/2001; 91/493/EEC) .

1. Average value should not be higher at 100ppm
2. Of the samples collected from each lot, two of them may have histamine value between 100-200ppm.
3. None on the samples may be over 200ppm.

Histamine poisoning is often referred to as scombrotoxin poisoning because of the frequent association of the illness with the consumption of spoiled scombroid fish (*Scombriedae*) such as tuna and mackerel. However, non–scombroid fish such as family *Clupidae* (herring) and *Engraunlidae* (anchovies) may also be involved (Huss *et al.* 2004).

3 MATERIAL AND METHODS

In this project modified Quality Index Method (QIM) scheme was used to evaluate freshness changes of herring with storage time as described in QIM Eurofish (2004), with exception of belly attribute having two demerit points instead of three demerit points. Sensory evaluation of cooked herring was done by Torry score (modified Torry score sheet for fat fish, - adapted to herring) to determine the end of shelf life. The sensory results were correlated with other methods, such as microbial and chemical analyses which are used as indicators of spoilage during shelf life study.

3.1 Experimental design

The herring (*Clupea harengus*) was caught off the coast of Westman Islands and after landing the herring was sampled randomly from the catch and packed in ice in polystyrene boxes and transported to the institute. After two days from capture 70 fresh/iced ungutted herring arrived to IFL. Each fish was weighing between 150-190 g. The fish was divided equally into two groups and each group was iced and kept in the cooling room at two different and controlled temperatures of -1.5°C and +1.5°C. In both groups herring was arranged in layers not to be in contact. Sampling was done every 2 or 3 days; on day two (same temperature), fifth, seventh and ninth storage day after the capture. After each evaluation, ice was added in the boxes containing herring to keep it in good condition.

Temperature data logger was used during the storage period of chilled storage of iced herring (Stow away®, Onset Computer corporation (USA)), to monitor the temperature of the ambient storage environment. One logger was located at the top of polystyrene box in cooling room to follow the environment variations of storage condition. Temperature recordings were at 3 minutes intervals. Temperature data logger was not set in chamber with -1.5^{o}C.

3.1.1 Quality Index Method (QIM)

Sensory evaluation of whole raw herring was done in the sensory laboratory by QIM. The fish was placed on the white clean table at room temperature under white fluorescent light and each fish was coded with a number consisting of two digits. For this evaluation (four sessions) five herring from each storage temperature were evaluated. The 6-9 trained panellists from IFL assessed changes in appearance, skin, colour, odour and texture of fish. All panellists evaluated all the herrings by moving from one fish to the other, and registered their evaluation for each quality parameter in a QIM scheme for herring (Jonsdottir 1992, Martinsdottir 2001) which was slightly modified before being used in this experiment (Table 3). The quality index (QI) was calculated as a sum of the score for all evaluated parameters. The average score of each panellist for each fish was calculated.

Table 3: Modified QIM scheme for herring –Modified 2006

Quality parameter		Description	Score
Appearance	**Skin**	**Very shiny**	**0**
		Shiny	**1**
		Mat	**2**
	Blood on gillcover	**None**	**0**
		Very little (10-30%)	**1**
		Some (30-50%)	**2**
		Much (50-100%)	**3**
	Loin consistency	**In rigor**	**0**
		Firm	**1**
		Soft	**2**
		Very soft	**3**
	Belly	**Firm**	**0**
		Soft and/or burst	**1**
	Odour	**Fresh sea odour**	**0**
		Neutral	**1**
		Not fresh, mushy, hint of rancid odour	**2**
		Spoilage odour, putrid, rancid	**3**
Eyes	**Brightness**	**Bright**	**0**
		Mat	**1**
	Shape	**Convex**	**0**
		Flat	**1**
		Sunken	**2**
Gills	**Colour***	**Characteristic red**	**0**
		Somewhat pale, mat, opaque	**1**
	Odour	**Fresh, seaweedy, metallic**	**0**
		Neutral	**1**
		Not fresh, mouldy	**2**
		Spoilage odour, putrid	**3**
Quality Index			**0-19**

*can be omitted in tank herring

3.1.2 Torry score

Sensory evaluation of the cooked herring by a modified Torry scheme for fat fish species (Table 3 below) was performed parallel to the QIM assessment. Each panellist evaluated samples from different storage temperature. Samples were collected from the loin part of 5 fish (from the same storage temperature) without skin. The samples were placed in aluminium boxes and cooked in a pre-warmed electric oven Convostar (Convotherm-German) by steam at 95-100^{o}C for seven minutes. Each sample was coded with three random digit numbers. The boxes were closed with plastic covers and then served to the panellist. Each sample from 2 different storage temperatures was evaluated by panellists in duplicate. Ten score of Torry scale was used as very good quality with description of sweet, metallic flavour as characteristic for the species, and the score three was the very bad quality with very rancid, bitter and putrid flavour. Score 5.5 was used as acceptable for human consumption, with trace of rancid flavour.

Table 4: Modified Torry score sheet for freshness evaluation of cooked herring

Odour	Flavour	Score
Boil cod liver, fat ,butter, characteristic for the species, boiled meat	**New cod liver, fatty, characteristic for the species, sweet, metallic**	**10**
Cod Liver, less meat odour	**Sweet, fat reminds of herring, metallic**	**9**
Faint odour of cod liver, meat	**Cod liver/fat, less characteristic flavour, meaty**	**8**
Loss of odour, neutral odour	**Loss of flavour, neutral**	**7**
Trace of mouldy odour, trace of rancid odour	**Insipid (towards "off-flavour"), trace of rancid, sour or bitter flavour**	**6**
Mouldy odour, rancid, hints of sour odour	**Slightly sour, trace of "off -flavour", rancid**	**5**
Rancid odour, acid, "old"	**Rancid flavour, slightly bitter, sour, spoiled fruit, "off-flavour"**	**4**
Very rancid, acid, sulphide, ammonia	**Very rancid, bitter, sour, putrid, rotten**	**3**

3.2 Microbiological analysis:

On each sampling day, three herring from each storage temperature were taken to the microbiological laboratory of IFL for microbial counts. The skin was disinfected with ethanol (70%) and the underlying muscle was minced. Sample of minced flesh weighing 25 g each, were placed in a stomacher bag containing 225 g a Butterfield's Buffer solution to obtain a 1/10 dilution. Blending was done in the stomacher for one minute. The following microbial counts, total plate count (TVC) and H_2S producing bacteria were done on Iron Agar (IA) as described by (Gram *et al.* 1987) with the exception that 1% NaCl was used instead of 0.5%. Surface plating was used and plates were incubated at 15^{o}C for 4-5 days. H_2S – producing bacteria form black colonies on the medium from sodium thiosulphate and/or cysteine.

