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QUALITY INDEX METHOD (QIM) FOR FROZEN-THAWED ATLANTIC MACKEREL (*Scomber scombrus*) STORED IN ICE: DEVELOPMENT AND APPLICATION IN A SHELF LIFE STUDY

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ABSTRACT

The sensory methods are the most accurate and widely used to evaluate fish freshness and the Quality Index Method (QIM) is an objective, rapid and reliable sensory method. The aim of the present study was to develop and evaluate a QIM scheme for frozen-thawed Atlantic mackerel (*Scomber scombrus*) stored in ice. After thawing, the mackerel was stored at 0 °C on ice up to nine days. The QIM scheme and a vocabulary for generic descriptive analysis (DA) were developed during a pre-observation and panel training. During storage, changes of raw whole fish and cooked fillets were observed and analysed using the developed QIM scheme and DA, respectively. Moreover, the amount of histamine, total viable counts (TVC) and counts of H_2S-producing bacteria were estimated. As a result of this study, a QIM scheme to evaluate freshness of frozen-thawed Atlantic mackerel storage in ice is proposed. A significant linear relationship between Quality Index (QI) and the storage time on ice was obtained. The maximum storage time on ice was four-six days according to DA of cooked fillets, mainly due to rancidity. The storage time could be estimated with an accuracy of ± 2.3 days using the QIM scheme. The TVC and H_2S-producing bacteria increase during the storage time (nine days), but their values were low. The histamine content was below 5 ppm during the nine days of storage.

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1 INTRODUCTION

The Republic of Cape Verde is an archipelago, which consists of 10 volcanic islands. It is located about 500 km off the west coast of Africa, with a total area of 4.033 Km^2 and a coastline of 1040 km. The EEZ is around 734 665 km (Bravo de Laguna 1985). The projection of the population of Cape Verde in 2009 was 508 633 inhabitants (INE 2009).

The fisheries sector plays an important socio-economic role in Cape Verde, because it provides a big part of the needed animal protein intake. It is also a source of income for families and the government creates revenues from fishing rights and fish export. The *per capita* consumption is 26 kg/year. Fisheries contribute in Gross Domestic Product (GDP) around 2% (INE 2009), employ 10% of labour force, and 30% of income exportation (Lopes *et al.* 2006). The main market of export is EU. To evaluate fish freshness, the Cape Verdean's Competent Authority of control and sanitary certification of fish and fish products use the EU scheme (*Portaria* 06/2001).

The pelagic species, like tuna and various smaller pelagic species, dominate the fisheries in Cape Verde, constituting to of around 80% of the total catch. The small pelagic big eye scad (*Selar crumenophthalmus*) and mackerel (*Decapterus macarellus*), which are particularly important for Cape Verdean's diet, are also utilized as raw material in the canning factories. These resources are taken with seine gears, both in the semi-industrial and artisanal fisheries. Due to insufficient supply of raw materials by local boats, the main canning factory in Cape Verde imports frozen scombridae, including Atlantic mackerel (*Scomber scombrus*), which later is exported to EU.

None of these pelagic has been caught in Iceland. However, for the past two years due to changes in the migratory patterns (possibly linked to increased sea temperature) the Atlantic mackerel *S.scombrus* has been caught in Iceland's EEZ.

In general, it has become more common to thaw fish before it is sold in recent years. Usually frozen fish has a lower price than frozen-thawed fresh fish, with may have the same price as the fresh just-caught fish (Baixas-Nogueras 2007). Several studies have been done on the effect of freezing on the quality of fish. However, less attention has been given to the changes of frozen-thawed fish (sensorial, microbial, chemical and physical) during storage in ice (Magnusson and Martinsdottir 1995).

Atlantic mackerel was chosen as a subject of this study, as it is a pelagic species similar to the mackerel that is found in Cape Verde, one of the most important fisheries resources in the country.

For sensory evaluation of the freshness of Atlantic mackerel, Andrade *et al*. (1997) developed a Quality Index Method (QIM) for fresh Atlantic mackerel. Considering the intrinsic differences between fresh and frozen-thawed fish a QIM scheme for Atlantic mackerel needs to be developed.

Atlantic mackerel is one of the fish that can develop histamine, scombrotoxin, as a result of temperature abuse during storage and can cause consumer illness. Certain bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with histidine, a natural occurring amino acid that is present in larger quantities in the following fish families: S*combridae*, *Clupeidae*, *Engraulidae* and *Coryphaenidae.*

The main aim of this project was to learn about sensory evaluation and to know how to apply sensory evaluation to Cape Verde fish and fish products. This work also contributes to an improved quality management system of seafood in Cape Verde, especially of the scombridae, for the local and export market.

This knowledge will be obtained through the development and evaluation of a QIM scheme to measure the freshness of frozen-thawed Atlantic mackerel, using a trained sensory panel. Moreover the aim was to study the quality changes of frozen-thawed Atlantic mackerel stored at 0°C, observing the sensory changes of the fish raw and cooked, the bacterial growth and histamine concentration changes and estimate the maximum shelf life of frozen-thawed Atlantic mackerel stored at 0 °C.

The project was carried out in two parts:

1) A pre-observation of frozen-thawed Atlantic mackerel was carried out using QIM schemes that had already been developed to evaluate the freshness of Atlantic mackerel (Andrade *et al.* 1997) and herring (Martinsdóttir *et al.* 2001). A draft QIM scheme was made for frozen-thawed Atlantic mackerel, and histamine was measured. The shelf life of cooked Atlantic mackerel storage at 0°C was estimated (approximately).

2) A full-scale shelf life study was conducted. Before the shelf life study, ten sensory assessors were trained using the draft QIM scheme and descriptive analysis (DA). During that time, the development of the QIM scheme for frozen-thawed Atlantic mackerel was finalised. In the shelf life study, the quality changes during the storage time were evaluated with DA, QIM, microbiology and chemical analysis of histamine, and the maximum shelf life of frozen-thawed mackerel storage at 0°C was estimated.

2 LITERATURE REVIEW

2.1 Atlantic Mackerel

The Atlantic mackerel *Scomber scombrus* (Linnaeus 1758) (Figure 1) is distributed in North Atlantic Ocean, including the Baltic Sea, eastern Atlantic including the Mediterranean and the Black sea, also in western Atlantic from Labrador to Cape Lookout. It is an epipelagic and mesodemersal species and most abundant in cold and temperate shelf areas (Collette and Nauen 1983). The maximum fork length is 50 cm, but the most common length is around 30 cm. Females become bigger than males (Collette and Nauen 1983).

The most important fishing areas for *S.scombrus* are the Northwest and Northeast Atlantic, Mediterranean and Black Sea. The mackerel school is mainly caught with purse seines. The Atlantic mackerel is traded fresh, frozen, smoked and canned (Collette and Nauen 1983).

Figure 1: Atlantic mackerel (*Scomber scombrus*) used in this project.

The chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning (Huss 1995). The edible portion of mackerel is composed of 18.7% protein and 11.4% fat (FAO 1989). The Atlantic mackerel is considered a fatty fish species.

Usually, small and medium size fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is in part that a large number of small fish are caught at the same time and because of problems with discoloration and the acceleration of rancidity. In general these species are chilled or frozen whole soon after capture. Storage time depends on the fat content of the fish and the amount of food in the gut. Shelf life of fat fish species is generally shorter (around 2-8 days) than of low fatty fish species (7-15 days) (Huss 1995, Martinsdóttir *et al*. 2001)

2.2 Fish freshness

Fish freshness refers to the condition of fish that has recently been caught and used in the context of unfrozen or unprocessed, but sometimes 'fresh frozen' is used for fish that is frozen shortly after catch/very fresh (Bremer 2002).

Because the fish is a very perishable product, and due to the short shelf life of fish, supplies of fish can be unstable. Freshness is the one of the most important aspects of fish and fish products and it is a key element in the quality assessment of fish by consumer (Luten and Martinsdottir 1997, Martinsdottir 2004). The freshness of the raw material used for processing is essential for the overall quality of the final product (Oehlenschlager and Sorensen 1997). The freshness of seafood can be evaluated by chemical, physical, microbiological or sensory methods (Olafsdottir *et al.* 1997). However, the sensory analysis has been the primary way to evaluate seafood freshness by the fisherman, producers, researchers and consumers.

Temperature and handling practices are the most important factors in determining the shelf life of the fish. If the fish is handled carefully and the temperature kept low and stable the shelf life is extended. The rate of bacterial spoilage and the enzyme breakdown is depending on the temperature (Doyle 1995).

Certain species deteriorate faster than others mainly due to the presence of chemical constituents in their body tissue (Jhaveri *et al.* 1982). Handling of fresh mackerel has remained a problem because of its soft flesh, high lipid content and the skin of fatty pelagic fish is often very thin, this allows enzymes and bacteria to penetrate more quickly (Hyldig *et al.* 2007).

