The United Nations University
FISHERIES TRAINING PROGRAMME
PO Box 1390, Skulagata 4
120 Reykjavik, Iceland

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Shelf life of red fish stored in ice and modified atmosphere (MA) and some aspects on the development of a Quality Index Method (QIM) scheme for red fish stored in MA

Estela Chichava Juliao Mausse
Ministry of Fisheries - Beira
Mozambique

Supervisors: Ms. Tyri Valdimarsdottir
Ms. Kolbrun Sveinsdott

ABSTRACT

The objectives of the project were to study the shelf life of red fish stored in ice and in modified atmosphere (MA). In addition, to analyse and define the parameters to be used in the development of a Quality Index Method (QIM) scheme for red fish stored in modified atmosphere.
Samples of iced red fish (*Sebastes marinus, S. mentella*) caught south of Iceland were stored in ice and in a modified atmosphere (60% CO_2: 40%N_2) two days after capture and investigated with sensory analysis, chemical measurements and microbial counts, during 4 sampling days.
Differences in total volatile bases (TVB), trimethylamine (TMA) and trimethylamine oxide (TMAO) values between red fish stored in ice and modified atmosphere were not significant. The pH of MA samples remained lower than in ice samples.
The Total Viable Counts and *Pseudomonas* in ice samples were much higher than in MA samples.
Significant differences in sensory changes during storage of ice and MA samples were found. The colour of eyes of fish stored in MA had higher scores than iced samples (changes from black to grey colour), and skin colour of fish stored in MA was more yellow.
The iced fish samples reached the limit of acceptance in day 19 according to sensory evaluation of cooked fish, whereas fish stored in MA reaches that limit in day 21. MA gave a 2 days shelf life extension of red fish.
The QIM scheme for red fish stored in ice is not suitable for red fish stored in modified atmosphere. A slightly modified QIM scheme specific for red fish stored in MA is needed.

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1. INTRODUCTION

Consumer goods are the main products exported by Mozambique. About half of the value of exports comes from fisheries products such as shrimps, lobsters, crabs and fish, which are exported to Europe.
Regulations in the European Union require freshness grading of most fish to be marketed within the Union. The Fish Inspection Department has to perform quality inspection of fish, prior to export in order to issue a health certificate in accordance with EU regulations (EU Directive 1991). On the other hand, the demand for chilled fresh fish is rising as improved refrigeration and transport enables good quality chilled fresh products to become available to larger sections of the population. The rapid deterioration of chilled fish makes special precautions necessary during processing and distribution. Modified Atmosphere Packaging (MAP) with refrigeration has been shown to increase the shelf life of chilled fish (Church 1998, Huss 1995).
The freshness of fish deteriorates with time, until the product is no longer acceptable to the consumer. Considering that consumers are the ultimate judges of the quality, realistic determination and accurate prediction of shelf life of fresh and lightly preserved seafood are important to meet consumer demands and to comply with legislative requirements. In this way, sensory evaluation such as the Quality Index Method (QIM) can be an important tool to assess fish freshness by systematic assessment of odour, flavour, appearance and texture of food. Recent studies have shown that QIM has to be developed for each species and the schemes are made in storage studies (Huss 1995). The aim when developing QIM for each fish species is to have a linear increase in the Quality Index (QI) with storage time in ice.
The development of QIM schemes for fish species caught in Mozambican territorial waters is a goal for the Mozambican Fish Inspection Department in order: i) to follow laws and regulations, ii) to increase the value of exports by increasing the proportion of high quality fish, iii) to limit losses because of fish spoilage. By using QIM, the fish inspector can give an estimate of the past and remaining storage time. In addition, the knowledge of shelf life of Mozambican fish species would allow fishermen, processors and retailers better control of their markets.

The two main objectives of the present project were:
- To study the shelf life of red fish (*Sebastes mentella* and *S. marinus*) stored in ice and in a modified atmosphere
- To analyse and define the parameters to be used for the development of a Quality Index Method (QIM) -scheme for red fish stored in MA

The main research questions to be answered were:
- What are the effects of modified atmosphere storage of ungutted red fish on chemical, microbial and sensory changes compared to red fish stored in ice?
- Can modified atmosphere increase the shelf life of red fish?
- Is it necessary to develop a QIM scheme for red fish stored in MA separately from the one existing for red fish stored in ice?

This project work was carried out as part of an on-going project (FAIR CT-97- 3833) at the Icelandic Fisheries Laboratories (IFL) called “Implementation on board of systems of atmospheres with variable composition applied to fresh fish. Continuation on shore of the modified atmosphere chain”.

2. LITERATURE REVIEW

2.1 Shelf life of fish

The shelf life of food is defined as the 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 is no longer fit to eat (Huss 1995).
Most fish are caught in nets, or with lines with baited hooks. Hence it is difficult to control the initial quality of the raw material with any degree of repeatability. 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, spoil 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. At the end of the shelf life, odour, flavour, texture, and appearance have become unpleasant.

2.1.1 Sensory changes

The first sensory changes that occur in fish during storage are concerned with the appearance and texture. The characteristic flavour of fish normally develops in the first couple of days of storage (Church 1998).
It has been estimated that the characteristic sensory changes in fish vary considerably depending on species and storage method (Huss 1995, Church 1998). Fish spoilage can be divided into four distinct phases, according to Huss (1995) and Church (1998):

- Phase 1: Very fresh, sweet, seaweed and delicate taste
- Phase 2: Loss of characteristic odour and taste, flesh neutral (no off-flavours), texture pleasant
- Phase 3: There is a sign of spoilage and a range of volatile, unpleasant-smelling substances are produced depending on the fish species and type of spoilage (aerobic, anaerobic). One of the volatile compounds may be trimethylamine (TMA) derived from the bacterial reduction of trimethylaminoxide (TMAO). There is a very characteristic “fish” smell. Production of volatile unpleasant-smelling odours/flavours starting with slightly sour, fruity and bitter off-flavours. During later stages sickly sweet, cabbage like, ammonia, sulphurous and rancid smells develop. The texture becomes either soft and watery or dry and tough
- Phase 4: The fish is spoiled and putrid

In phases 1 and 2 the major changes are due to autolytic reactions, but in phases 3 and 4 the major changes are due to bacterial activity.

2.1.2 Autolytical and chemical changes

pH fall
The glycogen levels of fish muscles are lower than those of mammalian muscles, mainly due to the stress of capture. As a result, the pH of fish muscle remains high after death (>6.0) favouring microbial growth and enzymatic activity (Church 1998, Gram and Huss 1996).

Autolytic changes
Autolysis or “self-digestion” of fish is due to enzyme activity. Depending on the type of enzymes and the substrate where it actuates, the changes that can be encountered

are for instance, belly-bursting, gapping of fillets, softening and loss of fresh fish flavour.

Nucleotide degradation
The levels of adenosine triphosphate, the muscle energy carrier, fall after death. Depending on temperature, fish species and packaging atmosphere, most of the ATP will be degraded to inosine monophosphate (IMP), as shown below:

ATP-ADP-AMP-IMP----HX-further metabolism-uric acid

As the degradation sequence continues, inosine (I) and later hypoxantine (HX) are produced. Accumulation of IMP is of particular significance since it is an important flavour component in fresh fish, and helps mask bitter flavours. HX has a mild bitter flavour, and high levels can make fish taste unacceptable (Church 1998, Huss 1995).