3.3 Chemical analysis:

3.3.1 TVB-N and TMA method (steam distillation)

Steam distillation method was performed using a Kjeldahl type distillator to analyse TVB-N and TMA for the whole iced herring. Sample of 100 g minced herring was mixed with 200 ml of 75% aqueous trichloroacetic acid solution, homogenized by the blender for 1 min and filtered. Next, 25 ml of filtrate were transferred into a distillation flask and 6ml 10% NaOH was added. The distillate was collected into the beaker containing 10 ml 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator. The distillation was performed by a Kjeldahl-type distillatory (Struer TVN) for 4 minutes. The boric acid solution turned green when alkalinized by the distilled TVB-N which was titrated with aqueous 0.02820 N sulphuric acid solution ($H_2SO_{4)}$ using 0.05 ml graduated burette. Complete neutralization was obtained when the colour turned pink on the addition of a further drop of sulphuric acid. To determine TMA the same method was used as for TVB-N but 20 ml of formaldehyde was also added to the filtrate sample to the distillation flask to block primary and secondary amines. Calculation (mgN /100g):

$$\frac{14\ \text{mg/mol} \times a \times b \times 300}{25\text{ml}}$$

a: ml of sulphuric acid
b: Normality of sulphuric acid

3.4 Data analysis

Microsoft Excel 2003 was used to calculate means for some of the measurements (QIM, TVB-N, TMA,) and to plot graphs for all results against the storage time. The same was used to fit linear regression and correlation equation on QIM graph. T-test Paired two sample for means was used to see if there was any significant difference between means (QIM, Torry, TVB-N and TMA) of two storage groups on the same storage day, at significance level $p < 0.05$.

4 RESULTS

4.1 Temperature data logger

Temperature data logger was used during all period of chilled storage of iced herring (Stow away®, Onset Computer corporation (USA)), to monitor the ambient temperature. Through storage time some variations were confirmed in the temperature, mainly between the days 10 - 15. 12. 2006 of our experiments. On this interval the temperature which was supposed to be +1.5°C was often lower than 0°C and sometimes it was around -2.5°C at days 10, 12, 13, and 15.12.2006 (Figure 2) below. That period represented the storage life of the herring from day 4 to day 9. Because of that failure, results in present study were different from what we would have expected. The results from the higher temperature were better than results from the lower temperature.

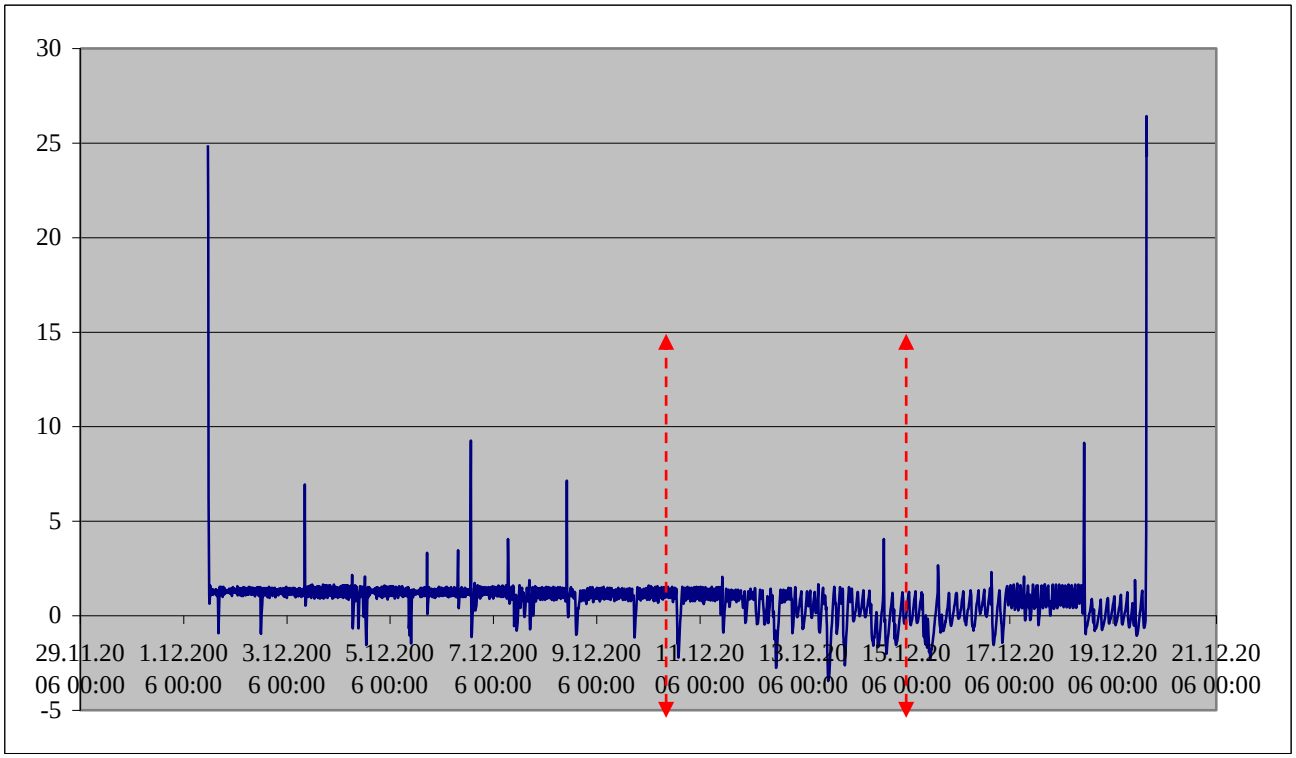

Figure 2: Temperature logger used in culling room with herring stored at +1.5°C shows temperature fluctuation during the storage time.

4.2 Sensory analysis

4.2.1 Sensory observation results

Changes were observed in the appearance of herring during second, fifth and seventh day of storage life (Figure 3). The changes on parameters skin, texture and blood on gillcover were clear on the pictures. With storage time loin consistency became softer, which signals were on the last picture. The blood on gillcover increased from day 2 to day 7 (figure 3). On three different storage days, skin of herring didn't change much from day 2 to day 7, appearance of skin presented shiny skin (Figure 3 below).