Shelf life of food is defined as the maximum length of time a given product is fit for human consumption. Shelf life of fish is the time from when it is caught until it is no longer fit to eat. In marketing, the shelf life of fresh and frozen fish is a very important consideration

(Doyle 1995). Information on the shelf life of fresh Atlantic mackerel indicates that it can range from 5–21 days depending on how it was kept. Jhaveri *et al.* (1982) reported that Atlantic mackerel could keep for 9 days on ice, but Xing *et al.* (1993) indicated that the shelf life was only 5-7 days at the same storage conditions, as by that time the stage of deterioration would probably be unacceptable in the North American market. However, for Atlantic mackerel fillets stored at -2 °C and under CO_2 modified atmosphere (MAP) the shelf life can be extended up to 21 days (Hong *et al.* 1996). Frozen mackerel, properly glazed and kept in cold storage at -30°C, will keep in good condition for at least 6 months. However, cold storage life can be extended up to one year when mackerel is packed in polyethylene bags topped up with water and frozen in vertical plate freezers. The block is doubly protected against deterioration in store by the encasing ice and by the packaging (Keay 1979). According to the same author gutting before freezing is not necessary, and may be a disadvantage.

2.3 Sensory Evaluation

The human senses have been used for centuries to evaluate the quality of foods. We form judgments about foods whenever we eat or drink. But this does not mean that all judgments are useful or that anyone is qualified to participate in a sensory test (Lawless and Heymann 1999). Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of food as perceived through the sense of sight, smell, taste, touch and hearing (Stone and Sidel 1985; Huss 1995). Subjective tests are based on a measure of preference or acceptance. They can be applied in the fields such as market research and product development where the reaction of the consumer is needed (Huss 1995).

The principles and practices of sensory evaluation involve each of the four activities mentioned in definition above, quoted from Lawless and Heymann (1999):

1) "to evoke" – give guidelines for the preparation and serving of samples under controlled conditions to minimize the biasing factors;
2) "to measure" – numerical data are collected to establish lawful and specific relationship between product characteristics and human perception;
3) "analysis" - proper analysis of data is a critical part of sensory test (there are many sources of variation in human responses that cannot be completely controlled in a sensory test);
4) "interpretation of results" – because it is an experiment, data and statistical information are only useful when interpreted in the context of hypotheses, background knowledge, and implication for decisions and actions to be taken.

The analytical objective test used in quality control can be discriminative tests or descriptive tests. Discriminative tests are used to determine if a difference exists between samples (triangle test, ranking test) and descriptive tests are used to determine the nature and intensity of the differences (profiles and quality tests) (Lawless and Heymann 1999, Huss 1995).

Sensory evaluation is the most important method today for freshness evaluation in the fish sector (Olafsdottir *et al.* 1997, Martinsdóttir 2004) and the fish inspection services. Seafood is also inspected by sensory methods when is imported. Sensory evaluation of the fish is usually done by assessing the appearance, texture and odour of raw fish, and

also flavour when fillets are cooked. In the fish sector, it is very important to study sensory changes during different conditions of storage of various fish species (lean/fat/flat fish).

There are many methods developed to evaluate the freshness of whole fish and fillets. The most common methods are the European Union (EU) scheme, Quality Index Method (QIM) scheme, Torry scheme and generic descriptive analysis (DA).

EU scheme

The European Union (EU) adopted an official method for sensory evaluation of raw fish freshness, named EU scheme (Regulation (EC) 2406/96). This method is applied to evaluate the freshness of fish in Cape Verde.

The EU scheme employs parameters, which can be assessed through the three senses of sight, smell and touch: skin colour, flesh texture, the shape of the eyes and the colour and odour of gill. The grade is express through tree freshness category: E = high quality, A = good quality, B = low quality and C = fish are not fit human consumption and are therefore discarded.

There are different versions for certain groups of products: whitefish, bluefish, cephalopods and crustaceans. This method does not take into account the differences between species as it only uses general parameters and there are also problems with mixing subjective and objective sensory in the scheme (Luten and Martinsdottir 1997**,** Martinsdottir 2002, Hyldig *et al.* 2007**).** Also in the EU scheme there is a risk that one or more description dominate the overall result (Martinsdottir *et al.* 2001).

Quality Index Method (QIM)

The Quality Index Method (QIM) is a sensory evaluation method based upon a scheme originally developed by the Tasmanian Food Research Unit in Australia (Martinsdottir 2004) that has several unique characteristics for sensory evaluation of the fish freshness.

This method is based on characteristic changes that occur in raw fish, in appearance (eyes, skin, and gills), odour and texture, and a well defined score system from 0 to 3 demerit (index) points. The descriptions of each score for each parameter are listed in the QIM scheme. The panellist need evaluate all the parameters defined in the scheme. The scores for all of the characteristics are summarised to give an overall sensory score, named Quality Index (Nielsen 1997, Martinsdottir 2002).

The Quality Index increases linearly with storage time in ice so this information can be used in production management and may be used to predict the remaining shelf of fish (Luten and Martinsdottir 1997). The QIM is well suited to teach inexperienced people to evaluate the fish freshness, train assessors and monitor their performance (Martinsdottir 2002). This sensory method has great advantages; can be very fast, reliable, non-destructive on raw fish and no expensive instruments are needed (Martinsdottir 2002). Further, the QIM method has advantages when compared to the EU scheme, the QIM method is specific for each species and the fluctuation between assessors is diminished (Olafsdottir *et al*. 1997)

QIM-schemes have to be developed for each fish species (Martinsdottir 2004). The unique QIM-scheme for fresh Atlantic mackerel was developed by Andrade *et al.* (1997).

Development of QIM scheme

The methodology to develop and evaluate a QIM scheme is described by Sveinsdottir *et al.* 2003 and Bonilla *et al.* 2007, as below:

- A preliminary QIM scheme for sensory evaluation of fresh fish is designed during a pre-observation. The changes occurring in fish from the first day storage until spoiled are observed and registered.

- The fish of varying freshness are observed during the first days of training. In this phase the panellists know the freshness of the fish and a preliminary scheme is explained.

- In the days following the panellists receive further training and the scheme was developed. The storage time of each fish is unknown to the panellists. The panel uses the scheme developed during pre-observation of the fish and some changes are made in the scheme during the training sessions. In the end the panel is informed about the storage time. All suggestions of improvements by the panellists are considered during the development of the QIM scheme.

During the final training session, the final version of the scheme is presented to the panel. The next step is conducting a full-scale shelf life. Throughout the storage trial, each time a new sample is taken. The panel evaluates blind coded samples in random order using QIM scheme. At same time, during storage time, chemical and microbiological indices might be measured to follow the spoilage pattern and may be use for comparison. In parallel, sensory evaluation of cooked samples should be performed to estimate the reasonable maximum shelf life. Analysis of the results from the shelf life studies is an important part of the development and linearity of the Quality Index with storage time should be checked (Martinsdottir *et al.* 2009).

Descriptive Analysis (DA)

Generic Descriptive Analysis (DA) is a sensory method that can be very simple and used for assessment of a single attribute of texture, flavour and appearance (Huss 1995). However, descriptive analysis can also be used to provide a complete sensory description of products and be used as a basis for determining the sensory attributes most important for consumer acceptance (Stone and Sidel 2002). The maximum storage time of fish can be determined by the sensory evaluation of cooked samples using DA. The results of this evaluation may be used as a reference to estimate shelf life when developing a QIM scheme for fresh fish (Sveinsdottir *et al.* 2003)

When the DA profiles are created the panellists make a list of attributes describing the product under the guidance of a panel leader. They are trained in using an unstructured scale for each of the attributes, before participating in a sensory analysis of the product to be tested (Sveinsdottir *et al.* 2003).

2.4 Histamine content

Histamine poisoning is a food-borne chemical intoxication resulting from the ingestion of food that contains unusually high levels of histamine. Histamine in foods occurs from the amino acid, L-histidine, by an enzymatic decarboxylation reaction catalyzed by histidine decarboxylase. The scombroid fish are commonly involved in histamine poisoning because they possess large amounts of free histidine in their muscle tissues that serve as a substrate for bacterial histidine decarboxylase (Okuzumi *et al.* 1982, Taylor 1986).

The consumption of scombridae (tuna, mackerel, skipjack) and scomberosocidae (Atlantic saury, mackerel pike) families and other marine fish such as mahi mahi can result in histamine poisoning. This can happen as a result of time and temperature abuse and inappropriate handling. To prevent this intoxication, the fish must be rapidly cooled down to a temperature as close to 0°C as possible after catch, and a high standard of handling (Good Manufacturing Practices and Good Hygiene Practices) during processing. The first part of curing period for fish that accumulate histamine should be done at temperatures between 0°C and 5°C to prevent development of histamine (Codex 2008).

It is not possible to eliminate the histamine when it has developed in the fish. Any lot that has demonstrated elevated levels of histamine should be destroyed or diverted to a non-food use.

Fish have been implicated in most of the outbreaks of histamine poisoning and the majority have been from scombroid fish. Tuna, mackerel and skipjack are most frequently involved, but this partially due to the greater consumption of those fish worldwide (Taylor 1986, Emborg 2007).

Only sporadic incidents of histamine poisoning were recorded in the first half of last century, probably because histamine poisoning was an unrecognized illness at that time (Taylor 1986). Only a few countries keep official records on incidents of histamine poisoning, and it can be assumed that many incidents in those and other countries, like Cape Verde, are not reported. However, since 1970 the countries with the most reported incidents of histamine poisoning are Japan, the U.S., and Great Britain and the less frequents incidents have been reported in various other countries like Canada, New Zealand, France, Norway, Australia, and South Africa (Taylor 1986, Emborg 2007).

Table 1 shows the data about the incidents after ingestion of seafood with high concentration of histamine.