Oxidation
Consumers perceive rancidity as an unacceptable taste: Typically soapy, stale and linseed oil flavours are detected. There are two types of rancidity, hydrolytic and oxidative. In the case of hydrolytic rancidity, the off-flavour is caused by free amino acids and is generally caused by a combination of micro organisms and moisture. Oxidative rancidity is a much more common problem than hydrolytic rancidity. Oxygen attacks unsaturated fatty acids giving hydro peroxides which degrade into off-flavour compounds (Church 1998).

Bacteriological changes
The first stage of fish spoilage is dominated by endogenous enzymes, whilst the bacterial flora of the gut cavity, gills and skin adapt to the changing environmental conditions (Church 1998).
The wide range of fish species, the vastly different environments from which they are harvested and the variety of the microbiological sampling techniques used, has resulted in widely ranging reports in numbers of organisms on fish. The bacterial flora of cold water fish is dominated by the psychrotropic Gram negative genera. Organisms involved belong to the genera *Acinetobacter, Flavobacterium, Moraxella, Shewanella* and *Pseudomonas*. Members of the *Vibrionaceae* (*Vibrio* and *Phosphobacterium*) and the *Aeromonadaceae* (*Aeromonas spp*.) are also common aquatic bacteria, whilst Gram-positive organisms such as *Bacillus, Micrococcus, Clostridium* and *Lactobacillus* can also be found in varying proportions (Huss 1995). Shewan (1962) concluded that the gram-positive *Bacillus* and *Micrococcus* dominate in fish from tropical waters. However, others have found that the micro flora on tropical fish is very similar to that on temperate species but with a slightly higher load of Gram-positive and enteric bacteria (Huss 1995). Many of the organisms present on spoiled fish play no active role in spoilage (Huss 1995).
A clear distinction should always be made between “spoilage flora” and “spoilage bacteria”. Spoilage flora refers to the bacteria present on the fish when considered spoiled, whereas spoilage bacteria are the bacteria responsible for producing the off-odours and off-flavours in the spoiled fish (Church 1998). Specific spoilage flora for cod stored at different temperatures is given in Table 1.

Table 1: Specific spoilage bacteria for cod and bacterial spoilage compounds (Church 1998).

Storage temp.	Pack Atmosphere	Specific Spoilage Bacteria	Spoilage compounds
0°C	Aerobic	*S.putrefaciens* *P.seudomonas*	TMA, H_2S, HX. CH_3SH. ketones. esters. aldehydes
0°C	Vacuum	*S.putrefaciens* *P.phosphoreum*	TMA, H_2S, HX. CH_3SH. ketones. esters TMA. HX
0°C	MAP	*P.phosphoreum*	TMA. HX
5°C	Aerobic	*S.putrefaciens*	TMA, H_2S, HX. CH_3SH, ketones, esters
5°C	Vacuum	*S.putrefaciens.* *Aeromonas spp*	TMA, H_2S, HX, CH_3SH, ketones, esters -
5°C	MAP	*Aeromonas spp*	-

2.2 Methods to evaluate fish freshness

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.

2.2.1 Sensory evaluation

Sensory testing must play an important part in any food quality evaluation programme since the ultimate criterion for judgement is the human response. Sensory evaluation is based on evaluation of appearance, texture, odour and flavour of food. According to Hall (1992), measurements of sensory quality are influenced by three variables: (i) the sample under investigation; (ii) the assessment method; and (iii) the judges.
Studies have shown that to have a sensory evaluation panel as a precise tool, the judges have to be carefully selected, the proper physical facilities must be available and statistical analysis must be used (Larmond 1967). According to the same study. the testing environment may influence the results. Thus, it is generally recommended that a special room, in which as many variables as possible is controlled, be used. This room should be noise and odour free and equipped with individual booths to minimise distraction.
For difference testing, it is important to select a cooking method which best permit detection of a difference, and to avoid preparations which may add flavour to the samples.
According to Larmond (1967) studies have shown that training reduced differences in scoring levels between judges. Kramer (1952) recommended that judges should be selected based on how well they can detect differences, how consistent they are and how they compare with the panel average.
Sensory methods can be either subjective or objective. The subjective methods are based on the panellist's preference for a product and bias among panellists is high. Subjective methods are often applied in market research, consumer tests or product development. Objective methods, on the other hand are used in quality inspection. Sensory methods used in inspection of freshness quality of raw fish usually involve

grading of the fish quality parameters. The methods must be precise, technically correct and objective rather than subjective. During the last 50 years many schemes have been developed for sensory analysis of raw fish. The first modern and detailed method was developed by the Torry Research Station in Scotland. Today the most widely used method for assessment of raw fish in Europe are the EU-scheme and the Quality Index Method (Huss 1995, Luten and Martinsdottir 1997).

EU-scheme

In the EU scheme three grades of freshness are laid down; E, A and B, corresponding to various stages of spoilage. E or extra is the highest possible quality and fish that does not make grade B, is not fit for human consumption. The EU-schemes list descriptive terms that fit the description of many fish species. There are some speculations about the usability or reliability of the EU-scheme because it does not take into account the differences between species, and it only uses general parameters to describe quality characteristics of fish (Luten and Martinsdottir 1997). When evaluating the freshness quality of raw fish. It would be practical if a method could be used to predict the remaining shelf life in days. The EU-scheme does not predict the remaining shelf life because it is too general, applying to many different species that spoil at different rates (Huss 1995).

Quality Index Method (QIM)

New seafood freshness quality grading systems have been under development for various species. The QIM is one of these systems and is based on well-defined characteristic changes that occur in raw fish, such as outer appearance of eyes, skin, gills, and changes that occur in odour and texture. A score from 0 to 3 demerit (index) points is given for each feature. On a developed QIM scheme the score increases linearly with storage time in ice. If the maximum storage time in ice is known the ideal demerit curve can be used to predict remaining storage time in ice. The ideal demerit curve begins at the zero and its maximum is where the two curves intersect i.e. where the Torry scale has rejected the cooked product (Figure 1). The advantages of the QIM scheme are that it is an objective method, non-destructive, rapid, cheap to use and its application requires little training. In addition the method can be used to predict remaining storage time. Good training, in combination with detailed descriptions of the methods, schemes, sampling plan and illustration materials, may facilitate the use of sensory evaluation in different parts of the fishery chain.

Development of QIM

The first step in developing a QIM is to perform a parallel sensory analysis of raw fish and cooked fish. The sensory analysis of cooked fish is based on judgement of taste, texture and odour by a trained panel using the Torry scale. The purpose of sensory evaluation of raw fish is to describe (literally) all detectable aspects of change on/in the fresh fish during cold storage in ice. This involves a detailed description of all possible changes or deviations of sensory parameters such as appearance, texture and odour. Any change for a specific parameter has to be described for each evaluation during the storage trials (Luten and Martinsdottir 1997).

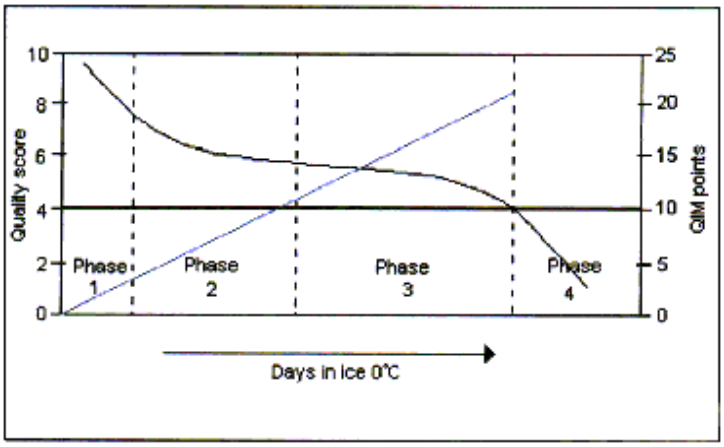

Figure 1: Combination of grading of raw fish with QIM and assessment of cooked fillet fish (modified from Hyldig and Nielsen 1977).