Day 2

Day 5

Day 7

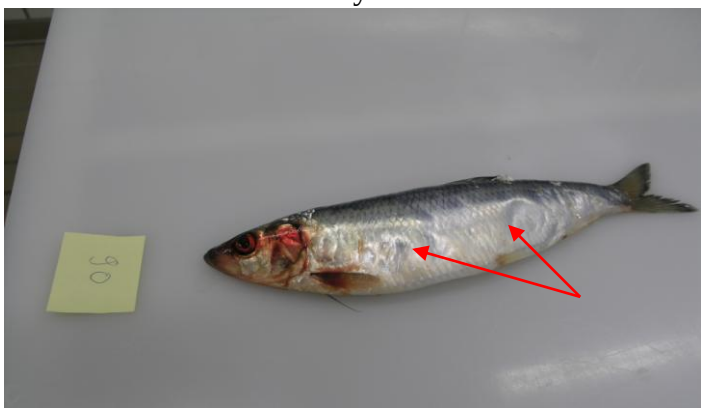

Figure 3: Herring used in this study at days 2, 5 and 7 of shelf life shows blood in gillcover, skin and texture changes

4.2.2 Quality Index Method

The Quality Index Method (QIM) developed for herring (Jonsdottir 1992 and Martinsdottir 2001 *et al*.) was slightly modified just when this experiment started, by IFL sensory experts, experienced in sensory evaluation of whole herring. One of the reason for modifying the QIM was due to the low correlation in the previous QIM manual (R^2 = 0,740) (Martinsdotir 2001 *et al*.). The sensory attribute modified was the belly score suggested by Nielsen and Huldig (2004) and Mai (UNU-FTP 2002), estimated by IFL experts on sensory evaluation of herring using the QIM scheme. On that quality parameter two description scores (soft and burst) was combined into one description score referred to soft and/or burst. QIM scheme for iced herring (Table 2) consists of nine parameters groups on three main categories, with total of 19 demerit points instead of 20 in previous QIM scheme (Appendix 3). Description of parameters related to odour and gills odour were modified to words that better described the same parameters. The Quality Index (QI) based on average of the whole panel was calculated for each storage day of the herring, and formed linear relationship with storage time (Figure 4). QI for both groups (+/-) had a high correlation between the average QI and storage days on ice, though the -1.5°C group had higher lineal correlation (R^2 = 0,914). Quality index was around 12 on the day 8 of storage which was the day the panel judged the herring unfit for human consumption by Torry score.

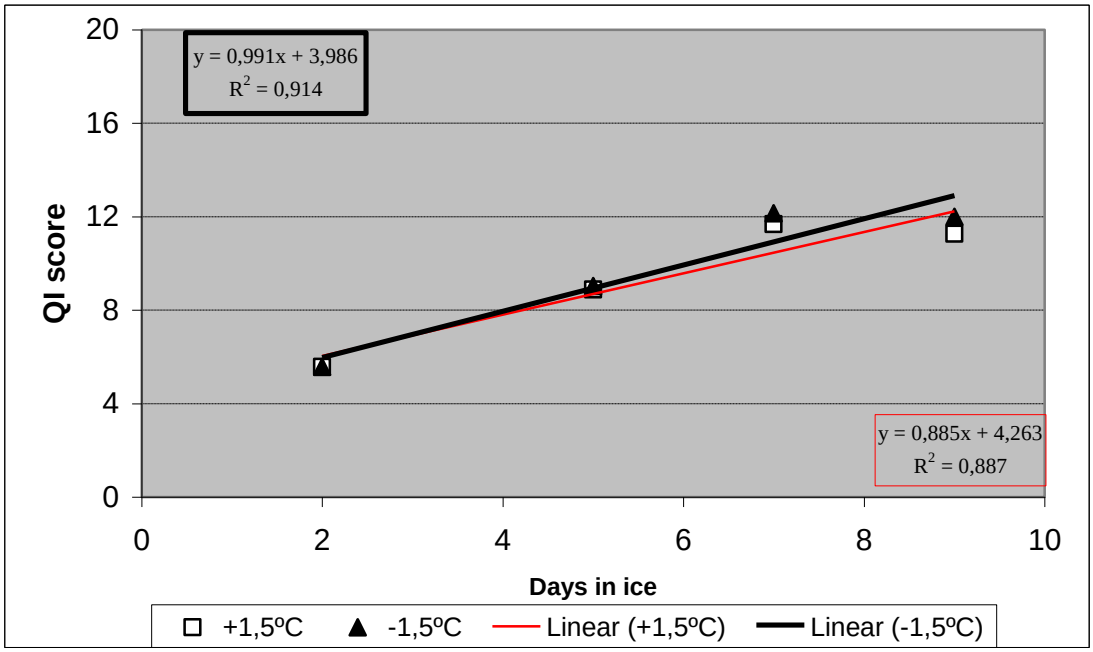

Figure 4: Average Quality Index (QI) for iced herring stored at different temperature (+1, 5ºC with R^2 = 0, 887 and -1, 5ºC with R^2 = 0, 914) during the storage time in ice.

The results on each quality attribute are presented as averages of all panellists. QIM assumes that score for all quality attributes increase during the storage time of iced herring, for both storage groups. The rate of increase was variable for different attributes, in some of them the increase was less pronounced than the other such as skin and loin consistence, and belly for positive temperature from day two to day five. For the rest of attribute the growth was more pronounced.

The changes on the skin appearance was very slow between day two and day five, increased more between days five to seven and decreased after that, in both storage conditions. The average skin score for both groups was around score 1, during storage life of herring. Blood on

gillcover, eyes brightness and gills odour increased considerably from day 2 to day 7 and than decreased or stagnated to the end of shelf life. Loin consistence, fish odour and gills colour, increased linearly during storage life of herring, but increase of loin consistence was lower than the other two parameters and average scores throughout the storage time were from 1-1,6.The changes on eyes form were insignificant with average scores being 0,9-1,2 from the beginning (see Figure 5).