The primary symptoms of histamine intoxication are cutaneous (rash, urticaria, oedema, localized inflammation), gastrointestinal (nausea, vomiting, diarrhoea), hemodynamic (hypotension) and neurological (headache, tingling, oral burning and blistering sensation etc.) (Taylor 1986, Huss *et al.* 2003). More serious complications such as cardiac palpitations are rare (Huss *et al.* 2003).

Table 1: Development of histamine fish poisoning (HFP) after ingestion of seafood with high concentration of histamine. The data are reported incidents where the concentration of histamine, the number of patients and persons at risk were provided (Emborg 2007).

		Number of people		Histamine	
Country	Year	Consuming product	Developing HFP symptoms (%)[a]	in product (ppm)	Product
France	1941	28	22 (79)	1,000-5,000	Tuna
Japan	1943	850	85 (10)	980	Mackerel
Japan	1953	11	11 (100)	5,220	Dried saury
Japan	1954	400	90 (23)	970-1,070	Dried saury
Japan	1995	111	50(45)	1,920-1,940	Tuna
USA	1968	9	8 (89)	4,255	Tuna
USA	1985	26	5 (19)	2,500	Bluefish
Japan	1998	40	21 (53)	400-7,300	Escolar
USA	2003	56	42 (75)	2,000-3,800	Escolar
Denmark	2003	16	8(50)	7,100-9,100	Tuna

[a] attack rate calculated as: cases * 100 / persons at risk.

Concerning food safety, the maximum level of histamine permitted in Cape Verde and EU is 100 ppm (regulation 25/2009 and regulation 2073/2005, respectively) and the analytical reference method is the HPLC (High-Performance Liquid Chromatography). To determine histamine it is necessary to take nine samples of each lot to fulfil the demands. From the nine samples collect the average should not be higher at 100 ppm; two of them may have histamine value between 100-200 ppm and none of the samples may be over 200 ppm. However, the US FDA (United States, Food and Drug Administration) use the maximum level of histamine allowed to 50 ppm. It is a defect action level, because the histamine is generally not uniformly distributed in a decomposed fish. Therefore, if 50 ppm is found in one section, there is the possibility that other units may exceed 500 ppm (FDA 1996).

2.5 Microbiology analysis

The objective of microbiology analysis of fish products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of temperature abuse and hygiene during handling and processing (Huss 1995). In a newly caught fish, microorganisms are present on skin and gills, the muscle is sterile consequently the proportion and activity of specific spoilage bacterial in microbiological flora determines remaining shelf life (Oehlenschlager and Sorensen 1997).

The total number of organisms varies greatly. A normal range on skin surface is 10^2-10^7 cfu/cm^2. The gills and the intestines both contain between 10^3 and 10^7 cfu/g. 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 (Huss 1995).

Microbiological food poisoning is characterized by rapid onset of the illness (typically symptoms are nausea and vomiting) as the toxins are already formed in the food before consumption. Ingestion of viable bacteria is not a prerequisite for the induction of the

disease. Most often intoxications require that the toxin-producing bacteria have grown to high numbers (10^5 - 10^8 cfu/g) in the food before it is consumed (Huss *et al.* 2003).

The microbial measurements can be used to evaluate the freshness of fish. When such microbiological measurements are needed it is recommended to use the numbers of specific spoilage organisms (SSO) as well as classical total viable counts (TVC) measurements (Olafsdottir *et al*. 1997). With regard to fish freshness, it is recommended to collect flesh samples to estimate microbial counts as the microbial counts within the flesh have higher correlation to sensory evaluation of freshness.

3 METHODS

3.1 Mackerel samples

Atlantic mackerel caught off the south east coast of Iceland on August 2009 was kept in frozen storage at -24°C until they were used in these experiments started. Totally 162 fish were used for the experiments. After different storage time samples of mackerel were thawed at 3°C for about 24 hours and later stored on flake ice in boxes, covered with plastic sheets and stored in a chamber at 0 to 1°C. During the thawing and storage in ice, the temperature was monitored using loggers (Micro-T DS1922L from NexSens Technology - Dayton, OH, USA).

The storage time (days) on ice of the thawed Atlantic mackerel used for the pre-observation, panellists training and shelf life study is shown in Table 2. One hour before the sensory analysis, the mackerel was removed from cooling room.

Table 2: The storage time of frozen-thawed Atlantic mackerel used for sensory analysis on pre-observation, training and shelf life study.

Type of session	Date of sessions	Number of evaluated fish	Storage days
Pre-observation	December 7, 2009	3, 3	3, 5
	December 9, 2009	2*	7
	December 10, 2009	3,3	2, 6
	December 11, 2009	2*	9
	December 14, 2009	3,3,3	1, 6, 10
Panellists training	January 12, 2010	5, 5, 5	1, 4, 8
	January 14, 2010	5, 5, 5	3, 6, 10
	January 15, 2010	7, 7, 7	2, 4, 7
Shelf life study	January 19, 2010	4, 4, 4	1, 4, 8
	January 21, 2010	5, 4, 5	2, 6, 9

* Only two fish was used because from the sample A, one fish was used for weighing the other was spoiled.

For the QIM training and sensory evaluation during the shelf live study, raw frozen-thawed Atlantic mackerel were placed on white clean table at room temperature, under fluorescent light and absence of foreign odours. During the sensory evaluation the storage days were unknown to the panellists; each fish was coded with random three-digit number unrelated to storage time (Figure 2).

During the training and the shelf life study using the QIM scheme, the panellists evaluated skin colour and texture; gills colour, mucus and odour; eyes pupil and shape and viscera solution.

Figure 2: Atlantic mackerel coded with three-digit number and sensory evaluated by a panellist using QIM scheme.

For generic descriptive analysis (DA), training and sensory evaluation during the shelf life study, fillets were trimmed from belly part and tail part (Figure 3). The rest of fillet was cut in three or four small parts, depending on the size of the fish. Each piece was placed in aluminium boxes coded with three digit random numbers. The samples were cooked at 100 °C for six minutes in a pre-heated oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) with air circulation and steam. The panellists evaluated the samples in separated booths under fluorescent light, free of foreign odours and no interruption or distraction.

Figure 3: Fillet without the belly part and tail part to be cooked.

3.2 Sensory evaluation of raw frozen-thawed Atlantic mackerel (QIM)

This sub-chapter describes the methodology used to develop and evaluate the QIM scheme. The following method is based on the QIM scheme described by Martinsdottir *et al.* (2001) and Sveinsdottir *et al.* (2003).

Pre-observation

The objective of pre-observation was to get an idea about the sensory chances of frozen-thawed mackerel storage at 0 ºC. During five sessions, two persons observed and registered all changes occurring in thawed whole mackerel until spoiled.

The changes of general appearance, eyes, gills, belly, peritoneum and viscera were evaluated, with the aid of existing QIM schemes for similar products, such as fresh Atlantic mackerel (Andrade *et al.* 1997), fresh herring (Martinsdottir *et al.* 2001) and fresh chub mackerel (Barbuzzi *et al.* 2009). All changes in appearance, texture and odour during the observation were registered and pictures were taken of each parameter. Based on these observations, each description received a score in which 0 corresponded to fresh frozen-thawed Atlantic mackerel and the score increased according to spoilage with a maximum score of 3 for each parameter. This information was used to make a preliminary QIM scheme.

Training sessions

During this part of the experiment, 10 sensory panellists, employees of MATIS with years of experience and trained according the ISO 1993, were trained during three sessions using the preliminary QIM scheme. In each session, 9-15 mackerel of three different storage times (ranging from 1 to 9 days in ice) were observed.

In first training session, the panel used the scheme developed during pre-observation. The panel leader explained how to use the scheme and how to evaluate each quality parameter. During the evaluation the panellists had opportunity to ask questions concerning the evaluation at any time during the session.

After each session, the panel leader recorded the suggestions given by the panellists. Improvements of the scheme were made according to these suggestions. The panel was notified about these changes at the next session.

During the first two sessions the panel were informed about the storage time of the Atlantic mackerel. At the third session the panel used the last version of the scheme to evaluate the freshness of the fish. The samples were blind-coded with a random 3-digit number.

The final version of the scheme was finalized.

QIM in a shelf life study

The QIM scheme finalized at the training part was used to evaluate the Atlantic mackerel. The QIM evaluation was carried out during two days, in the morning and in the afternoon (4 sessions). During each session eleven panellists evaluated 2-3 blind-coded samples of

three different storage times. It was decided for two sessions per day the order to reduce the number of fish analysed in each session.

3.3 Sensory evaluation of cooked fillets (DA)

Training sessions

The same panel used for QIM was trained during three sessions to evaluate cooked Atlantic mackerel fillets with the generic descriptive analysis (DA) method (Stone and Sidel 2004) and was carried out parallel to the QIM. At the first two sessions the panel developed a vocabulary to describe odour, flavour, appearance and texture of the thawed cooked fillets of Atlantic mackerel under guidance of panel leader. During the last session the panellists described the intensity of each attribute using an unstructured scale (from 0 to 100%).

DA in shelf life study

The generic Descriptive Analysis (DA) was conducted in parallel to the QIM sessions. The trained panel evaluated during two days (4 sessions) Atlantic mackerel fillets, using the list of attributes developed during training. The cooked fillets were served blind-coded in a random order and a computerized system (Fizz, Version 2.10C Biosystemes) was used for data recording.