Figure 1 shows both the assessment of cooked fish and the Quality index. The selection of parameters of QIM is determined as a combination of the best descriptors for the spoiling fish and fulfilling the aim that the sum of points-grading shall give a straight line with respect to stored days in ice (dashed line).
The figure also shows the four phases of fish spoilage. In phase1 the fish is very fresh, sweet, seaweed and has a delicate taste and in phase 2 there is loss of characteristic odour and taste, flesh neutral and texture pleasant. During phase 3 there is a sign of spoilage but fish is still suitable for human consumption and finally in phase 4 the fish is spoiled and putrid and rejected by cooked assessment. Bold line shows the limit of acceptability (score 4 for cooked assessment).

Torry scale
The maximum shelf life of fish can be determined by sensory evaluation of cooked samples. A descriptive 10-point scale developed at the Torry Research Station is often used for this purpose. This scale is often referred to as the Torry scale and has been developed for lean, medium fat and fat fish species. The Torry scale is based on the four phases described in chapter 2.1.1. Scores range from 10 (very fresh in taste and odour) to 3 (spoiled). Figure 1 illustrates how the sensory evaluation of cooked fish may be combined to freshness grading with the Quality Index Method.

2.2.2 Microbiological measurements

The number of specific spoilage bacteria is related to the remaining shelf life, which can be predicted from such numbers (Huss 1995). Different peptone-rich substrates containing ferric citrate have been used for detection of H_2S-producing bacteria such as *Shewanella putrefaciens*, which can be seen as black colonies due to precipitation of FeS (Huss 1995).
When stored aerobically, levels of 10^8-10^9 cfu/g of specific spoilage bacteria in the flesh are required to cause spoilage in iced fish. A much lower level (10^7 CFU/g) of *P.phosphoreum* is needed to spoil chilled fish packed in MA (Gram and Huss 1996).

2.2.3 Chemical analysis

Valuable information can be gained from knowing the pH of fish flesh. Measurements can be done using a pH-meter by placing electrodes either directly into the flesh or into a suspension of fish flesh in distilled water.
Most marine fish contain a substance called trimethylamine oxide (TMAO). Certain bacteria that occur naturally on the skin and in the guts of fish and in seawater can break down TMAO to trimethylamine (TMA). The amount of TMA produced is a measure of spoilage activity.
Total amount of ammonia, dimethylamine and trimethylamine formed during spoilage of fish is a commonly used estimate of spoilage. A range of methods are used to measure total volatile bases (TVB), but in all of them the fish or a extract of the fish, is made alkaline, the bases are distilled off, collected and measured by titration. The commission has fixed a reference method for determination of TVB-N based on a water steam distillation of a perchloric acid extract (Oehlenschlager 1997).

2.3 Modified atmosphere packaging (MAP)

Packaging of semi-prepared and prepared fresh food, have become part of the every day life of modern consumers. They want to see food on their plates as fresh as when it was first prepared. This interest in fresh foodstuffs is a strong driving force behind the development of new shelf-life enhancing methods, whereby artificial additives and preservatives are no longer acceptable. Nowadays, efforts are being made to meet consumer demands for naturally preserved quality food, handled and processed as little as possible (AGA AB 1997).
MAP is the packaging of a perishable food product in an atmosphere, which has been modified so that its composition is other than that of air (Ooraikul and Stikes 1991). Early work in 1930 demonstrated that 10-20% CO_2 in atmosphere surrounding a foodstuff will suppress the growth of *Pseudomonas spp* and certain other spoilage organisms, provided that the temperature is maintained at or below 4°C. This physiological effect of CO_2 provides the fish technologist with a method for controlling the growth of *Pseudomonas spp* in chilled fish, and consequently increases its shelf life (Hall 1992).
. Using MAP can also improve overall cost-effectiveness because it:

- Increases sales by satisfying the growing demand by consumers for naturally preserved quality food, without additives and preservatives
- Increases shelf life in the distribution chain by days or even weeks, which increases the availability of fresh food to consumers
- Reduces the return of spoiled foodstuffs
- The correct gas mixture in MAP maintains high quality by retaining the original taste, texture and appearance (AGA AB 1997).

In spite of all these benefits, some studies have reported problems such as *pack collapsing* caused by reduction of pressure within a pack, which manifests itself as a concave surface of the lid of rigid base packs. In high CO_2 concentrations problems such as discoloration of whole fish; texture changes and drip losses can occur (Church 1998). MAP used in retail packs is an expensive technique and it can not replace good chilling or good hygienic production conditions (Huss 1995). In addition, toxin production of *Clostridium botulinum* is increased under anaerobic conditions which may be of importance for the safety of packed fish (Church 1998, Huss 1995).

Potential problems with MAP and a variety of techniques adopted to minimise them are discussed in detail by Church (1998).

2.3.1 Characteristics of CO_2, O_2 and N_2 gases

The MAP gas mixtures usually consist of normal air gases: carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). CO_2 is the most important gas in the field of MAP technology. Micro organisms, in particular aerobic bacteria, are strongly affected by CO_2. CO_2 inhibits microbial activity by effectively dissolving into the food's liquid phase, thereby reducing its pH and causing changes in permeability and function. Nitrogen is an inert gas; it is primarily used to replace oxygen in packaging and thereby prevents oxidation. Owing to its low solubility in water, N_2 also helps to prevent package collapse by maintaining internal volume. For most foodstuffs, the package should contain as little oxygen as possible to retard the growth of aerobic micro organisms and reduce the degree of oxidation (AGA AB 1997).

2.3.2 MAP of fish and fish products

Studies of several species of fish in MA have shown that shelf life can be extended (Table 2).

Table 2: Examples of shelf life of fishery products stored in modified atmosphere (Church 1998, * Rehbein et al. 1994, ** Dalgaard et al. 1997).

Storage Temp. (°C)	Product	MA	Shelf life MA (days)	Shelf life ice (days)
5	Various types of white fish	40% CO_2 30% O_2 30% N_2	9	6
4	Whiting fillets	100% CO_2	15	7.5
26	Whiting	100% CO_2	2	2
0	Mackerel	60% CO_2 40% N_2	6.5	3.5
0	Scampi	40% CO_2 30% O_2 30% N_2	4.5	3.5
4	Scallops	100% CO_2	22	12
4	Cooked cray fish	80% CO_2	21	14
0	Red fish	60% CO_2 40% N_2	21*	21**
4	Brown shrimp	100% CO_2	14	4.5

2.3.3 Packaging materials and machines

According to AGA AB (1997) packaging materials are of decisive importance for food quality and shelf life. In order that MA will be maintained during the lifetime of the package, several different plastic materials are often combined into a multi-layered structure, each layer having it's own function. Thus, different plastic materials can therefore be chosen and combined to achieve: mechanical strength; water vapour barriers to prevent dehydration and weight loss; gas barrier and gas permeability; anti-fogging properties (the inside of the material should have a surface that does not allow the formation of water droplets, which reduce transparency); and sealing properties. i.e. capable of sealing into a tight package while retaining material properties even along a weld seam.
There are 5 main groups of packaging machines used with MAP technology depending on the type of product (packaged product, packaging material and product volume).
Although these machines are based on different principles, the basic working operation is the same: First, a package is formed (or prefabricated packages are used) and filled with the food product. Then, the air in the package is replaced by MA. Finally the package is sealed. These three steps take place either manually or automatically. The methods used to modify the atmosphere include gas flushing or vacuum extraction and then gas injection (AGA AB 1997).