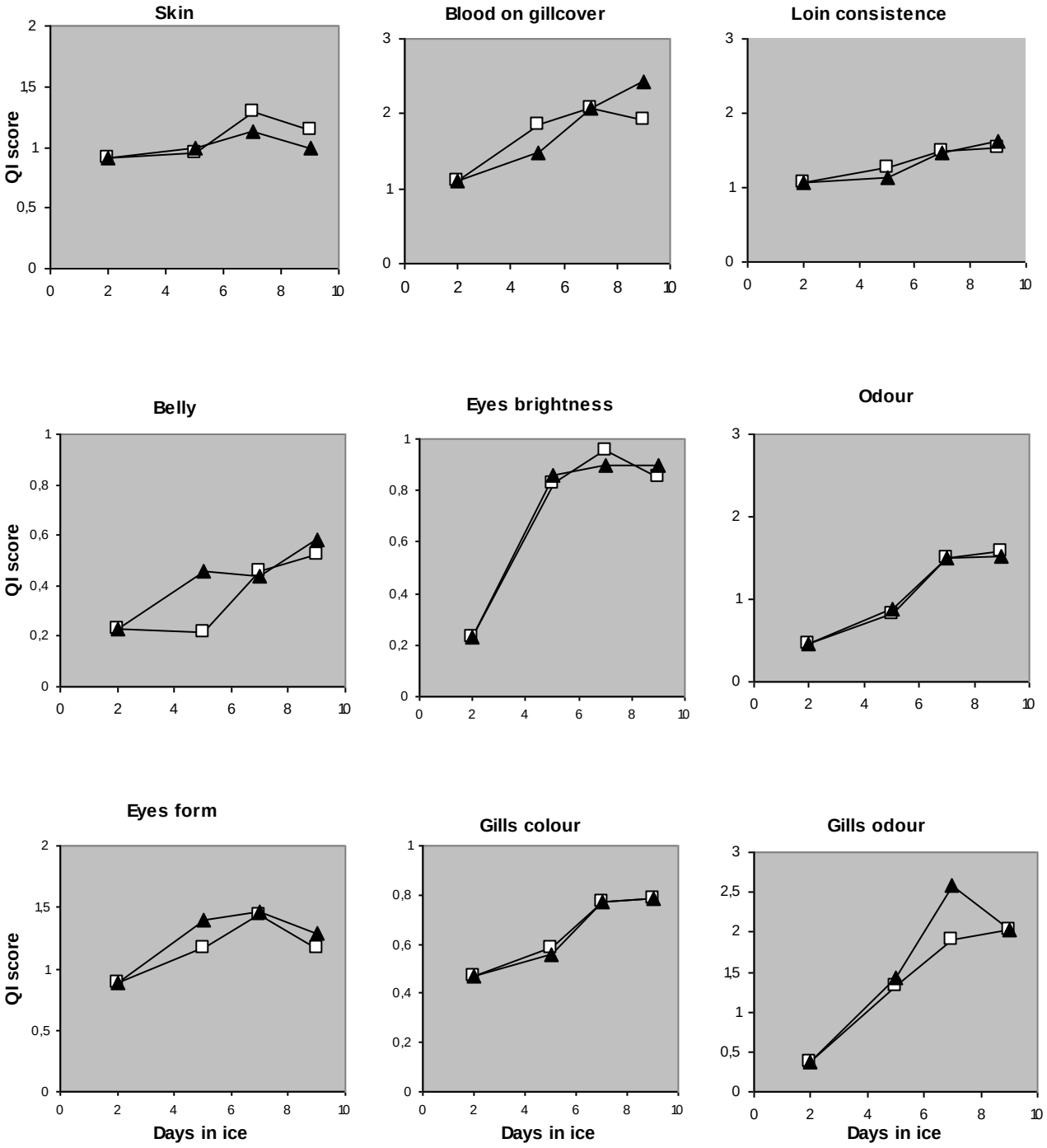

Figure 5: Average score for each quality attribute assessed with QIM scheme for iced herring stored at two different□mperatures ▲ +1, 5°C and -1, 5°C against days in ice.

The pictures below (Figure 6) show changes in eyes shape with storage time. There were no major changes in the eyes shape on the two different storage days evaluated.

Day 5 Day 7

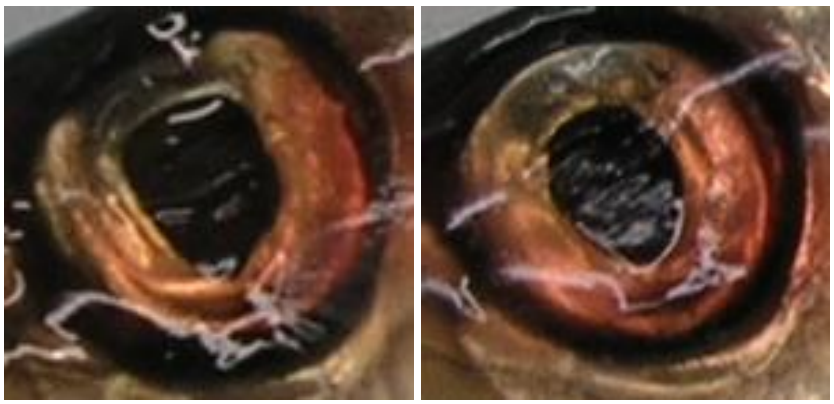

Figure 6: Picture of eyes shape of iced herring (stored in chilled condition) at the day 5 and 7 of shelf life

Figure 7 shows the gills colour on two different storage days of iced herring (day 5 and day 7). The gills colour as expected lost the characteristic red colour with storage time, as observed in Figure 7, on day 5 the red colour was more pronounced than on day 7 which was somewhat opaque.