3.4 Chemical measurements

Before the DA evaluation, the histamine was performed in duplicate samples (two fillets from two fish of the same freshness/storage time). The histamine concentration was determined according the MATIS Laboratory HPLC (High-Performance Liquid Chromatography) method (Corbin *et al.* 1989, Gouygou *el al.* 1987), as described below:

Extraction of biogenic amines

Approximately 25 g of muscle of mackerel (sampled along the fillet) was weighted into a glass container and homogenized for 60 minutes in ca. 50 ml 10% TCA – Trichloroacetic acid. The ultra turrax homogenizer was used to homogenize the sample. The extract was filtered through Whatman 542 filter paper under vacuum, made in a volumetric flask and filtered through a 0.45 µm filter (millipore).

Derivatization

0.25 ml of sample filtered before was added 0.5 ml OPA reagent (o-phthaldialdehyde) in a test tube with a screwed cap. The solution was kept in dark for exactly 3.5 minutes. Then 2 ml ethylacetate is added and vortexed for 1 minute, until phase separation was completed. An aliquot from the top phase was pipetted in a vial.

HPLC analyse

The sample was run through reversed phase chromatographic column, using acetonitrile/natriumdihydrogenfosfat solvent gradient. After 40 minutes the results was obtained.

3.5 Microbial analysis

Muscle from two samples of mackerel with the same storage time was collected in parallel to QIM evaluation. The microbial load for 6 different storage times was determined.

First the skin of the fish was sterilized and samples of minced muscle, weighing 20 g each, were placed in a stomacher bag containing 180 g peptone water to obtain a 10-fold dilution. Blending was done in the stomacher for one minute. The plates were incubated at 17°C for five days. TVC and selective counts of H_2S-producing bacteria were done on iron agar (IA) by the spread plate technique.

3.6 Data analysis

The averages for all data and the equation of best fit and correlation coefficient (r) of QI were calculated using the Microsoft Office Excel 07. The statistical programme Unscrambler (Version 9.7; CAMO, Trondheim, Norway) was used to predict the uncertainty of days on ice from the QIM, using partial least-square regression (PLS). The results of generic descriptive analysis DA were treated with analysis of variance (ANOVA) to analyse if some difference existed between samples. Multivariate comparisons were calculated using Duncan's Multiple-Comparison Test. In the ANOVA $p < 0.05$ was used to indicate a significant difference between samples. This statistical treatment was performed using the software NCSS 2000 (NCSS, Kaysvill, Utah, USA).

The software PanelCheck (V1.3.2) was used to study performance of sensory assessors and study scores (samples) and loadings (sensory attributes) of frozen-thawed Atlantic mackerel cooked fillets as evaluated by trained panel.

4 RESULTS

4.1 Development of a QIM scheme for frozen-thawed Atlantic mackerel

A QIM scheme for frozen-thawed Atlantic mackerel was developed during the pre-observation (Table 3) and training sessions. The scheme was then tested in a shelf life study.

4.1.1 Sensory evaluation of raw frozen-thawed Atlantic mackerel

Pre-observation

The quality parameters to describe the changes of frozen-thawed Atlantic mackerel stored in ice are listed in a preliminary scheme (Table 3). Each parameter was assigned scores (0 to 1, 0 to 2 or 0 to 3) according to descriptions. The total sum of score was 38 points.

Table 3: – Preliminary QIM scheme developed after pre-observation session for frozen-thawed Atlantic mackerel.

Quality parameter		Description	Score
Appearance, texture	Skin, back	Strong blue and iridescent Greenish and loss of iridescent Pale	0 1 2
	Skin, abdomen	Pearly/white colour Slightly golden Golden tint	0 1 2
	Texture, back	Firm and elastic Slight soft Soft Very soft	0 1 2 3
Eyes	Pupil	Black Slight grey Grey	0 1 2
	Cornea	Bright Slight cloudy Cloudy	0 1 2
	Shape	Flat Slightly sunken Very sunken	0 1 2
Gills	Colour	Liver red Brownish Pale brown (and grey/greenish/yellow)	0 1 2
	Mucus	Slime Abundant slime	0 1
	Filaments	Close - coherent Split parted	0 1
	Odour	Seaweed, metallic, ocean Rancid, sour grass (or grassy) Strong rancid, acid milk, wet dog	0 1 2
Abdomen	Texture	Slightly soft Soft, stretch-marks Very soft	0 1 2
Peritoneum		Adherent Fairly adherent Torn	0 1 2
Viscera	Solution, bones	Whole and bright (bone embedded within flesh) Beginning to dissolve and less bright (bone start loss from flesh) Dissolved and dull (bone loss from flesh)	0 1 2
Quality Index			**(0-38)**

The Figures 4, 5, 6, and 7 illustrate the quality parameters of thawed Atlantic mackerel observed with different storage time in ice.

The skin tends to lose its colour with the storage time, turning from iridescent blue to pale blue (dorsal part) and from pearl/white colour to golden tinge (ventral part) (Figure 4).

At the beginning of storage the eyes were flat/slightly sunken and the pupil slightly grey. They became sunken/very sunken and the pupil grey (light spot in the centre) at the end of the storage (Figure 5).

A – Appearance at the beginning of storage time. The dorsal part is blue and iridescent. The ventral part has a pearly/white colour.

B – Appearance at the end of storage time. The dorsal part is pale. The ventral part is golden tint.

Figure 4: Skin appearance of thawed Atlantic mackerel after different storage time in ice.

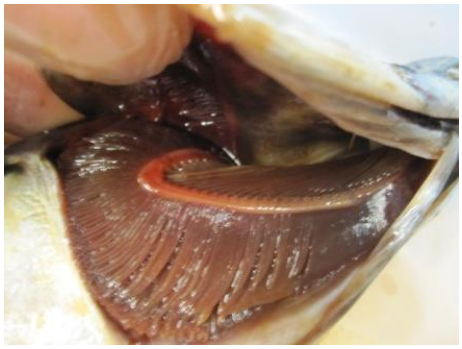
A – The eyes are flat/slightly sunken, the pupil slightly grey.

B – The eyes are sunken/very sunken, the pupil grey (light spot in the centre).

Figure 5: Eyes appearance of thawed Atlantic mackerel after different storage time in ice.

The appearance of the filaments and the colour of the gills during storage changed; started with a liver red colour and closed filaments, turning to grey/yellowish with split filaments and lamellas (Figure 6).

A – The gills are liver red and the filaments are closed (stick together).



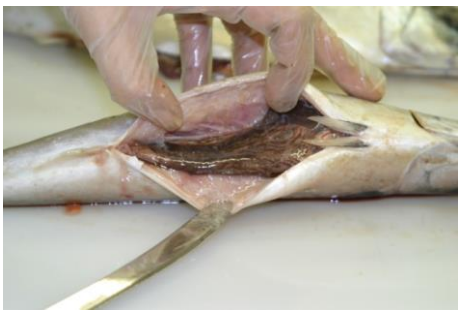
B – The gills are brownish and the filaments are not closed (adhered in groups).

C – The gills are pale brown (grey-yellowish) and the filaments are split (adhered in groups).

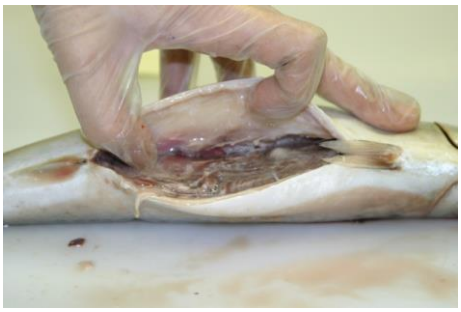
D – The gills are grey/yellowish and filaments very split.

Figure 6: Gills appearance of thawed Atlantic mackerel after different storage time in ice

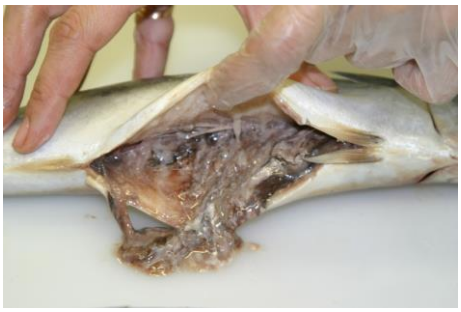
The viscera during storage changed from being whole and bright to dissolved viscera. At the end of storage the bones are loose from the flesh (Figure 7).



A – The viscera is whole and bright



B – The viscera has begun to dissolve, is less bright and bones start to get loose from the flesh.



C – The viscera have dissolved, are dull and bones loose from the flesh.

Figure 7: Viscera solution appearance of thawed Atlantic mackerel after different storage time in ice.

Picture 8 illustrates two fish with two and six storage days that were spoiled. These fish represented 7% of the Atlantic mackerel analysed during pre-observation.

Panel training

During the training the panellists started the sensory analysis using the preliminary scheme and the suggestions provided by the panel were considered. The descriptions of appearance of the skin (back), texture (back), pupil, mucus, gills filaments, gills odour, and viscera solution and bones were modified to better define the changes.

The quality parameters for the cornea, abdomen texture and peritoneum appearance were removed. After the changes the scheme was completed, the quality index was reduced

from 0-38 to 0-19 points. Table 4 shows the QIM scheme developed for the frozen-thawed Atlantic mackerel.