3. MATERIALS AND METHODS

3.1 Red fish

A total of 270 iced red fish (*Sebastes spp*) were used in this experiment. The fish were caught in pelagic haul off the south coast of Iceland and chilled to 0°C in ice. The fish arrived at laboratory on 16/11/00 iced in a container 2 days after capture. The batch of 270 fish was divided into 3 groups: 160 fish were stored in MA, 100 fish were stored in ice and 10 fish were used for sampling day 2.
For storage in modified atmosphere the 160 fish were iced in 8 plastic boxes (20 fish in each) with holes to allow ice melt drainage and air exchange. The 8 boxes were put in a big container where a 59.7 % CO_2: 40.3% N_2: 0.123% O_2 gas mixture was injected to modify the atmosphere. Prior to gas injection, the container was closed and the two holes (one for oxygen extraction and other for gas mixture injection) were checked.
For ice storage, the 100 fish were iced in a container in alternating layers of ice and fish. Afterwards, the two containers were placed in a cold storage at 0-2°C.The containers were opened 3 times for sampling and ice addition. The gas mixture composition was adjusted each time in the MA storage. The temperature of the fish was measured by locating thermometers in the boxes.

3.2 Sampling plan for all measurements

Two trials were carried out in the experiments. In trial one, whole red fish was stored in ice, and in trial two whole iced red fish was stored in modified atmosphere (60% O_2: 40% N_2).

The sensory evaluation of raw (QIM) and cooked (Torry scheme) red fish were carried out parallel on the sampling days along with measurements of microbial counts, pH and chemical analysis. Each sampling day, 8 red fish from each trial were collected. Three fish were analysed whole with QIM, 2 filleted and cooked for sensory evaluation with the Torry scheme, 1 for photographing and 2 for pH, microbial counts and chemical analysis. The flowchart of the fish used in the experiment is shown in Figure 2.

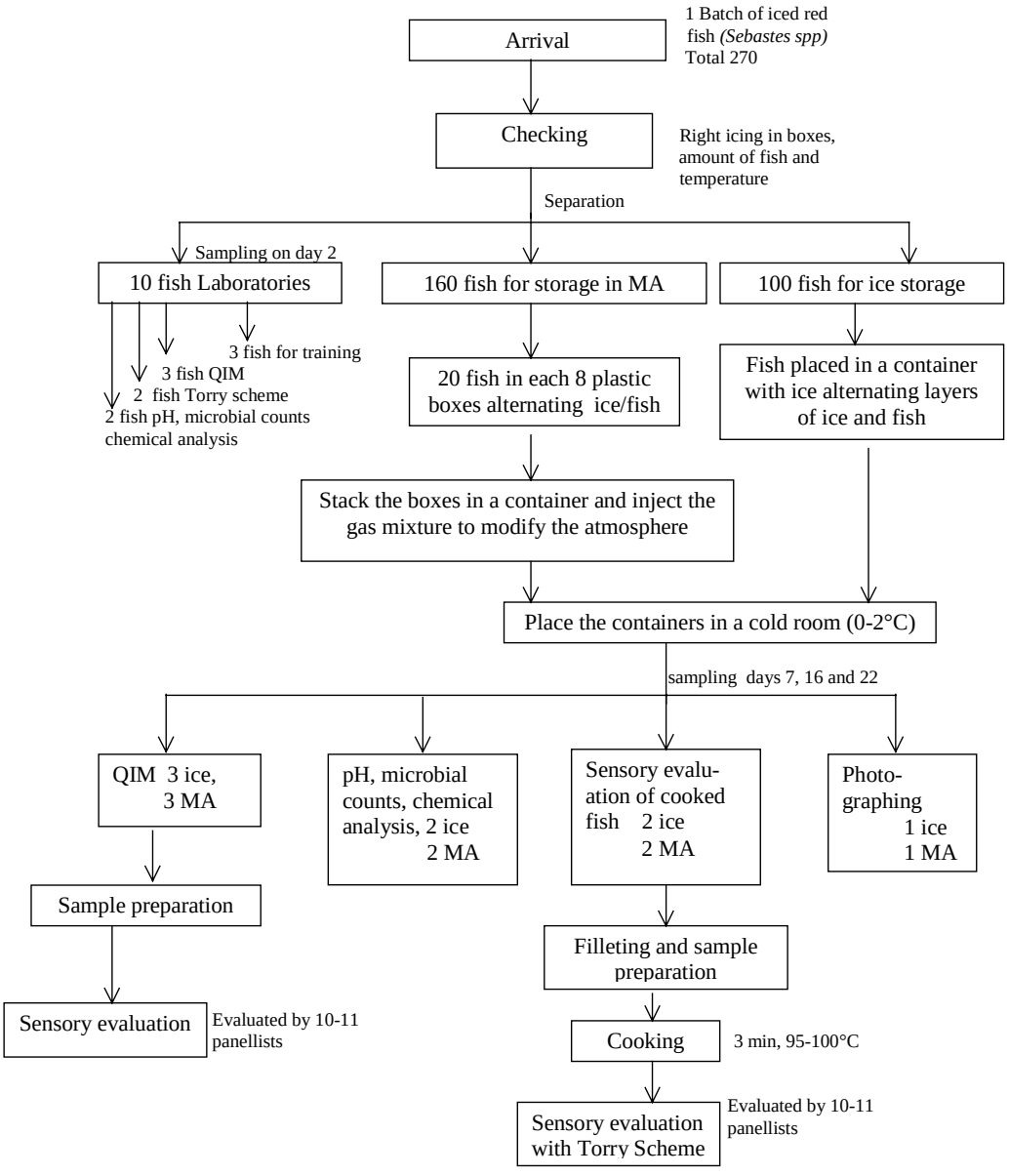

Figure 2 : Flowchart of fish used in the experiment.

3.3 Quality Index Method (QIM)

A total of 24 red fish were analysed with QIM during the training and evaluation period.

Training of judges
The training of 11 QIM judges was carried out in one session. The judges were all employees at the Icelandic Fisheries Laboratories and had experience in assessing fish with QIM. The judges were introduced to the scheme developed earlier for red fish (*Sebastes marinus, S. mentella*) stored in ice (Appendix 1).
The procedure of evaluation was introduced to the judges and each parameter evaluated was discussed. The judges were informed of the plan to develop a QIM scheme for red fish stored in MA, and were asked to comment on the QIM scheme for iced red fish. The judges observed red fish (the storage time in ice was given) and the scheme was explained to them at the same time.

Sample preparation and QIM evaluation
The fish were collected from the iceboxes and placed on a clean table. The belly side was opened to access the internal organs. Each red fish was coded with a number consisting of 3 digits that did not indicate the storage time or condition of the fish. For QIM evaluation (6 sessions over 4 sampling days), 21 fish from the two trials at different storage time were evaluated. Fish from both trials (MA and iced) were evaluated each time. All observations of fish were carried out under standardised conditions; always in the same room, with as little interruption or distraction as possible, at room temperature and under electric light.
The QIM scheme for red fish *(Sebastes mentella, S. marinus*) was applied for the sensory analysis of the raw fish. Ten to eleven QIM judges evaluated the fish individually, and registered their evaluation for each quality parameter in the scheme. The judges had no information about the storage time in ice and MA before the evaluation and were asked to make comments. The evaluation took 20-30 minutes each time.

3.4 Sensory evaluation of cooked red fish

Training of judges
Prior to the experiment, the panel was trained during one session in the use of the Torry scheme for sensory evaluation. They were all familiar with and had experience in sensory evaluation of cooked red fish from previous experiments.
Sensory analysis of cooked red fish using the Torry scheme for medium fat fish was carried out parallel to the QIM evaluation. The rejection limit score 5.5 was used for the Torry scheme. A total of 16 red fish was used for training and assessment with the Torry scheme (see Appendix 2).