Day 5 Day 7

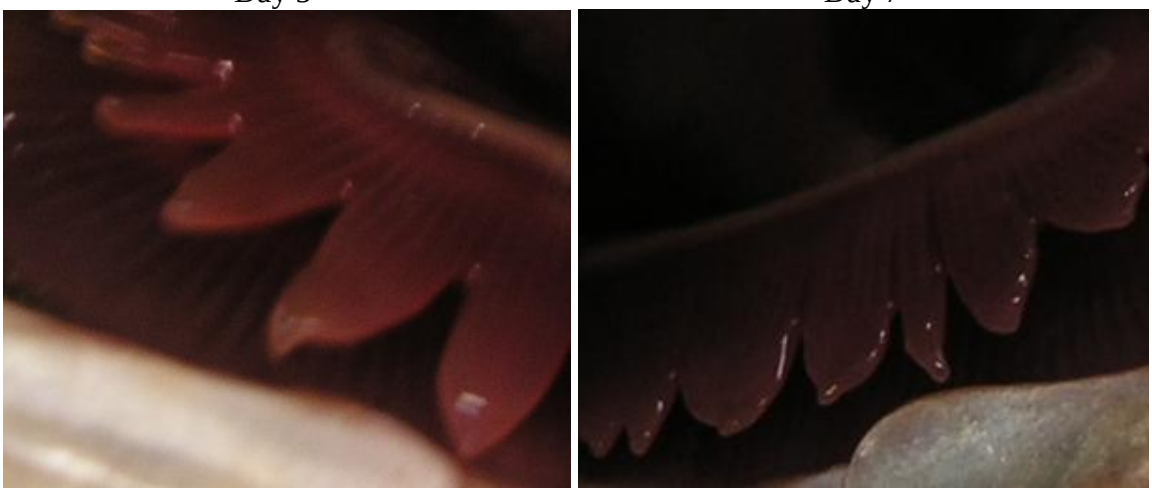

Figure 7: Changes on appearance of gills colour of iced herring (stored in chilled condition), on the day 5 and day 7 of storage time.

4.2.3 Torry score

Torry scheme used in this study was slightly modified based on prior experience working on herring projects at IFL. The sensory characteristics of herring did not fit well with the Torry score sheet for fat fish species and thus needed modification. The Torry score was used to evaluate quality of freshness and storage time of herring. The Torry scores ranges from 10 (very fresh) to 3 (very spoiled) and score 5.5 is used as the limit for human consumption. Torry scores reduced with storage time during evaluation of cooked herring samples. On day 2 of storage, cooked samples received an average score of 7.5. After storing at two different temperature conditions, on day 5 both groups had similar score (around 7.4) although herring stored at plus temperature appeared to have higher scores on that day and successive days during the study (Figure 8). There after both groups received noticeable decrease up to the last day of storage (day 9), the average score for all panellists was less than 5.5 as shown in Figure 8.

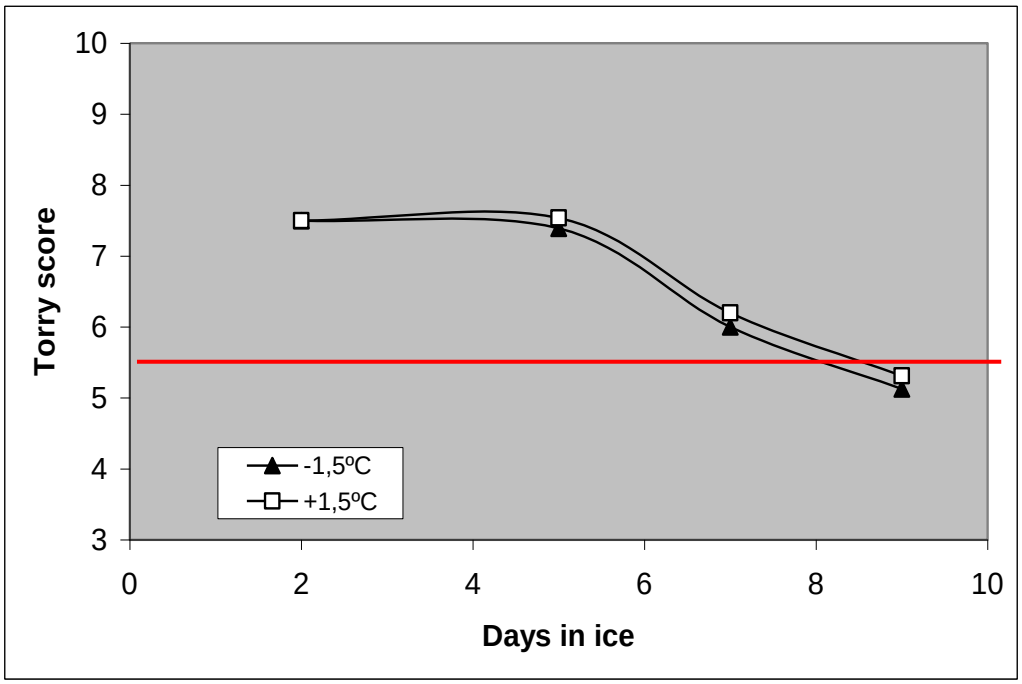

Figure 8: Average Torry score for flavour for iced herring, kept at different temperatures (chilled and superchilled).

4.3 Microbiological results for TVC and H_2S producing bacteria

During the chilled and superchilled storage of herring very low numbers of microorganisms were detected (Figure 9). Fish stored at chilled temperature, had almost the same TVC and H_2S - producing bacteria, from day two to day five of storage. The number increased from log 3 to log 4.3 for TVC and from log 2.6 to log 4.2 for H_2S producing bacteria, from day five to day nine of storage. Herring stored at superchilled condition, TVC and H_2S producing bacteria increased lineally with storage days, reaching maximum of log 5 and log 4.4 for TVC and H_2S producing bacteria respectively at the end of storage time (day nine). Counts of H_2S – producing bacteria were at all points slightly lower than TVC. At the end of storage, H_2S – producing bacteria were about 32% of the TVC in the group at – 1.5°C and 57% in the group at +1.5°C.