Figure 8: Fish with torn abdomen and pulp flesh.

Table 4: Quality Index Method scheme developed for frozen-thawed Atlantic mackerel (*Scomber scombrus*).

Quality parameter		**Description**	**Score**
Appearance, texture	Skin, back	Strong blue and iridescent colour More mat, loss of iridescent colour Mat, pale blue colour	0 1 2
	Skin, abdomen	Pearly/white colour Slightly golden Golden tinge	0 1 2
	Texture, back	In rigor Slight soft, fingermark disappears rapidly Soft, fingermark disappears slowly Very soft, fingermark doesn´t disappear	0 1 2 3
Eyes	Pupil	Slightly grey Grey (light spot in the center)	0 1
	Shape	Flat Slightly sunken Very sunken	0 1 2
Gills	Colour	Liver red Brownish Pale brown (grey/greenish/yellow)	0 1 2
	Mucus	Clear mucus Dry, very little mucus	0 1
	Filaments	Whole, lamellae coherent Slightly split parted Split parted	0 1 2
	Odour	Fresh seaweed, metallic, ocean Rancid, grass, sour grass Strong rancid, sour milk, wet dog, table cloth, smelly feet	0 1 2
Viscera	Solution, bones	Whole and bright, bone embedded within flesh Beginning to dissolve and less bright, bone a little loose from flesh Dissolved and dull, bone loose from flesh	0 1 2
Quality index			**(0-19)**

In order to ensure proper application of this QIM scheme a practical Guide was developed (Table 5). Guidelines to the evaluation are important, especially for new assessors.

Table 5: Practical Guide of QIM for frozen-thawed Atlantic mackerel.

Parameter	Description to evaluate
Appearance skin	Analyse separately the skin colour of the back and the abdomen.
Texture	Deformability of the muscle when pressured with the finger. How fast does the flesh recover. When the pressure signs disappear immediately and completely the texture is considered firm and elastic.
Eyes shape	Since being frozen-thawed fish only flat and sunken eyes are observed.
Gill filaments	Analyse if the gill lamellae are coherent or separate. When the fish is fresh, the lamellae are coherent and gill filaments are whole. With increased storage, the lamellae separate and the filaments split.

Evaluation of QIM scheme in shelf life study

The QIM evaluation was carried out with 11 panellists. For the data analysis one result from one of the panellists were removed because the panellist did not participate in all sessions.

The Quality Index (QI) based on average of the panel (10 panellists) was calculated for each trial day of storage (1, 2, 4, 6, 8 and 9). There was a linear relationship with significant correlation (R^2= 0.8553) between the average QI for each storage day and storage time in ice (Figure 9).

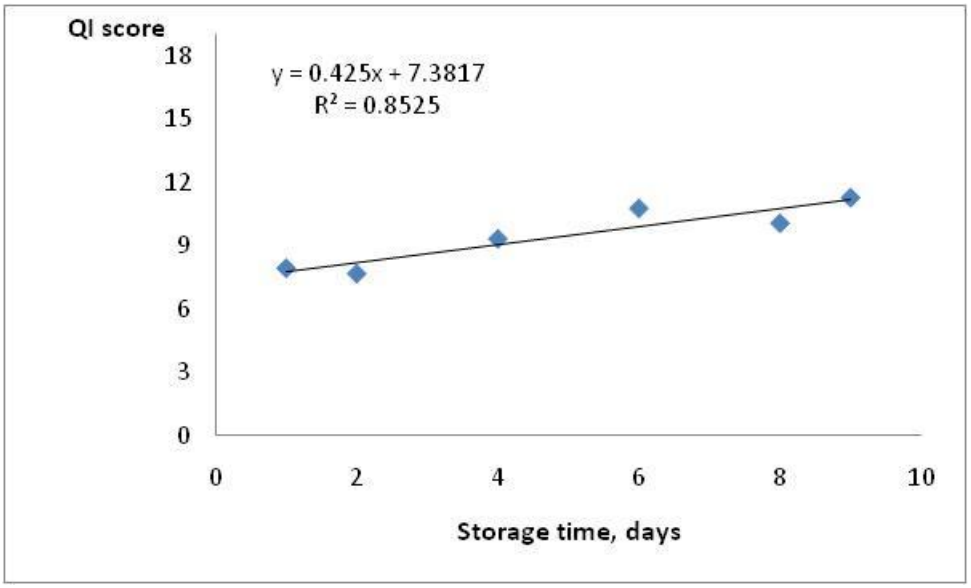


Figure 9: Correlation between Quality Index score and storage days of frozen-thawed Atlantic mackerel.

There was a variation in the QI obtained by the 10 panellists (Figure 10). Some panellists scored higher or lower at the same storage time than the others and this was most evident during early storage. However, the evaluation trend was similar for all panellists, except for panellists 8 and 9. During the last two days, the panellists were in more agreement (lower variation between panellists). This indicated that the panellists agreed more when evaluating the samples at the end of storage time.

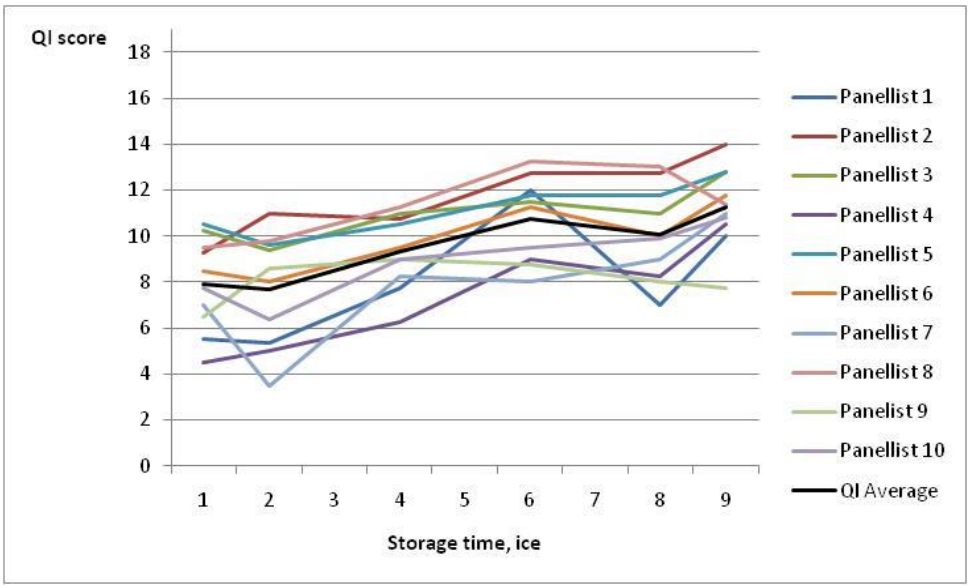


Figure 10: Quality Index score by panellists per storage time.

QIM assumes that scores for all attributes increase with storage time in ice. The attribution of demerit points for each QIM attributes is shown in Figure 11. None of the attributes started with zero demerit point on average at the beginning of the storage and the range of score was low during the storage time, with the exception of the viscera solution. The eyes and gills attributes show a fluctuation of the score during the nine days of storage, while the score of appearance and texture of skin and viscera solution increased during this period.

The correlation (r) between the average scores for each attribute for the Atlantic mackerel and the storage time in ice is shown in Table 6. This correlation coefficient was different between the attributes. According to Chatfield (1983), for sample size of six the critical value for the correlation is 0.83 (95 per cent critical points for the absolute value of correlation coefficient). Thus, was detected no significant correlation between the days in ice and the eyes and gills attributes, however for the other attributes there was significant correlation, with highest correlation for the viscera solution attribute.

During the study, a significant difference between frozen-thawed Atlantic mackerel for the same storage time in ice was observed in some cases.