Sample preparation and sensory evaluation
The samples were collected from the fillets under the dorsal fin. From each fish 6 samples were collected and altogether 12 samples were prepared for each session. The samples were placed in aluminium boxes. Each sample was coded with a number consisting of 3 digits that did not indicate storage conditions and were cooked at 95-100°C in a pre-warmed oven, with air circulation and steam for 7 minutes. The boxes were closed with plastic covers and served to the panel in warm glass containers. Each panellist evaluated 2-3 samples in duplicates per session. For every sample, the panellists evaluated the attributes using a computer software program (HyperSense 1.6 © 1993-1996, Icelandic Fisheries Laboratories, Reykjavik, Iceland) for data collection. The evaluation was carried out in 6 sessions over the sampling days. The

panellists had no information about the storage conditions (storage time, MA or ice) of the samples.

3.5 Photographs

After the fish had been analysed with QIM, one fish from each trial was iced in boxes, where they were stored until photographed (within 3 hours). Thus red fish stored from 7 to 22 days in MA or ice were photographed by a professional photographer. In the photography emphasis was made to highlight the colour and mucus of the skin, colour and form of the eyes and colour and mucus of the gills. Selected photos are intended to be used with the QIM scheme for red fish stored in MA.

3.6 Microbial counts

In each sampling day, 2 samples from each trial were taken to the Microbiological Laboratory of IFL for microbial counts, performed by the staff. The following microbial counts were carried out: Total plate count on L&H, total and H_2S producing bacteria on Iron Agar and *Pseudomonas* counts on CFC-agar.

3.7 Chemical analysis

TMAO, TMA and TVB from each trial were determined using Flow Injection Gas Diffusion Technique (Sadok et al. 1996). The pH of the samples was determined as well. These measurements were carried out at the Chemistry Laboratory of IFL by the staff.

3.8 Data analysis

For comparison of two samples a t-Test was applied to test the hypothesis that means from two or more samples are equal, using Microsoft Exel 97. Averages, variance, standard deviations and correlation coefficient were calculated using Microsoft Exel 97.
The Torry results were analysed using the HyperSense 1.6 software© 1993-1996, Icelandic Fisheries Laboratories, Reykjavik, Iceland. Interaction of judges and samples was assumed and statistical analysis was done using two factor design with interaction in the analysis of variance (ANOVA) to observe if a significant statistical difference existed between samples for each quality attribute assessed. The programme calculates multiple comparisons using Tukeys test.

4. RESULTS

The results from sensory evaluation, chemical measurements and microbial counts are presented in relation to storage time of red fish in ice and modified atmosphere.

4.1. QIM

The results from the sensory evaluation of ungutted red fish with QIM scheme are shown in Figure 3. The figure shows the linear relationship between the average QI of red fish stored in ice and MA.

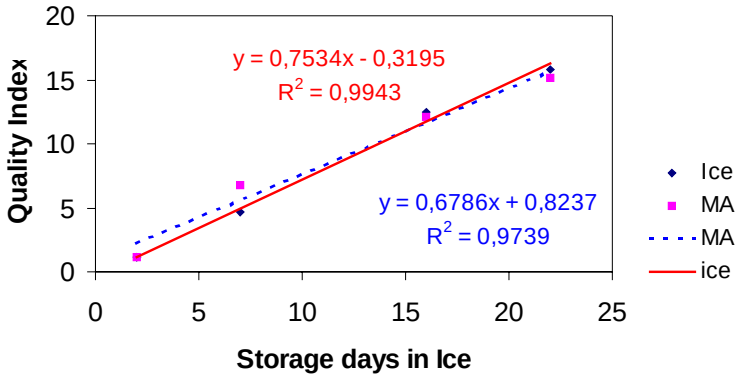

Figure 3: Average Quality Index scores of ungutted red fish stored in ice and MA based on results from 10-11 judges. On sampling day 2 the samples were the same (iced fish).

There was a not statistical difference between average QI scores during storage time of iced and MA samples, except on sampling day 7 (Table 3).

Table 3: Statistical analysis of Quality Index scores of ungutted fish stored in ice and MA (average QI scores with standard deviation).

Storage days in ice	Average QI score Ice	Average QI score MA	Significance (P)
2	1.16 (0.24)	1.16 (0.24)	-
7	4.70 (0.58)	6.78 (0.39)	0.003
16	12.46 (0.84)	12.07 (1.14)	NS *
22	15.81 (1.91)	15.18 (0.94)	NS *

*NS: Not significant (p> 0.05)
The table shows the significant differences between average QI scores of iced and MA samples on sampling day 7.

The main sensory differences between the two trials commented by judges for the MA fish were yellow colour of skin and grey colour of eyes (Table 4 and Figure 4).

There was not a significant difference for the appearance (colour of skin) between ice and MA samples except on day 16 (Figure 5 and Table 5). when 4 of 11 judges commented on yellow skin colour of all red fish stored in MA.
There was a significant difference in eye colour between the two trials during storage time (Figure 6 and Table 6). The MA fish samples received higher scores for colour of eyes.

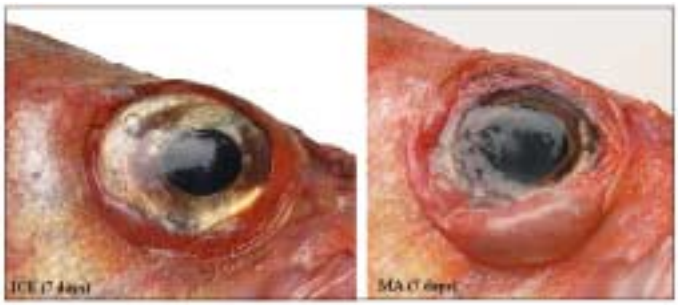

Figure 4: Black colour of pupil and convex form of iced fish eye (left) and opaque colour of pupil and flat form of MA eye (right) on sampling day 7.

Comments from judges (regarding development of QIM scheme for red fish stored in MA)

Table 4: Comments from judges about the main differences (sensory assessment of raw fish) between the two trials.

Storage day	Iced samples	MA samples
7	no comments	Pink gills (1 out of 11 judges) Grey eyes (2 out of 11 judges) Gills faint odour of 1/3 of samples (1 out of 11 judges) Mat and pink colour of skin in 1/3 samples (1 out of 11 judges)
16	no comments	Yellow, yellowish, pale and mat colour, with red stains in the skin (4 out of 11 judges) Grey eyes (1 out of 11 judges)

Based on these comments, a few attributes were looked into in more details as shown in Figures 5 and 6. The figures show how the scores of skin appearance and eye colour of fish stored in ice and MA increases with storage time.

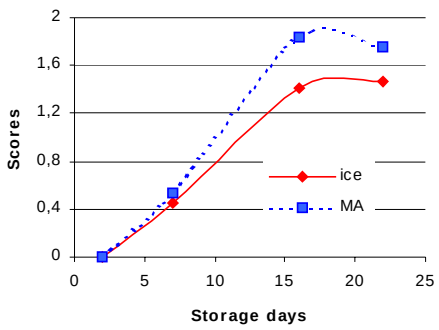

Figure 5: Average scores from QIM scheme given for appearance of skin. On day 2, fish were scored 0 with bright iridescent pigmentation; on day 7 both fish trials were scored 0-1. Score 1 meaning that the fish skin pigmentation is becoming discoloured. On days 16 and 22, both fish trials were scored 1-2 which means the fish skin appearance was becoming discoloured or dull. The MA fish skin presented higher scores than fish stored in ice on days 16 and 22.