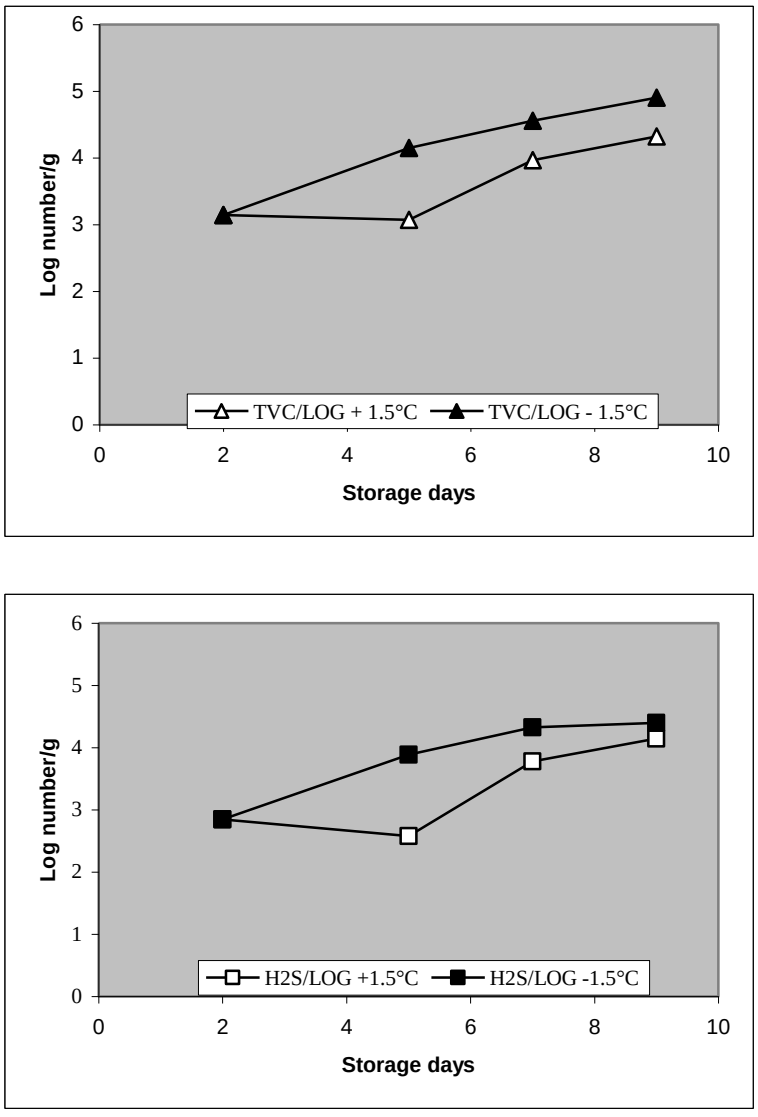

Figure 9: Changes in total count and H_2S –producing bacteria during storage time of iced herring at the temperature +1,5°C and-1,5°C.

4.4 Chemical results for TVB-N and TMA

Measurements of TVB-N for both storage groups were different throughout the storage period of herring (Figure 10). TVB-N values for iced herring stored at plus temperature decreased from day two, with the value of 17.4 mgN/100 g to day seven of storage, where the TVB-N value was 15.5 mgN/100 g. There after it increased to the end of shelf life (day nine), where TVB-N value was 18.6 mgN/100 g. For storage at lower temperature, TVB-N value increased from day two (17.4 mgN/100 g) to day seven, where TVB-N value was 18.6 mgN/100 g, and decreased there after to day nine (16.9 mgN/100 g). No significant difference was found between iced herring stored at different conditions. Measurements of TMA for both storage groups throughout storage time of herring increased linearly (Figure 10). TMA value for negative temperature (-1.5^{o}C) appeared to be higher than for plus temperature, although the final value for both groups at the end of storage time (day nine) was approximately 2.0 mgN/100 g.

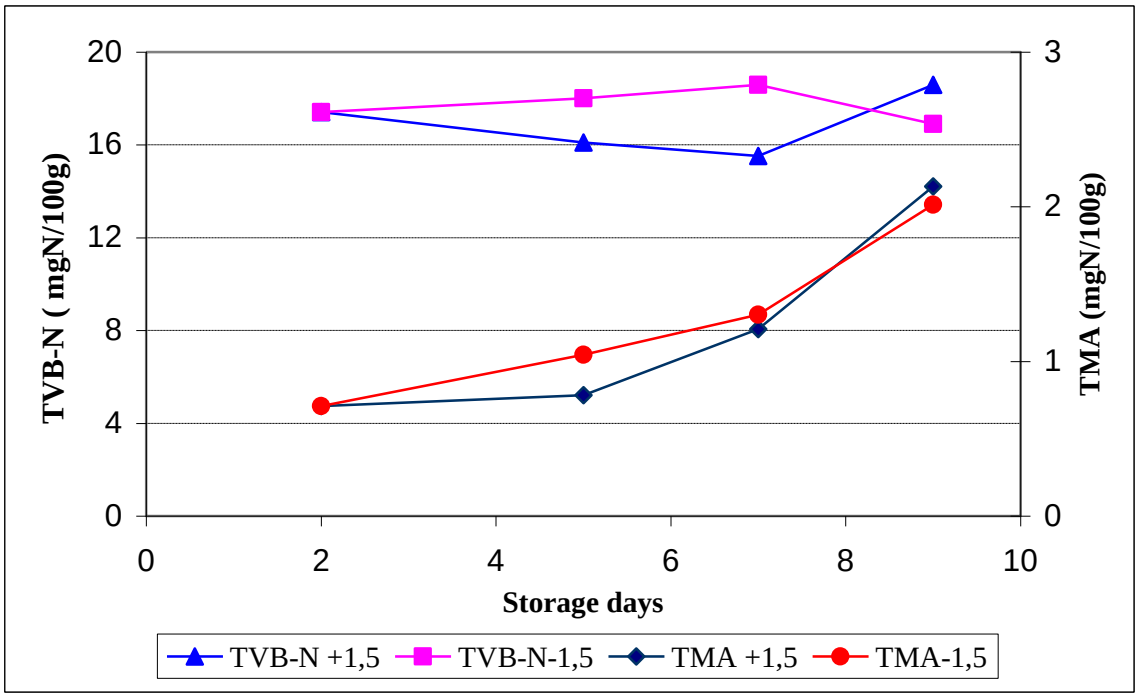

Figure 10: Changes in concentration of TVB-N and TMA during storage time of iced herring under different temperature +1,5^{o}C and -1,5^{o}C.

5 DISCUSSION

5.1 Sensorial analysis using the Quality Index Method (QIM)

The quality parameter belly was modified because the belly is a very sensitive part of herring and can burst very easily, with bad handling or icing. Effect in the form of belly bursting can be a direct cause of the shorter shelf life of herring in some case. That is because the rejection of fish could be before sensory indication of microbiological deterioration and signals of rancidity. The belly bursting doesn't mean that the herring is not of good quality. The modified QIM scheme for herring used in this study, showed higher linear correlation between the total QI score (sum of all attributes) and the storage time of herring (R^2 = 0,887 and 0,914), compared to previously published QIM scheme (Martinsdottir *et al*. 2001). A high correlation between the QI and the storage time shows that the attributes gradually deteriorated with time.