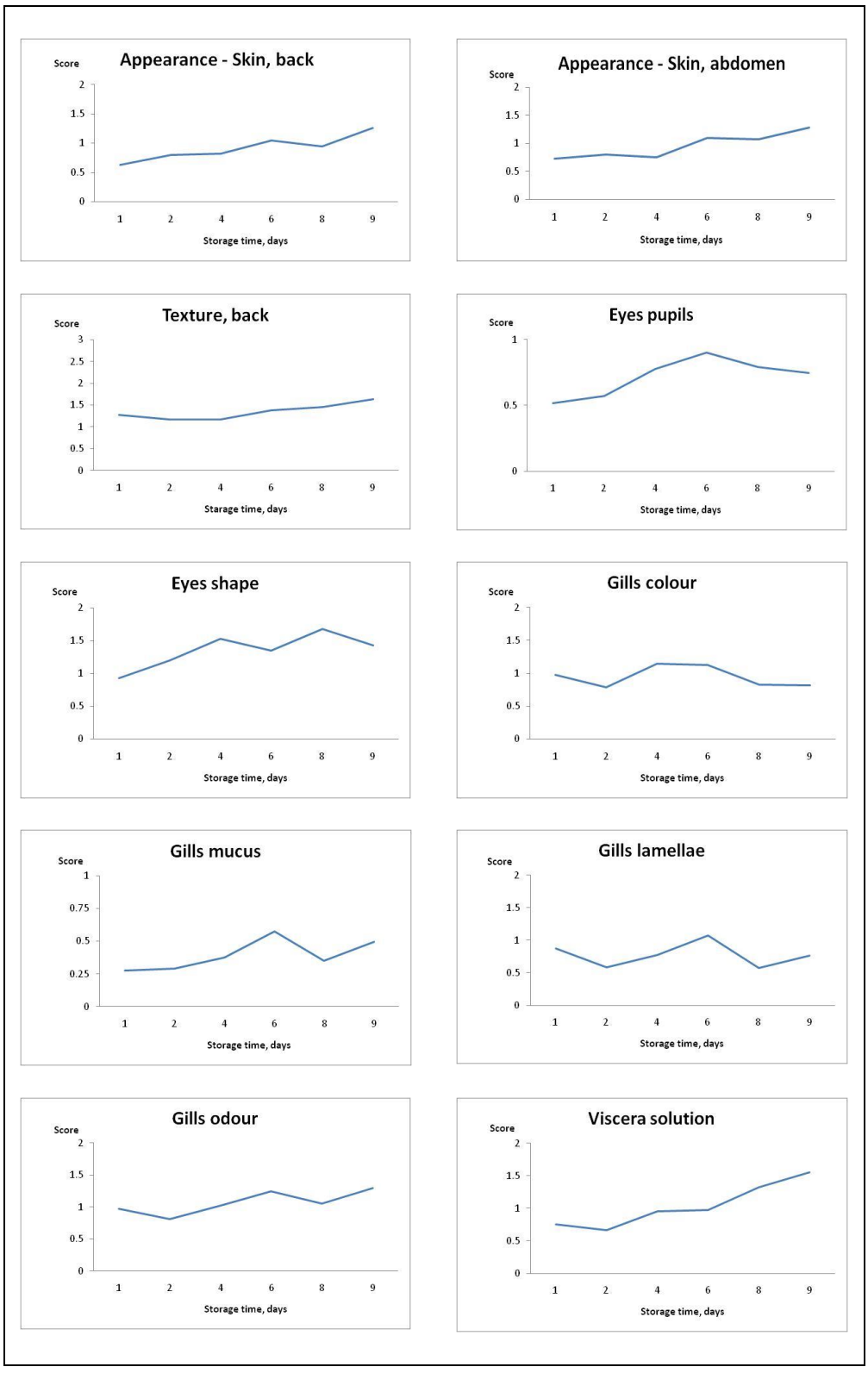


Figure 11: Average score for each quality attributes evaluated with QIM scheme for frozen-thawed Atlantic mackerel (N=4/5) against days in ice.

Table 6: Average scores for each attribute assessed with the QIM scheme for frozen-thawed Atlantic mackerel storage in ice and the correlation to days in ice**.**

Days in ice	**1**	**2**	**4**	**6**	**8**	**9**	**r**
Skin appearance, dorsal	0.63	0.79	0.83	1.05	0.95	1.26	0.90*
Skin appearance, ventral	0.73	0.80	0.75	1.10	1.08	1.29	0.92*
Texture stiffness, back	1.28	1.17	1.18	1.38	1.45	1.63	0.85*
Eyes pupils	0.52	0.57	0.78	0.90	0.79	0.75	0.73
Eyes shape	0.93	1.20	1.53	1.35	1.68	1.43	0.24
Gills colour	0.98	0.78	1.15	1.13	0.83	0.82	0.21
Gills mucus	0.28	0.29	0.38	0.58	0.35	0.50	0.66
Gills lamellae	0.88	0.58	0.78	1.08	0.58	0.77	0.05
Gills odour	0.98	0.81	1.03	1.25	1.05	1.30	0.77
Viscera solution	0.75	0.67	0.95	0.98	1.33	1.56	0.95*

*** Significant correlation (r>0.83)**

The results were analysed with Partial Least Square regression (PLS) to examine how well the QI could predict the storage time in ice (Figure 12).

The Standard Error of Performance (SEP) value for the QI was 1.7 (Figure 12). The SEP may be used to evaluate the precision of predictability of the QI. Because the QI is a sum of 10 parameters values, the measurement error may be assumed to be normally distributed. The predictions may then be considered as *t*-distributed, and for the 95% confidence interval (Sveinsdottir *et al.* 2003), by SEP x *t* (df = 5) = 1.77 x 2.571 = 4.55. Therefore, it was estimated that the QI for 4/5 Atlantic mackerel from the same batch could predict the storage time with an accuracy of ± 2.3 days.

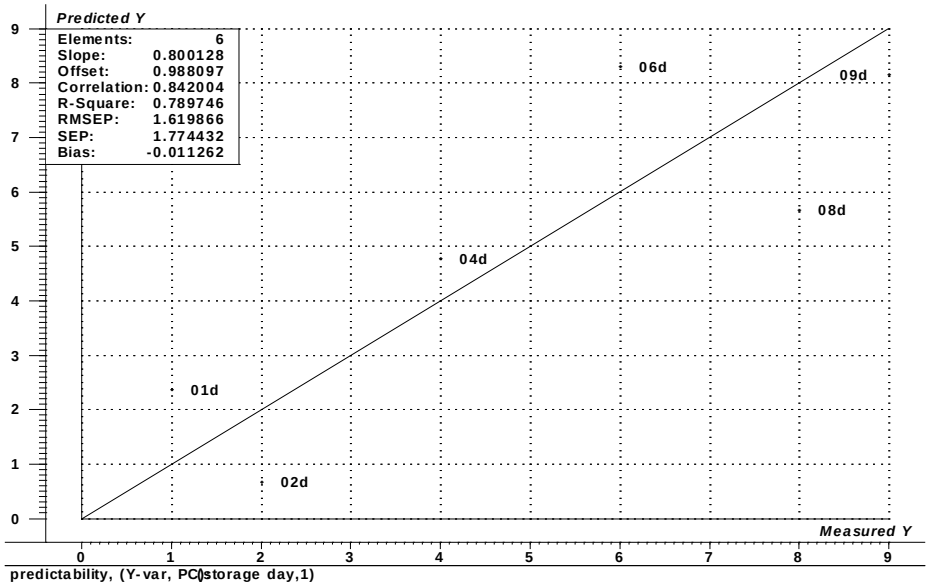


Figure 12: PLS modelling of QIM data from frozen-thawed Atlantic mackerel stored in ice using full cross validation: measured versus predicted Y values. Average QI for each storage day based on assessment of 4/5 mackerel used to predict storage time in days.

4.1.2 Sensory evaluation of cooked fillets (DA)

The sensory panel produced a list of words that was used to describe the quality parameters of appearance, odour, flavour and texture of cooked Atlantic mackerel fillets (Table 7).

Table 7: Sensory attributes of Atlantic mackerel fillets assessed by generic DA method.

Sensory attributes		**Description of attribute**
Odour	fresh oil	Fresh fish oil
	metallic	Metallic odour
	sweet	Sweet odour
	sour	Sour odour, fresh
	musty	Musty, earthy
	butyric acid	Butyric acid, smell of sweaty feet
	rancid	Rancid
Appearance	colour	Excluding lateral line: Light: light grey. Dark: dark grey / dark grey-brown
	yellow liquid	How yellow is the liquid in the box
	white precipitation	White precipitation on the sample surface
	lateral line	Clear: dark and distinct. Unclear: light and indistinct.
	flakes	Sample splits into flakes when pressed with a fork
Flavour	fresh oil	Fresh fish oil, fresh fish liver oil
	metallic	Metallic flavour
	sweet	Sweet flavour
	sour	Sour flavour
	musty	Musty, earthy
	acrid	Acrid
	rancid	Rancid
Texture	softness	Assessed in the first bite
	succulence	When chewing. Dry: draws liquid from mouth. Succulent: releases liquid.
	tenderness	After chewing a few times.
	adhesiveness	Adheres teeth when biting.

<u>Descriptive analysis of cooked mackerel</u>

The changes in odour, flavour, appearance and texture of frozen-thawed Atlantic mackerel cooked fillets by storage time in ice are shown in Figure 13.

The DA attributes characteristic for mackerel at the beginning of storage (freshness attributes) to describe the odour and flavour during the first four storage days were stable, however from the sixth storage days these attributes (fresh oil, metallic, sweet) become lower and the attributes describing indications of spoilage (spoilage attributes), like sour, musty butric acid, acrid and rancid, increase at the same time.

The attributes rancid odour and rancid flavour seemed to increase more after the fourth storage day compared to the other spoilage attributes.

Except for white precipitation and rancid flavour attributes, there was no statistical difference between average DA scores during storage time in ice (Annex 1).

Hints of the spoilage attribute rancid flavour were detected during the first four days of storage and increased significantly until day 8. After six days, the rancid flavour was obvious, but after eight days of storage, it dominated the flavour profile of the fish. The rancid odour did not increase significantly during the storage time (p= 0.187).

The texture attributes did not change significantly during the storage time.