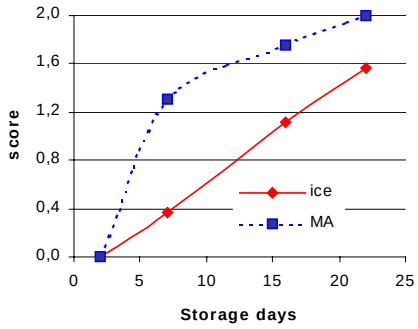

Figure 6: Average score from QIM scheme given for colour of eyes. On day 2 fish were scored 0 (black colour of eyes pupil); On day 7, 16 and 22 eyes of fish stored in MA were scored 1-2 (opaque to grey pupil colour). On day 7 fish stored in ice were scored 0-1 and 1-2 on days 16 and 22 respectively. Score 1 means opaque pupil eye colour. Score 2 means grey pupil eye colour. Fish stored in MA presented higher scores than fish stored in ice.

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Table 5: Statistical analysis of Quality Index scores given for appearance of the skin using the QIM scheme (average QI scores with standard deviation).

Storage days	Average score ice	Average score MA	Significance
2	0 (0.00)	0 (0.00)	-
7	0.44 (0.24)	0.54 (0.25)	NS
16	1.41 (0.05)	1.84 (0.06)	0.01
22	1.47 (0.34)	1.75 (0.43)	NS

There was not a significant difference for the appearance (colour of skin) between ice and MA samples except on day 16 where skin colour score of MA samples is higher than in iced samples.

Table 6: Statistical analysis of Quality Index scores given for colour of the eyes using the QIM scheme (average QI scores with standard deviation).

Storage days	Average score ice	MA	Significance (p)
2	0.00 (0.00)	0.00 (0.00)	-
7	0.37 (0.12)	1.30 (0.28)	0.01
16	1.11 (0.38)	1.76 (0.10)	NS
22	1.58 (0.16)	2.00 (0.00)	0.04

The table shows significant differences in eyes colour between the two trials. MA fish samples received higher scores than iced fish samples.

4.2 Torry

The results of assessment of cooked fish (Figures 7 and 8, Tables 7 and 8) show that there is a statistical difference between Torry scores for flavour and odour of the two trials at storage day 16 and 22. Fish stored in MA maintained better eating quality (higher Torry scores) than fish stored in ice during storage days 7 and 22. Fish stored in ice maintained better eating quality than fish stored in MA on day16.

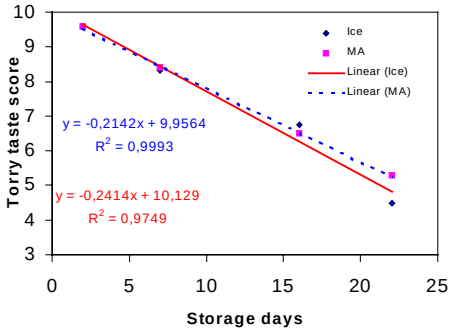

Figure 7: Average Torry flavour scores of cooked red fish fillets stored in ice and MA based on evaluation of 10-11 judges. The figure also shows the linear relationship between Torry score and storage time in ice and MA.

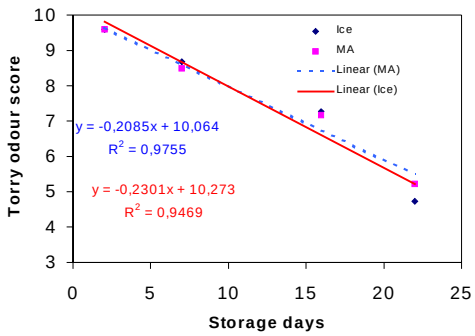

Figure 8: Average Torry odour scores of cooked red fish fillets stored in ice

and MA based on evaluation of 10-11 judges. The figure also shows the linear relationship between Torry score and storage time in ice and MA.

Table 7: Statistical analysis of Torry sensory scores for flavour of cooked red fish fillets stored in ice and MA (averages with standard deviation).

Storage days in ice	Average score ice	Average score MA	Significance (P)
2	9.58 (0.41)	9.58 (0.41)	-
7	8.33 (1.02)	8.40 (0.87)	NS *
16	6.76 (1.30)	6.50 (1.48)	0.001
22	4.50 (1.36)	5.28 (1.27)	0.010

* Not significant

The table shows significant differences of flavour between the two trials on days 16 and 22. Fish stored in MA had better eating quality (higher scores) than fish stored in ice on day 22, whereas on day 16 fish stored in ice maintained better eating quality (higher scores) than fish stored in MA.

Table 8: Statistical analysis of Torry sensory scores for odour of cooked fish fillets stored in ice and MA (averages with standard deviation).

Storage days in ice	Average score ice	Average score MA	Significance (P)
2	9.60 (0.50)	9.60 (0.50)	-
7	8.68 (0.65)	8.50 (0.84)	NS *
16	7.26 (1.47)	7.15 (0.99)	0.021
22	4.74 (1.26)	5.21 (1.07)	0.011

* Not significant.

The table shows significant differences of odour of fish stored in ice and MA on days 16 and 22. Iced fish maintained better odour than MA samples on day 16. MA samples maintained better odour than iced fish on day 22.

The shelf life of fish stored in ice was 19 days (Figure 9) whereas shelf life of fish stored in MA was 21 days (Figure 10).
Figures 9 and 10 show the combination of grading raw fish using QIM scheme and assessment of cooked fish using Torry scale during storage days. A score of 5.5 is used as the limit of acceptability as shown with the dashed line.

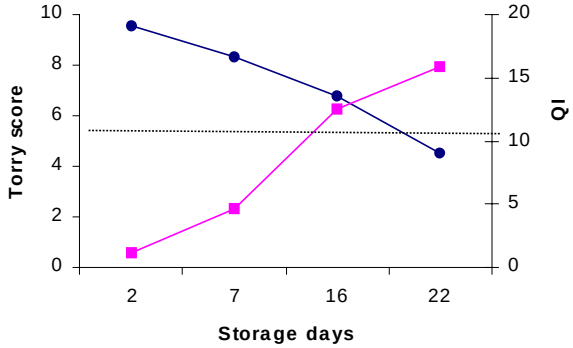

Figure 9: Combination of grading raw fish (squares) and assessment of cooked fish stored in ice (circle). Eating quality of the fish was regarded as unacceptable on storage day 19, when the Torry score reached the limit of acceptability (5.5), shown as a dashed line.

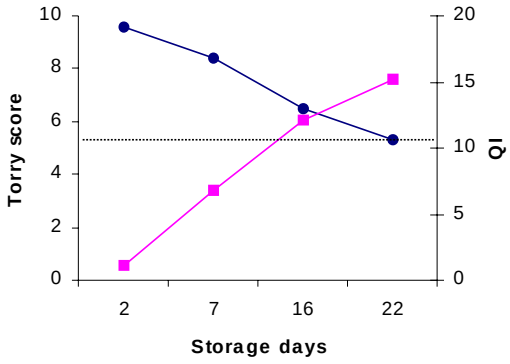

Figure 10: Combination of grading raw fish (squares) and assessment of cooked fish stored in MA (circle). Eating quality of the fish was regarded as unacceptable on storage day 21, when the Torry score reached the limit of acceptability (5.5), shown as a dashed line.