Quality Index of 12 was the rejection limit in this study, corresponding to 8 days in ice or Torry score of 5.5 (limit for human consumption). This shelf life of herring is in agreements with previous UNU-FTP study (Mai 2002) and Martinsdottir *et al*. (2001). In a study by Jonsdottir (1992) the rejection limit for herring was a QI of 16 corresponding to 6,5 days in ice. This value was not reached in present study even on day 9 in ice and neither by Nielsen and Hyldig (2004), even after 10 days in ice, when the fish was unfit for human consumption.

At the end of shelf life of herring there was no significant difference between results in two storage groups. This observation may be due to temperature fluctuation in the cold chamber with iced herring on the chilled temperature, especially between day 4 and day 9 of storage (Appendix 1). This could be the main reason for why there was no difference between the results of herring in both groups in all analyses (QIM, Torry, TVB-N and TMA).
Some of individual attributes did not show strong correlation with storage time of herring, such as appearance of skin and shape of eyes. Description of these parameters like in Nielsen and Hulding study of herring (2004), need special attention and possible revision.

5.2 Torry score

End of shelf life is usually determined when sensory attributes related to spoilage, such as slightly sour, trace of "off-flavour", rancid flavour and odour become evident. When the average Torry score for those attributes was above 5.5 (on the scale 10 to 3), most panellist detected rancidity, which indicated that the samples were approaching the end of shelf life. Lipids of the iced herring are particularly sensitive to oxidation (Huss *et al*.1992). Lipid oxidation seems to have a main role in the quality changes during storage life of iced herring in this study. Rancidity was first noticed with some sensory panellists after 5 days of storage probably due to accumulation of aldehydes and ketones (strong rancid flavour) in fish at that time. In present study rejection of herring by panellists was probably because of rancidity like in the study by Kolarowska *at al.*, in (Huss *et al.* 1992) when the rancidity was noticed after 6 days.

Total shelf life of herring was when Torry score was below 5.5. Based on this, the expected shelf life should be 8 days, considering that on day 7 the Torry score was 6.1 and on day 9 of storage average Torry score was less than 5.5 for both storage groups. This is almost the same shelf life compared to earlier studies reported for herring where the shelf life of herring was reported to be between 5 and 10 days, (Kolakowska *et al.* 1992, Jonsdottir 1992, Martinsdottir *et al*. 2001, Nielsen and Hyldig 2004).

5.3 Microbiology analysis using TVC and H_2S producing bacteria

Total viable counts (TVC) and H_2S producing bacteria of the herring were found to be low during the 9 days of storage. This could be due to lower initial bacterial load on the fish and little temperature abuse during handling on board because of low environmental temperatures and/or proper icing may have retarded bacterial/ microorganism multiplication. Similar growth pattern was observed in both TVC and H_2S –producing bacteria at each storage condition. No increase was recorded until after day 5 in herring stored at chilled temperature. Temperature fluctuation in this cooling chamber might have played a parte here.

5.4 Chemicals analysis using TVB-N and TMA

Analysis of TMA and TVB-N (Figure 10) using the Kjeldahl type distillatory, showed a slow increase during storage time and the TMA and TVB-N were still very low at the last sampling day. TMA is not a particularly good indicator of edibility of herring quality (Huss 1995). The herring in this experiment did not reach more than 2 mgN/100g of TMA at the last sampling day (day 9) in both storage groups, when the sensory panel had judged the fish unfit for human consumption. This was not in accordance with (Huss 1988) and (Connel 1995) who found out that fish with less than 1.5 mg TMA-N/100g is considered of good quality while 10-15 mg TMA-N/100g or 35-40 mg TVB-N/100g is considered the limit of acceptability. The low production of TMA might be explained by the low proportion of H_2S - producing bacteria (*Shewanella putrefaciens*) in the flesh as these bacteria are known to be TMA producers. The low TMA values in present study suggest that TMA is not a good spoilage indicator for herring. It was concluded by Magnusson and Martinsdottir (2001) that TMA is not a good spoilage indicator for cod fillets kept in ice.

The TVB values at day 9 indicate though that the fish was still in good condition since the values are only from 17 to19 mgN/100g in both storage groups. It demonstrated that TVB values are lower than the limits values which are about 30 mgN/100g, according to Icelandic regulation number 233 from 1999.
In previous study, IFL concluded that TMA and TVB values do not increase significantly in most seafood until after 8-10 days of storage, when the signs of spoilage become clearer using other methods of assessment (Sigurdur Einarsson 2001).

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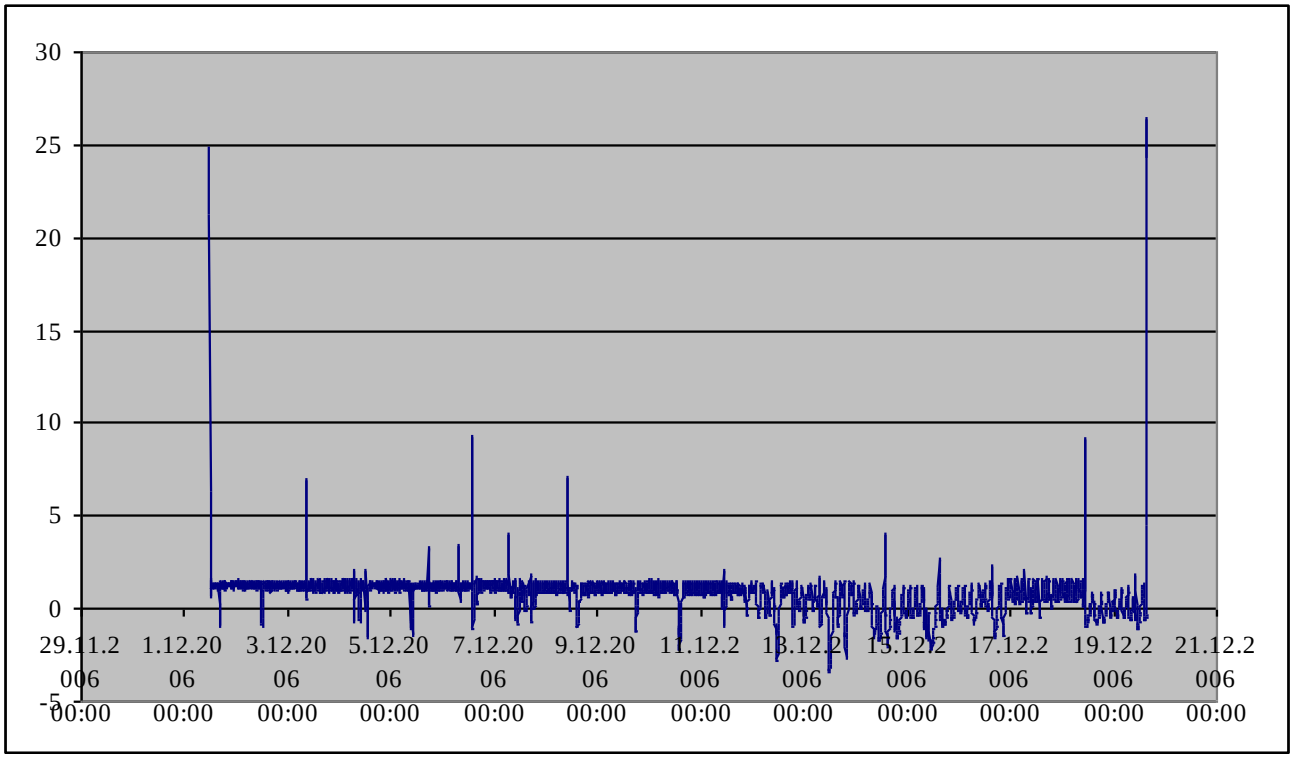
APPENDIX:

Appendix 1: Temperature logger used in the cooling room with +1.5°C during the storage time of herring.



Appendix 2: Torry score sheet for freshness evaluation of cooked fat fish

Odour	Flavour	Score
Butter, margarine	Meaty, shellfish flavour, slightly bitter, slight garlic flavour	10
Fatty odour, peppery	Fat reminds of herring, metallic – but meaty	9
Fatty odour "baked" odour, peppery	Spiced meat, garlic, peppery	8
Caramel, boiled potatoes, musty	Neutral, slight sweet flavour	7
Metallic, slightly sour	Insipid (towards "off-flavour"), slightly rancid, sour or bitter.	6
Milk jug odours, reminiscent boiled clothes	Slightly sourness, trace of "off -flavours", rancid	5
Sour beer, TMA-ammonia, spoiled cheese	Bitter, sour, traces of TMA, rancid, "off-flavour"	4
Ammonia, very sour, drain-odour	Strong bitterness, sour, spoiled fruit, rancid	3

Appendix 3: Quality Index Method (QIM) for herring (Martinsdottir *et all*., 2001)

Quality Index Method (QIM) Scheme for Herring

Quality parameter		Description	Score
Appearance	**Skin**	**Very shiny**	**0**
		Shiny	**1**
		Mat	**2**
	Blood on gillcover	**None**	**0**
		Very little (10-30%)	**1**
		Some (30-50%)	**2**
		Much (50-100%)	**3**
	Consistency	**Hard**	**0**
		Firm	**1**
		Yielding	**2**
		Soft	**3**
	Belly	**Firm**	**0**
		Soft	**1**
		burst	**2**
	Odour	**Fresh sea odour**	**0**
		Neutral	**1**
		Slightly secondary odour	**2**
		Strong secondary odour	**3**
Eyes	**Brightness**	**Bright**	**0**
		Somewhat lustreless	**1**
	Shape	**Convex**	**0**
		Flat	**1**
		Sunken	**2**
Gills	**Colour**	**Characteristic red**	**0**
		Somewhat pale, non-glossy, opaque	**1**
	Odour	**Fresh, seaweedy, metallic**	**0**
		Neutral	**1**
		Some secondary odour	**2**
		Strong secondary odour	**3**
Quality Index			**0-20**

Appendix 4: Average scores of individual quality parameters for herring stored at temperature +1,5°C and – 1,5°C during the storage in ice.

Quality parameters	Appearance										Eyes				Gills			
	skin		Blood on gillcover		Loin consistency		Belly		Odour		Brightness		Shape		Colour		Odour	
Day/Temp	+1.5°C	-1.5°C	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
2	0,9	0,9	1,1	1,1	1,1	1,1	0,2	0,2	0,5	0,5	0,2	0,2	0,9	0,9	0,5	0,5	0,4	0,4
5	1,0	1,0	1,9	1,5	1,3	1,1	0,2	0,5	0,8	0,9	0,8	0,9	1,2	1,4	0,6	0,6	1,3	1,4
7	1,3	1,1	2,1	2,1	1,5	1,5	0,5	0,4	1,5	1,5	1,0	0,9	1,4	1,5	0,8	0,8	1,9	2,6
9	1,1	1,0	1,9	2,4	1,5	1,6	0,5	0,6	1,6	1,5	0,9	0,9	1,2	1,3	0,8	0,8	2,0	2,0

Appendix 5: Average QI of herring for all parameters given by panelists for chilled and superchilled temperature during the storage time.

Storage days	Average Quality Index (QI)	
	+ 1.5°C	- 1.5°C
2	5,57	5,57
5	8,89	9,03
7	11,69	12,15
9	11,27	12,00

Appendix 6: Average values for Torry score (flavour and odour) for coked herring for storage temperature (+1.5 and -1.5°C) during the storage time.

Storage days	Flavour		Odour	
	- 1.5°C	+1.5°C	-1.5°C	+ 1.5°C
2	7,5	7,5	7,9	7,9
5	7,4	7,5	7,8	7,6
7	6,0	6,2	6,1	6,1
9	5,1	5,3	5,6	5,5

Appendix 7: Average values for TVB-N and TMA of herring stored at temperature + 1.5°C and - 1.5°C during the storage time.

Storage days	Average values for TVB-N mgN/100g		Average values for TMA mgN/100g	
	+ 1.5°C	-1.5°C	+ 1.5°C	-1.5°C
2	17,41	17,41	0,71	0,71
5	16,11	18,00	0,78	1,04
7	15,52	18,60	1,21	1,30
9	18,60	16,90	2,13	2,01

Appendix 8: Log of Total viable counts (TVC) and hydrogen sulphide (H_2S) producing bacteria of herring stored at temperatures + 1.5°C and - 1.5°C during the storage time.

Storage days	TVC/LOG		H_2S/LOG	
	+ 1.5°C	- 1.5°C	+ 1.5°C	- 1.5°C
2	3,15	3,15	2,85	2,85
5	3,07	4,15	2,58	3,89
7	3,97	4,56	3,78	4,32
9	4,32	4,90	4,15	4,40