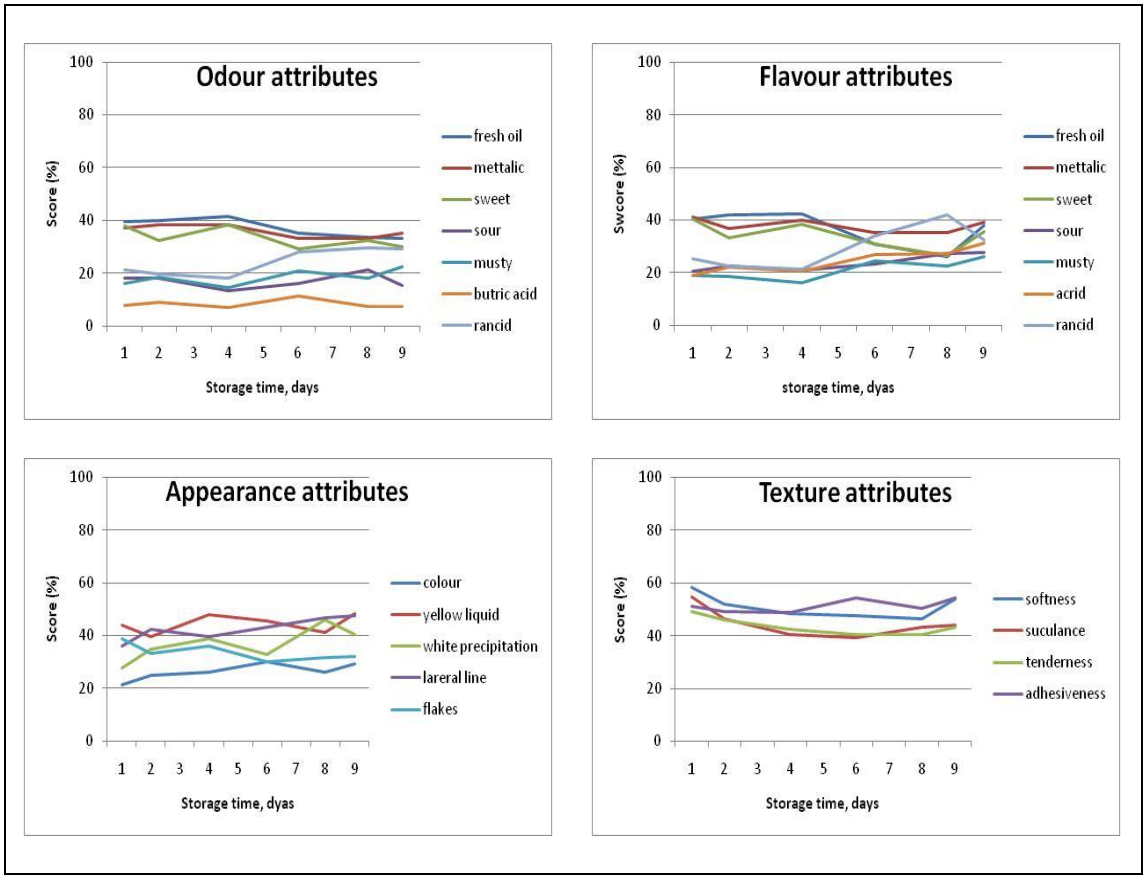


Figure 13: Changes in odour, flavour, appearance and texture of frozen-thawed Atlantic mackerel cooked fillets. The attributes were evaluated with DA using a unstructured scale (0 – 100%).

When applying principal component analysis changes from day one up to day eight can be seen (Figure 14). The first four days, at the beginning of storage time, the odour and flavour were characterized as sweet, fresh oil and metallic; at the end of storage (D6, D8 and D9) the samples were characterized by rancid odour and flavour.

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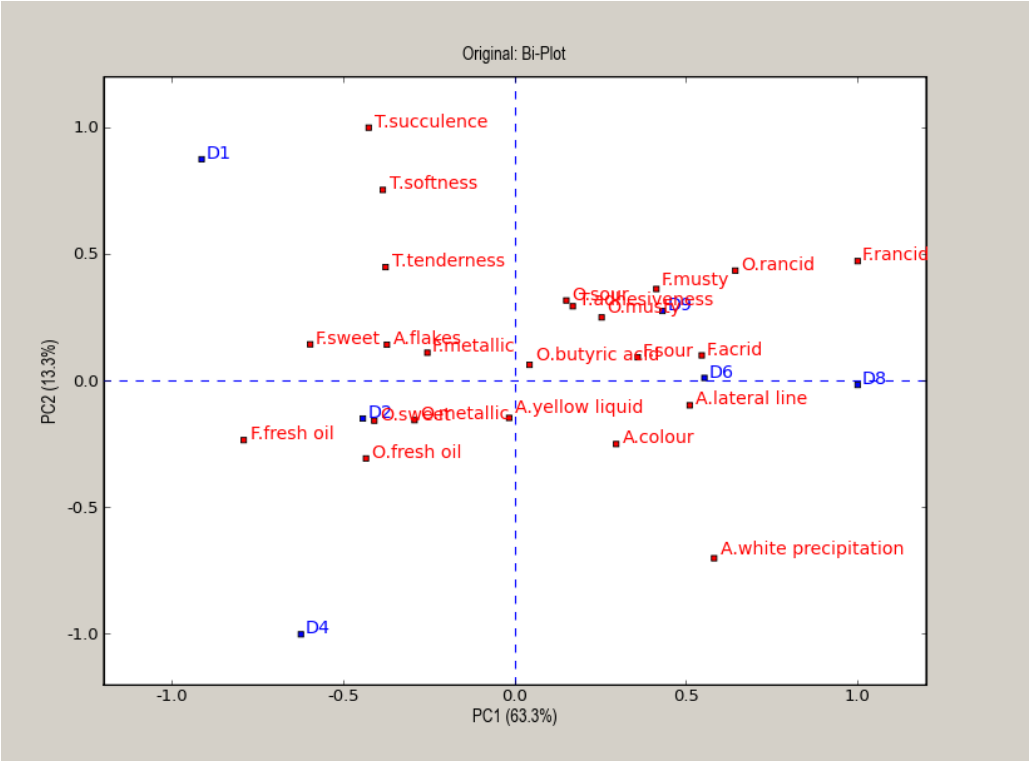

Figure 14: Bi-plot of score (samples) and loading sensory attributes of frozen-thawed Atlantic mackerel cooked fillets as evaluated by trained panel. O. = odour, A. = appearance, F. = flavour, T. = texture and D = days of storage).

4.1.3 Histamine content

The histamine of frozen-thawed Atlantic mackerel storage in ice during the whole storage time (nine days) was not detected, thus considered to be less than 5 ppm.

4.1.4 Microbial analysis

An increasing trend of the total viable counts (TVC) was observed during the storage time. On day 1 it was 1.7 log cfu/g and increased to 4 log cfu/g in the fish muscle on day 9 (Figure 15).

The H_2S-producing bacteria maintained constant (< 1.3 log cfu/g) during the first four days of storage and an increase was observed for the following days of storage, reaching 2.7 log cfu/g at the end of storage on day nine (Figure 15).

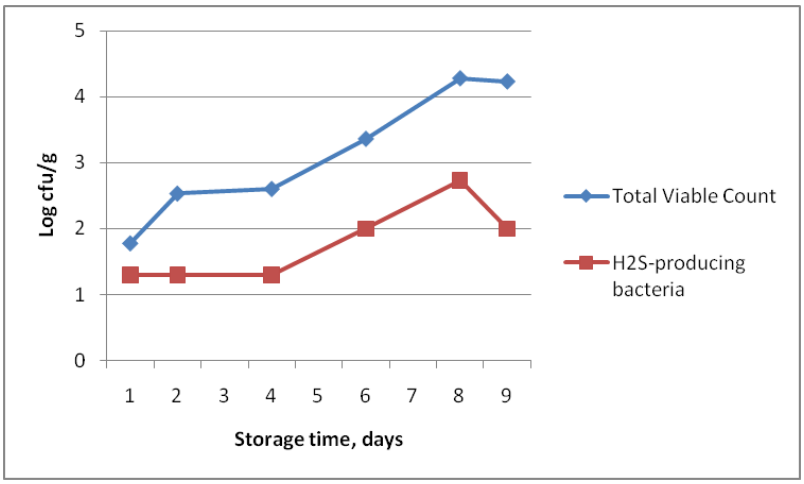

Figure 15: Total viable counts and counts of H_2S-producing bacteria expressed as log colony forming units (cfu) *per* gram in muscle of frozen-thawed Atlantic mackerel versus storage time in ice.

5 DISCUSSION

5.1 Development and evaluation of QIM scheme for frozen-thawed Atlantic Mackerel

During the pre-observation two fish with two and six storage days were spoiled, probably because of rupture of the mackerel stomach e.g. during handling or catch. Under these conditions the trypsin-like enzymes readily leak to the fish muscle, increasing their normal activity (Lauzon pers. comm.). As reported by Keay (1979) this also depends on the feeding conditions and the ambient temperature, rapid chilling to 0°C is essential to help control this problem. Fish with burst bellies should not enter the food chain and should be discarded.

The deterioration of frozen-thawed Atlantic mackerel was observed during the pre-observation sessions and a preliminary scheme with 38 points of total sum of scores was developed. During the training sessions the attribute appearance of cornea was excluded from the QIM scheme because no changes were detected during the storage time.

For practical reasons the attributes texture of abdomen and appearance of peritoneum were excluded because the viscera solution attribute changed more during the storage time. The panellists cannot evaluate these attributes simultaneously because it is necessary to cut the fish for viscera evaluation.