4.3 Microbiological counts

There was significant difference between the two trials regarding H_2S-producing bacterial counts on day 22 (Table 9), and *Pseudomonas* count on day 7 (Table 12). The H_2S-producing bacterial counts were very low at the beginning of storage in iced samples and absent in MA samples. On day 16 and 22 the counts were higher in iced samples than in MA fish (fFgure 11).
Total viable counts (TVC) in iced samples were higher than in MA samples (Figure 12). When the fish reached the limit of acceptability (sensory score 5.5 Figure 7) TVC in both trials had reached levels of 10^6-10^7 cfu/g in iced samples and 10^2-10^3 in MA samples.
Pseudomonas were absent on day 2. They were present from day 7 in iced fish and from day 16 in fish stored in MA. The *Pseudomonas* count was higher in fish stored in ice than in MA (Figure 12).

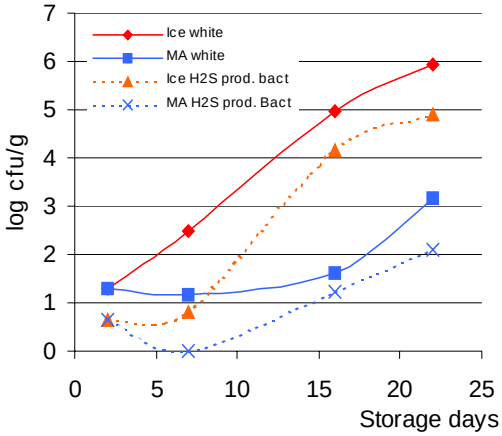

Figure 11: Relationship between growth of H_2S producing bacteria (spoilage bacteria) shown with dashed lines and non-spoilage micro organisms (white colonies) shown with solid lines, during storage of red fish in ice and MA. H_2S-producing bacterial counts in iced samples were low in the beginning of storage and absent in MA samples. In both trials the number of H_2S producing bacteria constituted a larger percentage of total counts at the end of shelf life and was higher in ice fish than in MA fish.

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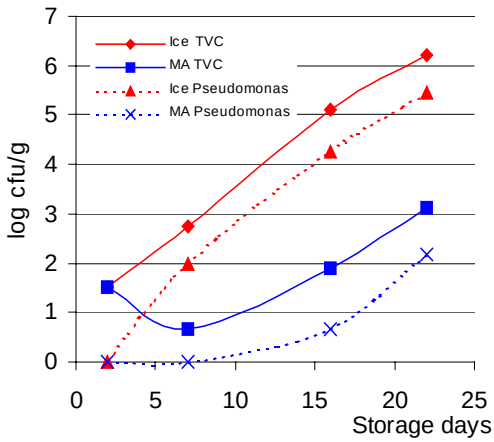

Figure 12: Changes in Total viable counts on L&H agar (solid lines) and *Pseudomonas* on CFC-agar (dashed line) during storage of red fish in ice and MA. On day 22 TVC in ice samples were higher than in MA samples (10^7cfu/g in iced fish and 10^2-10^3 cfu/g in MA fish). *Pseudomonas* were absent on day 2. *Pseudomonas* counts were present in iced fish from day 7 and from day 16 in MA fish. On days 6 and 22 *Pseudomonas* count were higher in fish stored in ice than in MAP.

Table 9 shows statistical differences on H_2S-producing bacteria counts on day 22 between fish stored in ice and MA. Fish stored in ice have higher counts (10^4-10^5) than MA fish (10^2-10^3).

Table 9: Statistical analysis of H_2S-producing bacteria counts (averages with standard deviation).

Storage days	average (log cfu/g) ice	average (log cfu/g) MA	Significance (p)
2	0.65 (0.92)	0.65 (0.92)	-
7	0.80 (1.13)	0.00 (0.00)	NS*
16	4.16 (0.65)	1.24 (1.75)	NS*
22	4.89 (0.16)	2.09 (0.12)	0.01

Table 10 shows that there was not a significant difference between the two trials regarding non-spoilage micro organisms (white colonies count).

Table 10: Statistical analysis of non-spoilage micro organisms (white colonies counts) on iron agar (averages with standard deviation).

Storage days	average (log cfu/g) ice	average (log cfu/g) MA	Significance (p)
2	1.30 (0.00)	1.30 (0.00)	-
7	2.49 (0.16)	1.15 (0.21)	NS*
16	4.97 (1.07)	1.63 (0.89)	NS*
22	5.95 (0.12)	3.16 (0.45)	NS*

Table 11: Statistical analysis of total viable counts on L&H agar (averages with standard deviation).

Storage days	average (log cfu/g) ice	average (log cfu/g) MA	Significance (p)
2	1.50 (0.71)	1.50 (0.71)	-
7	2.74 (0.11)	0.65 (0.92)	NS*
16	5.11 (0.33)	1.89 (0.83)	NS*
22	6.22 (0.14)	3.13 (0.45)	NS*

NS* Not significant

TVC was not significantly different between iced fish and MA fish during storage (Table 11).

Table 12: Statistical analysis of *Pseudomonas* counts on CFC agar (averages with standard deviation).

Storage days	average (log cfu/g) ice	average (log cfu/g) MA	Significance (p)
2	0.00 (0.00)	0.00 (0.00)	-
7	2.00 (0.06)	0.00 (0.00)	0.01
16	4.25 (0.06)	0.65 (0.92)	NS*
22	5.45 (0.19)	2.18 (0.67)	NS*

NS* Not significant

On day 7 there was a significant difference regarding *Pseudomonas* counts between fish stored in ice and MA, where iced fish had levels of 10^2cfu/g but were absent in MA fish.

4.4 Chemical measurements

Differences in TMA TMAO and TVB values between ice and MA red fish were not significant ($p>0.05$) (Tables 14, 15 and 16).
The pH of MA samples remained lower than in ice samples during the whole trial. Initial pH values decreased until day 7 and then increased with both trials except for MA samples which decreased again from day 16 (Figure 13).
TVB increased more rapidly and remained higher in the ice storage samples than in MA samples (Figure 15).
TMA values were similar in both trials until day 7. From day 16 TMA amount in iced fish increased whereas, decreased in MA fish (Figure 15).
TMAO in both trials decreased up to day 16. From day 16 TMAO started to increase slightly in ice but some in MA samples (Figure 14).

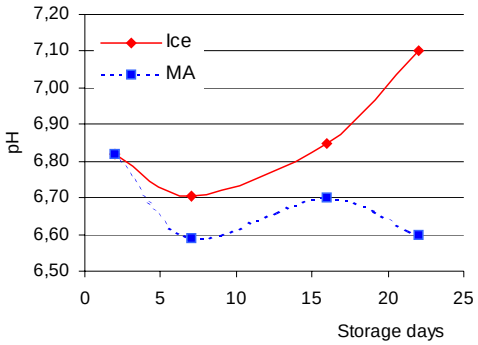

Figure 13: Changes in pH during storage of ungutted red fish stored in ice and MA. The pH values decreased until day 7 and then increased in both trials whereas, the pH decreased again in MA samples from day 16.

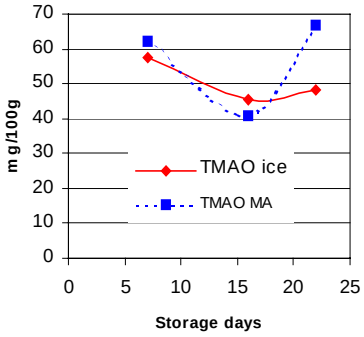

Figure 14: Changes in trimethyl-amineoxide (TMAO) during storage of ungutted red fish stored in ice and MA. The figure shows decreasing TMAO up to day 16 in both trials. From day 16 TMAO started to increase in fish stored in ice reaching levels of 48.04 mg/100g on day 22. From day 16 there is a slight increase in MA samples reaching levels of 66.77 mg/100g on day 22.