During the pre-observation the colour and the mucus of the gills did not have a coherent evolution with the loss of freshness. Also the eyes attributes were not related to storage. During the training sessions the samples were increased from three to five fish from each storage day because of individual differences of the fish within a storage day. The individual differences between fish of same storage time were verified during the training and evaluation sessions. Therefore, it is important to evaluate the appropriate number of fish, depending on the size of the fish and the size of the lot. It is recommended for small fish species, that 10 fish be evaluated from each lot in the fish industry (Martinsdóttir *et al.* 2001).

The final QIM scheme for frozen-thawed Atlantic mackerel consisted of 10 parameters (Table 3), resulting in a total of 19 demerit points. There was a linear significant relationship ($R^2 = 0.855$) between the average QI for each storage day and storage time in ice. Andrade *et al.* (1997) found a high correlation between QI and day in ice of fresh Atlantic mackerel, but not provide information about the maximum shelf life, such as according to microbial methods, chemical methods, or sensory evaluation of cooked samples.

The range of QI during the storage time was small (from 7.8 to 11.3) and the freshest fish started with a high QI, may be because the batch that was used for the study had been kept frozen for five months in boxes. Therefore, the fish was exposed to air, which may have contributed to faster lipid oxidation.

The QI may be used to predict the remaining storage time with an accuracy of ± 2.3 days if four to five fish from the same lot are evaluated by a trained panel. It can be considered high for a relative short storage time. According to the guidelines by Martinsdottir and Magnusson (2001), for freshness assessment of whole fish, a minimum of three (in large fish) to ten (in small fish) random samples should be taken to cover the biological difference it spoilage rate of fish and it could increase the precision of the prediction of storage time (Sveinsdottir *et al.* 2002). If the number of samples for the assessment was increased, it may contribute to a better prediction of the remaining storage time.

Even though the correlation coefficient between the Quality Index and storage time was significant it is based on a rather small part of the QIM scheme score system (QI between 7 and 11). Therefore, in order to improve its usefulness the QIM scheme for frozen-thawed Atlantic mackerel must be validated. A validation of the QIM scheme should be repeated using a newly frozen mackerel to observe the practical use of the scheme. It might be expected that after a very short frozen storage, the physical changes of mackerel are less than after five to six months of frozen storage. Therefore it could be assumed that a larger part of the QI system is used and the intercept is lower than 7.38 as in our case in this study. However, if the low increase of the QI with storage time persists, is necessary to adjust some of the parameters. The non-significant attributes, gills and eyes, should be removed.

5.2 Descriptive Analysis (DA)

During the first four days of storage the sensory attributes of cooked fillets of frozen-thawed did not change much, the following days the odour and flavour attributes increased reaching the limits of acceptance between eight and nine days, when the panellists had difficulties in evaluating the fillets, because of unacceptable odour and flavour related to spoilage.

The appearance and texture attributes changed less than other attributes according to the panel.

The result of lipid oxidation and hydrolysis on fatty fish produce unpleasant (rancid) taste and smell (Huss 1995). Lipid oxidation results in formation of secondary volatile compounds (aldehydes, ketones, alcohols etc.) that will give rise to rancid off-flavour formation.

During the sensory analysis of cooked fillets, the fresh characteristic flavour and odour, (oil, sweet and metallic) indicated a clear freshness while rancid flavour and odour indicated the beginning of spoilage. Also, the fish became less flaky with storage time. As reported by Licciardello and D'Entremont (1987) the physical-chemical changes (oxidative rancidity and textural toughening) during extended frozen storage impaired the quality of thawed fish during subsequent iced storage.

5.3 Histamine content

As expected, histamine was not detected in frozen-thawed Atlantic mackerel during the nine days storage in ice. These results are in accordance with the results of Barbuzzi *et al.* (2009), who did not detect histamine in Atlantic mackerel stored under refrigerated condition until 10 days. The Atlantic mackerel used in present study was stored at -24 ºC for six months. No histamine could be detected in mackerel stored four days in ice followed by frozen storage during weeks at -14 ºC, -21 ºC and – 29 ºC (Hardy and Smith 1976).

Many studies determining the histamine content in muscle of Atlantic mackerel have been published. Jhaveri *et al.* (1982) reported that initial histamine level (2 mg/100 g tissue) did not increase up to 10 days and rose to 10 mg histamine/100 g after 2 weeks of storage. Luten (1983) has shown that Atlantic mackerel storage at 0ºC the histamine content is constant and low (0.3-0.6 ppm) during the first thirteen days. According to Taylor (1986), all studies seem to agree that histamine formation is negligible in fish stored at 0 ºC. In study by Lokuruka and Regenstein (2007), the highest amount in ungutted Atlantic mackerel stored in ice was 39 ppm on the 13th day storage, and there was a haphazard variation in histamine for the first seven days of storage, although it increased after eight days of storage. Also, according these authors the ungutted mackerel is safe regarding histamine up to nine days of iced storage. The fish is relatively safe even beyond 13 days in ice as far as scombrotoxicity is concerned, as long as the initial microbiological quality and subsequent handling is good.

Shelf life and spoilage do not necessarily correlate with the amounts of scombrotoxic amines formed in edible fish tissue, since shelf life has more to do with spoilage than on scombrotoxin levels (Lokuruka and Regenstein 2007).

5.4 Microbial analysis

The total viable counts load (1.7 log cfu/g) was low and H_2S-producing bacteria load (1.3 log cfu/g) in muscle was stable and also low during the first four days storage in ice. The following days until the end of the storage the microbial load increased. As reported by Magnusson and Martinsdottir (1995) and Martinsdottir and Magnusson (2001) keeping fish frozen for an extended time prior to thaw and storing in ice might be an effective way of reducing the bacterial load. Licciardello and D'Entremont (1987) also reported reduction of bacterial load, which could contribute increased subsequent iced storage life.

Jhaveri *et al.* (1982) reported microbial counts on the tissue of fresh and gutted Atlantic mackerel stored in ice as the initial microbial load being 3.3 log cfu/g and after two weeks it was 6.8 log cfu/g. In present results for the frozen-thawed Atlantic mackerel stored in ice the results obtained were lower.

The principal bacterial species H_2S producer on fish flesh is *Pseudomonas putrefaciens* and is considered to be a major fish spoilage organism because of its proteolytic activity (ability to reduce trimethylamine oxide to trimethylamine, causing a fishy odour) and production of hydrogen sulphide. This microorganism was very sensitive to long-term freezing and in thawed samples held frozen for six months, there was no growth during storage in ice (Licciardello and D'Entremont 1987). This fact can explain that during the four days of storage the total viable counts and H_2S producer was low.

6 CONCLUSIONS

- A QIM scheme for frozen-thawed Atlantic mackerel (*S. scombrus*) was developed during this study.
- The total score for quality attributes (QI) had a linear relationship with storage time in ice.
- The storage time could be estimated with an accuracy of ± 2.3 days.
- This QIM scheme needs to be re-evaluated for Atlantic mackerel kept frozen for less than five months.
- The main limiting factor for length of shelf life was mainly due to sensory attributes describing spoilage. The shelf life, as measured by generic DA, was estimated to be between four and six days, due to a strong rancid odour and flavour.

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Annex 1: Mean score for frozen-thawed Atlantic mackerel storage in ice, samples (one, two, four, six, eight and nine days) evaluated by trained sensory panel with p-values.

Attributes		**Days of storage**						
		1	**2**	**4**	**6**	**8**	**9**	**Prob. Level**
Odour	fresh_oil	39.4	39.8	41.5	35.1	33.6	33.1	0.775
	mettalic	37.1	38.4	38.3	33.1	33.1	35.3	0.882
	sweet	37.8	32.4	38.4	29.1	32.2	30.1	0.126
	sour	17.9	18.0	13.1	16.3	21.2	15.4	0.539
	musty	16.2	18.6	14.5	20.8	18.1	22.6	0.568
	butyric acid	7.9	8.9	6.9	11.4	7.2	7.5	0.709
	rancid	21.1	19.6	18.1	28.1	29.4	29.3	0.187
Appearance	colour	21.4	25.1	26.3	30.1	26.2	29.4	0.341
	yellow liquid	44.1	39.4	47.9	45.5	41.3	48.4	0.678
	white precipitation	27.7	34.9	38.7	32.7	45.9	40.6	0.006*
	lateral line	36.2	42.3	39.6	43.3	46.6	47.6	0.459
	flakes	38.8	33.1	35.9	30.1	31.6	32.0	0.711
Flavour	fresh oilx	40.3	41.9	42.4	30.9	26.0	38.1	0.181
	mettalicx	41.1	36.7	39.9	35.3	35.1	39.4	0.913
	sweetx	40.4	33.2	38.5	31.0	26.3	35.7	0.094
	sourx	20.5	22.6	20.9	23.1	27.3	27.7	0.717
	mustyx	18.8	18.6	16.4	24.6	22.5	26.3	0.432
	acrid	19.0	22.1	20.6	26.8	27.3	31.3	0.201
	rancid	25.2	22.6	21.5	34.1	42.1	32.4	0.012*
Texture	softness	58.2	52.2	48.6	47.4	46.3	54.0	0.468
	suculence	54.8	46.6	40.6	39.3	43.3	44.3	0.128
	tenderness	49.4	46.1	42.6	40.3	40.6	43.4	0.610
	adhesiveness	51.2	49.4	48.7	54.4	50.5	54.3	0.888

* $p<0.05$