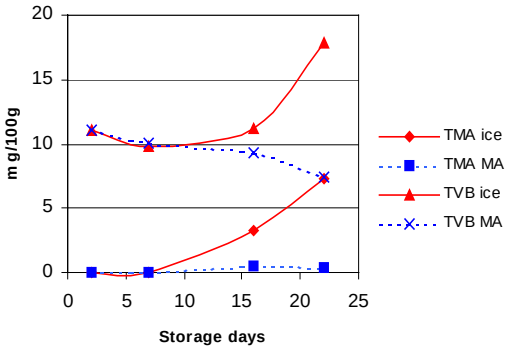

Figure 15: Changes in total volatile bases (TVB) and trimethylamine (TMA) during storage of ungutted red fish stored in ice and MA. The figure shows TVB values increasing during storage of iced fish and decreasing during storage of MA fish. TVB values in iced fish were higher than those on fish stored in MA. TMA values were similar in both trials until day 7. From day 16 TMA amount in iced fish increased whereas decreased in MA fish.

During storage time no significant difference between pH of iced fish and MA was found (Table 13).

Table 13: Statistical analysis of pH measurements (averages with standard deviation).

Storage days	average Ice	average MA	Significance (p)
2	6.8 (0.04)	6.8 (0.04)	-
7	6.7 (0.01)	6.6 (0.03)	NS*
16	6.9 (0.07)	6.7 (0.00)	NS*
22	7.1 (0.14)	6.6 (0.14)	NS*

NS* Not significant

No significant difference was found between iced fish and MA fish regarding TMA (Table 14).

Table 14: Statistical analysis of TMA amount (averages with standard deviation).

Storage days	average (mg/100g) ice	average (mg/100g) MA	Significance (p)
2	0.02 (0.00)	0.02 (0.00)	-
7	0.03 (0.00)	0.03 (0.00)	NS*
16	3.29 (1.79)	0.46 (0.02)	NS*
22	7.33 (4.75)	0.44 (0.13)	NS*

NS* Not significant

There were no significant differences on TMAO of fish stored in ice and MAP (Table 15).

Table 15: Statistical analysis of TMAO amount (averages with standard deviation).

Storage days	average (mg/100g) ice	average (mg/100g) MA	Significance (p)
7	57.39 (6.02)	62.17 (9.77)	NS*
16	45.63 (1.22)	41.01 (1.53)	NS*
22	48.04 (12.85)	66.77 (2.03)	NS*

NS* Not significant

There were no significant differences on TVB of fish stored in ice and MAP (Table 16).

Table 16: Statistical analysis of TVB amount (averages with standard deviation).

Storage days	average (mg/100g) ice	average (mg/100g) MA	Significance (p)
2	11.08 (1.36)	11.08 (1.36)	-
7	9.81 (0.45)	10.12 (0.00)	NS *
16	11.18 (0.79)	9.27 (0.35)	NS *
22	17.88 (5.49)	7.45 (0.99)	NS *

*NS Not significant

5. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The QI scores showed there was a significant difference between iced and MA fish on sampling day 7. The significantly higher QI scores given for MA fish in storage day 7 may be because of changes in skin colour and form of eyes as was reported by the panellists for the MA fish. These changes in colour of fish stored in MA are not surprising. Huss (1995) has reported discoloration as a negative effect of CO_2 on ungutted fish colour. Ooraikul and Stiles (1991) reported discoloration of beef as the effect of MA.

The higher scores given for the eyes of the fresh MA stored fish, implies that the descriptions and scores for colour of pupil by the QIM scheme for red fish, are not accurate enough for red fish stored in MA, whereas, the eyes of MA red fish looked spoiled (score 2) before they were actually spoiled. It can therefore, be concluded that the Quality Index Method (QIM) scheme for red fish (*Sebastes mentella, S.marinus*)

is not suitable for red fish stored in MA because of rapid changes in eyes and skin colour that occur during MA storage. To overcome this problem, it is necessary to apply different descriptors to describe the changes that occur in colour of eyes and skin of MA stored red fish.

The shelf life of red fish stored in ice was similar to what has been reported by Rehbein et al. (1994) for red fish (*Sebastes spp*) stored in ice, i.e. 3 weeks. The results reported here are also similar to what has been reported by Dalgaard et al. (1997) for the shelf life of MA stored red fish which was rejected at the 21st storage day.
In this experiment, MA gave a shelf life extension of 2 days over the ice fish. The high CO_2 resistance of *P. phosphoreum* explained the modest shelf life extension of MA cod. It can therefore, be concluded that MA can extend shelf life for red fish under the conditions of this experiment.
The level of *Pseudomonas* in ice samples was always higher than in MA samples. It supports the idea that under aerobic iced storage, the flora is composed almost exclusively of *Pseudomonas sp.* and *S. putrefaciens* (Gram et al. 1987).
The decrease of pH during the first days of storage could be explained by "*post mortem*" glycolysis that results in the accumulation of lactic acid, which in turn lowers the pH of the muscle. Huss (1995) has reported pH drops from 6.8 to 6.1-6.5 in cod and from 6.8 to 5.4-5.6 in tuna and halibut.
The higher increase of Total Volatile Bases (TVB) in fish stored in ice compared to fish stored in MA is not surprising since the TVB are produced mainly by H_2S producing bacteria which were in much higher amounts in iced fish than in MA fish. At the rejection time TVB had not reached the concentration of around 25 mg/100g. Commission decision of 8 March 1995 fixed this value as the limit for rejection of lots (Oehlenschlager 1997), whereas in this study TVB and TMA values are not a criterion for unspoilt fish.
It can be concluded that in this study, TVB and TMA measures have not provided a useful index of shelf life.

For MA stored red fish it is necessary to revise the existing QIM schemes for iced red fish. For development of QIM scheme for Mozambican species, it would be necessary to finish the construction and equipment of the new laboratories in Maputo, Beira and Quelimane which are scheduled to be ready by the first term of this year. In order to ensure defined characteristics for different storage time to be incorporated in the QIM, preliminary studies must be conducted in an appropriate way. It is also recommended to have manuals to support QIM in fish inspection and production. The manuals must contain the total plan for evaluation, explanation of the evaluation terms, and colour photos illustrating the different levels of freshness during the storage time of fish.

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Appendix 1

Quality Index Method (QIM) scheme for Redfish *(Sebastes mentella/ Sebastes marinus)*

Quality parameter		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
	Colour of pupil	Black	0
		Opaque	1
		Grey	2
Gills:	**Colour**	Blood red	0
		Reminds of beef	1
		Reddish areas	2
		Rusty, dark brown	3
	Smell	Fresh, seaweedy, metallic	0
		Neutral, grassy, musty	1
		Yeast, bread, beer, sour milk	2
		Acetic acid, sulphuric, very sour	3
	Mucus	Clear	0
		Milky	1
		Discoloured, rusty, brown, clotted	2
Viscera:	**Solution**	Whole	0
		Beginning to dissolve	1
		Viscera dissolved	2
Fillets:	**Colour**	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
Quality Index (0-23)			

Appendix 2:

Torry scheme for medium fat fish (such as red fish)

- a freshness score sheet for cooked fish-

Score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver Watery, metallic
9	Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver Sweet, meaty, characteristic
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity
7	Woodshavings, woodsap, vanillin	Neutral
6	Condensed milk, Boiled potato	Insipid
5	Milk jug odours, boiled clothes-like	Slight sourness, trace of "off"-flavours, rancid
4	Lactic acid, sour milk, TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid
3	Lower fatty acids (e.g. acetic or butyric acids) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid