

## **DEVELOPMENT OF QUALITY INDEX METHOD (QIM) SCHEME FOR POLLOCK (*POLLOCK VIREN*) FILLETS AND APPLICATION IN A SHELF LIFE STUDY**

Yanara Tamarit Pino  
yanaratamarit8@gmail.com  
Centre for Fisheries Researches, Cuba  
Quality Management

Supervisors:

Kolbrún Sveinsdóttir  
kolbrun@matis.is  
Aðalheiður Ólafsdóttir  
adalheiduro@matis.is  
Matis ehf.

### **ABSTRACT**

Freshness is one of main attributes affecting the overall quality of fish. Quality Index Method can be applied to evaluate the freshness of seafood raw materials and final products. Cuba imports pollock fillets without skin from Iceland. The aim of the study was to develop and evaluate a new Quality Index Method (QIM) scheme for pollock fillets (*Pollachius viren*) and apply it in a shelf life study. Fresh pollock fillets were stored at -1 and 2°C for up to 12 days. During storage, changes in quality attributes were evaluated by a trained sensory panel using the QIM scheme developed for pollock fillets. At the same time, the Torry scale was used to evaluate the freshness of cooked pollock samples, and measurements of Total Volatile Bases Nitrogen (TVBN), Total Viable Counts (TVC) and count of hydrogen sulphide (H<sub>2</sub>S) producing bacteria were conducted. A new 24-point QIM scheme for pollock fillets without skin was proposed from the experimental results. A high correlation was seen between the Quality Index (QI) and storage time at 2°C ( $R^2 = 0.9151$ ) but the correlation was lower at -1°C ( $R^2 = 0.8606$ ). The levels of TVBN and TVC and count of H<sub>2</sub>S producing bacteria increased during storage time at 2°C. Based on the Torry scores, TVBN and TVC counts, the shelf life of pollock fillets stored at 2°C was six days, and 10 days for fillets stored at -1°C.

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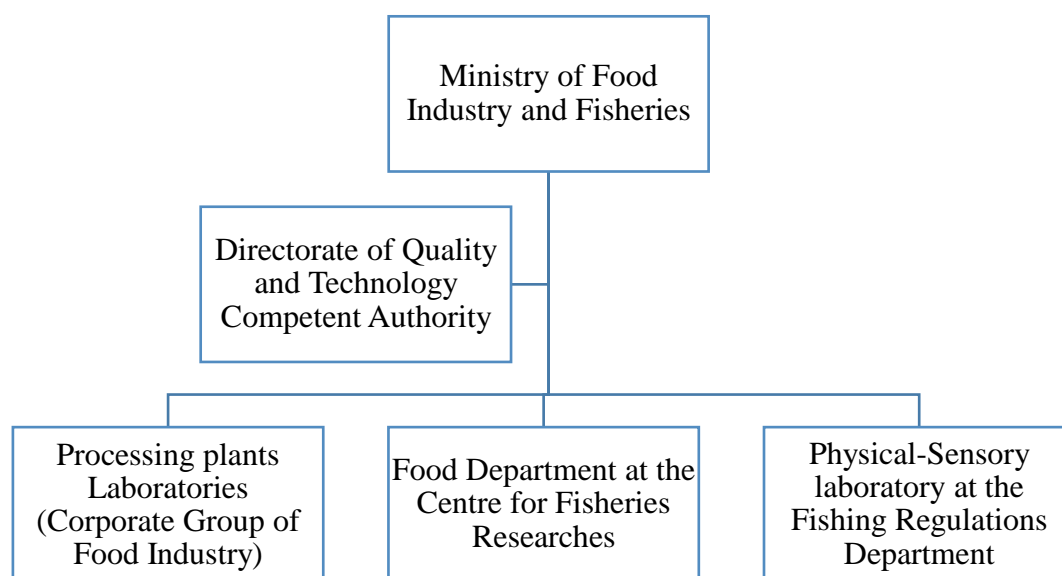
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## 1 INTRODUCTION

Fisheries and aquaculture are two of the main economic sectors contributing to Cuban food security with an annual per capita consumption of 5.5kg. It is an important factor driving economic growth, with export and import estimated between \$71 Million USD and \$25 Million USD respectively in 2013. Fisheries and aquaculture also drive employment, with 7,480 jobs in 2013 in fisheries sector (FAO, 2015). The FAO Fishery and Aquaculture Statistics (2012) reported 5.5% of animal protein come from fish, in apparent consumption in Cuba. The products are mainly traded with the European Union (EU), Asia and South America markets (Comex, 2015).

The Ministry of Food Industry and Fisheries is the main authority in Cuba for control the quality in process fish for export and is also responsible for imports of seafood. A subgroup under the Ministry, the Directorate of Quality and Technology acts as the competent authority for control the quality of fish and fisheries products. The quality control process in Cuba is outlined in Figure 1.



**Figure 1: Quality control process of fisheries products in Cuba**

The Corporate Group of Food Industry have seventeen fish processing plants, of which, ten plants have permission to export to the European Union with a HACCP system implemented, and ISO 9001 (2008) standard certification and quality control labs. Meanwhile, the Resolution 223 (2014) of the Ministry of the Food and Fishing Industries in Cuba states that the Food Department at the Centre for Fisheries Researches is the reference laboratory for the Competent Authority. This department is accredited by ISO / IEC 17025 (2005), in fourteen microbiological and chemical techniques, also responsible for the quality control of fisheries products imports and exports. The Physical-Sensory laboratory from the Fishing Regulations Department is the last lab in control for every seafood for export.

Major problems in the processing and commercialization of fisheries products in Cuba are related to deficient handling practices of fish after capture, lengthy unloading and transportation times to processing plants, lack of monitoring in the refrigeration and freezing chambers inside the processing plants, and lack of monitoring at the displays and storage at the market places. All these problems affect the final quality and shelf life of the products.

Freshness is one of main attributes affecting the overall quality of fish worldwide. Sensory evaluation is the method of choice for freshness assessment (Martinsdottir, et al., 2009) and the main method used to evaluate the freshness of seafood raw materials and final products ( Bernardi, et al., 2013).

Many schemes have been developed for sensory evaluation of raw fish. The Torry research (Shewan, et al., 1953) developed the first modern and detailed method to evaluate the freshness of fillets. Another freshness grading system for seafood is the Quality Index Method (QIM) which is based on a scheme originally developed by the Tasmanian Food Research Unit (TFRU) (Bremner, 1985) which has mainly been developed for whole fish, adapted to each species.

QIM has developed over the years and in 2001 Eurofish published a manual ( Martinsdóttir, et al., 2001), available in 11 languages. It contains QIM schemes for 12 fish species developed for whole cod, haddock, pollock, redfish, plaice, sole, brill, turbot, herring, fjord shrimp, deep water shrimp, peeled shrimp and farmed salmon. It is based on significant, well-defined characteristic changes of appearance attributes that occur in raw fish such as eyes, skin, gills and changes that occur in odour and texture with storage time.

A few QIM schemes have been developed for fillets of fresh cod (*Gadus morhua*) (Bonilla, et al., 2007), Arctic charr (Tran Thi , et al., 2013) and farmed tilapia (Cyprian, et al., 2014).

According to Bernardi et al. (2013) many studies on QIM schemes have been conducted. Between 2000 and 2011 close to 50 QIM schemes were developed for different fish species, available in the scientific literature, with the respective storage condition and estimated shelf life.

In Cuba every lab uses sensory tests for evaluation of the raw material and final products, but improvements are needed, and the current methods and procedures need to be replaced by new and more efficient methods. It is important to transfer this knowledge to the Ministry of the Food and Fishing Industries in Cuba.

The main aim of this project was to learn about sensory methods for seafood, with focus on freshness evaluation and shelf life and how to apply sensory evaluation tools to Cuban export and import seafood products. Further, the aim was to compare two different storage conditions. This was done through the development and evaluation of a QIM scheme to measure the freshness of pollock (*Pollachius viren*) fillets because it is the most important species imported from Iceland to Cuba. More specifically, the objectives of the project were achieved by:

- 1) Development of a QIM scheme for pollock fillets.
- 2) Training of panellists for the sensory evaluation of QIM and Torry scheme of pollock fillets.
- 3) Conducting a shelf life study of fresh Pollock fillets (stored at -1 and 2°C) using the developed QIM scheme, Torry scale, chemical and microbiological methods.

The developed QIM scheme was used to evaluate the freshness of the fillets in comparison to chemical and microbiological methods and sensory evaluation of cooked fillets with the Torry scheme for lean white fish.

## 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). Many factors related to the environment (e.g., catching ground and season), fishing practices, storage conditions or handling, including bleeding and gutting procedures, affect the fish quality (Dowlati, et al., 2013).

Many factors may affect the freshness of fish. These include method of catching, and overall handling and the processing of the fish (Massaquoi, 2011). According to Doyle (1995) “seafood shelf life is a function of temperature.” The temperature will control the rate of bacterial spoilage, enzyme activity and oxidation reaction. At low temperatures, the spoilage rate of fish is reduced, and the products remain edible longer. Temperature during shelf life studies can be determined using thermometers such as thermometer data loggers.

Microbiological, biochemical, and sensory methods have been used to assess the freshness and quality of fish during handling and storage in different conditions: whole tilapia (*Oreochromis niloticu*) in ice and ambient temperature (Ihuahi, et al., 2010), whole Mediterranean Sea common sole (*Solea solea*) in ice (Özoğul, et al., 2011), whole tilapia (*Oreochromis species*) in ice (Kapute, et al., 2013) and whole bayad catfish (*Bagrus bayad*) in crushed ice (Hussien & Adil, 2014).

Non-sensory methods based on biochemical, physical and/or microbiological analyses are also used to evaluate the quality of fresh fish. Biochemical and physical methods measure the amount of breakdown products from bacterial or enzymatic activity, and several spoilage indicators are commonly used, including total volatile basic nitrogen (TVB-N), trimethylamine, ammonia, production of amines, nucleotides, pH and K value (Ihuahi, et al., 2010; Özoğul, et al., 2011; Kapute, et al., 2013; Viji, et al., 2014; Abraham-Olukayode & Oramadike, 2015; Islamil, et al., 2015).

### 2.2 Sensory changes

The characteristic flavour of fish after catch normally develops in the first couple of days of storage, but other sensory changes that occur during storage are related to the appearance and texture (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). Rigor mortis is the most extreme change after the death of fish. The technological impact of this depends on if the fish is filleted before or in rigor. In rigor, the fish body is completely stiff, and the filleting yield will be very poor, and rough handling can cause gaping (Buttkus, 1963). If the fish is cooked pre-rigor the texture will be soft and pasty, if is cooked in rigor, the texture is tough but not dry and post-rigor the flesh will become firm, succulent and elastic (Huss, 1995).

Bonilla et al. (2005) studied fresh cod fillets with skin in ice. At the beginning of storage time the skin was bright with iridescent pigmentation, the slime was uniform, thin and transparent, and scales were transparent. The flesh texture was firm with fresh or neutral odour and the colour was white or greyish, transparent and bright. At the end of shelf life, the skin became dull with small bubbles and single scales were loose. The flesh texture became very soft with sour, acidic or ammoniac odour and the colour became yellow or dark pink and opaque bright.



According to Huss (1995) and Church (1998), fish spoilage can be divided into four different phases:

- 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.

A high correlation was found between bacteria counts in flesh and sensory changes of cod fillets stored, evaluated for Quality Index Methods, on ice at 0–1 °C,  $R^2 = 0.944$  with Log TVC cfu/g and  $R^2 = 0.975$  with Log H<sub>2</sub>S producing-bacteria cfu/g (Bonilla, et al., 2007).

Antoine et al. (2002), studied Mahi-Mahi (*Coryphaena hippurus*) stored at 7 °C, after 0, 2, 4, 6, 8, and 10 days of storage existed high correlation between TVB-N and odour intensity ( $r = 0.84$ ) and between odour and the log<sub>10</sub> APC ( $r = 0.91$ ) was found.

### 2.3 Microbiological changes

According to Gram and Huss (1996) the microbial spoilage of foods is manifested as changes in sensorial characteristics (Table 1).

**Table 1: Microbiological spoilage of foods and sensory manifestation (Gram & Huss, 1996).**

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odours and flavours
Production of extra cellular polysaccharide material	Slime formation
Growth of moulds, bacteria, yeasts	Large visible pigmented or non-pigmented colonies
CO <sub>2</sub> – form carbohydrate or amino acids	Production of gas
Production of diffusible pigments	Discolouration

On live and newly caught fish, the microorganisms are found on the skin, gills and in the intestines (Huss, 1988; Bataringaya, 2007). The microbial status of fish after catch depends on environmental conditions and the microbiological quality of the water (Shewan, 1977). Others factors that affect the microbial growth include temperature, salt content, natural bacteria flora (Feldhusen, 2000). Fish caught in very cold, clean waters carry lower numbers of bacteria compared with fish caught in warm waters. When a fish dies, the bacteria are allowed to proliferate, in the beginning on the skin and during storage they invade the flesh (Huss, 1988; Bataringaya, 2007).

The spoilage of marine temperate-water fish is characterised by the development of offensive, fishy, rotten, H<sub>2</sub>S off-odours and off-flavours. This occurs differently for different fish and for tropical and fresh water fish, fruity, sulphhydryl off-odours and off-flavours are more typical (Gram & Huss., 1996).

Only minor changes in processing and packaging of fish products can cause a drastic change in the development and composition of the spoilage association and a complete different type of spoilage (Gram & Huss, 1996). Table 2 shows the dominating microflora and the specific spoilage organisms in different fish products.

**Table 2: Dominating microflora and specific spoilage organisms (SSO) in different fish products (Huss, 1995; Gram & Huss., 1996; Lyhs, 2009).**

Fish product	Packaging atmosphere	Dominating microflora	Typical SSO
Fresh chilled fish (0–5 °C)	Aerobic	Shewanella putrefaciens, Pseudomonas spp., Moraxella, Acinetobacter	Shewanella putrefaciens, Pseudomonas spp.
Fresh fish (> 10–15 °C)	Aerobic	Shewanella putrefaciens, Vibrionaceae	Aeromonas spp., Shewanella putrefaciens
Fresh chilled fish (0–5 °C)	Vacuum	Shewanella putrefaciens, Vibrionaceae, Photobacterium	Shewanella putrefaciens, Photobacterium phosphoreum, Lactic acid bacteria
	Modified atmosphere	Photobacterium, Shewanella putrefaciens, Pseudomonas spp.	Photobacterium phosphoreum, Lactic acid bacteria
Cold-smoked fish	Vacuum	Pseudomonas spp., Enterobacteriaceae, Acinetobacter, Staphylococcus spp., Shewanella putrefaciens, Vibrionaceae, Photobacterium	Lactic acid bacteria (Lactobacillus, Lactococcus, Carnobacterium), Enterobacteriaceae (Serratia spp., Hafnia alvei, Enterobacter spp.), Photobacterium, Brochothrix thermospacta
Hot-smoked fish	Vacuum	Pseudomonas spp., Enterobacteriaceae, Staphylococcus spp., Lactic acid bacteria	Lactic acid bacteria (Carnobacterium)
‘Gravad’ fish	Vacuum	Data not available	Lactic acid bacteria (Lactobacillus, Leuconostoc, Weissella, Carnobacterium)
Semi-preserved marinated fish		Lactic acid bacteria	Lactic acid bacteria (Lactobacillus)
Lightly salted and fermented fish	Modified atmosphere	Data not available	Lactic acid bacteria (Lactobacillus, Enterococcus, Lactococcus)

Fernández-Segovia et al. (2007) studied the microbial changes during refrigerated storage of desalted cod (*Gadus morhua*) preserved by combined methods. No *Aeromonas* or sulphite-reducing *Clostridium* were isolated from any of the analysed samples. The lowest microbial counts of mesophilic, psychrotrophic, *Pseudomonas*, moulds and yeasts, were found in samples with additives in all kinds of packaging. These samples in vacuum packaging or modified atmosphere packaging maintained an excellent microbial quality throughout the 42 days of storage.

## 2.4 Chemical changes

One important aspect in fish quality is chemical composition, which affects the keeping quality and the sensory characteristics of the fish (Huss, 1988). The autolytic changes are responsible for the initial loss of quality in fresh fish but contribute very little to spoilage of chilled fish and fish products. Autolytic changes are however of great importance in frozen fish (Huss, 1994).

The chemical compounds developing in naturally spoiling fish and sterile fish has shown that most of the volatile compounds are produced by bacteria (Shewan, 1962). It is typical of many of the specific spoilage bacteria on fish that they can use TMAO as electron acceptor in an anaerobic respiration. The reduced component, TMA, which is one of the dominant components of spoiling fish, has a typical fishy odour (Dalgaard, et al., 1993).

Bacterial action is inhibited and TMAO is broken down by autolytic enzymes to dimethylamine (DMA) and formaldehyde (FA). The FA formed causes increased denaturation of fish tissue, changes in texture and loss of water retention capacity (Huss, 1994). Some of the compounds typically formed by bacteria during spoilage of fish are shown in Table 3 together with the substrate used for the formation (Huss, 1995; Gram & Huss., 1996).

**Table 3: Substrate and off-odour/off-flavour compounds produced by bacteria during spoilage of fish (Huss, 1995).**

Substrate	Compounds produced by bacterial action
TMAO	TMA
cysteine	H <sub>2</sub> S
methionine	CH <sub>3</sub> SH, (CH <sub>3</sub> ) <sub>2</sub> S
carbohydrates and lactate	acetate, CO <sub>2</sub> , H <sub>2</sub> O
inosine, IMP	hypoxanthine
amino-acid s (glycine, serine, leucine)	esters, ketones, aldehydes
amino-acids, urea	NH <sub>3</sub>

In cod and other gadoid fishes, TMA constitutes most of the so-called total volatile bases, TVB (also called total volatile nitrogen, TVN) until spoilage. These are good indicators, with sensory evaluation and microbial analyses, in predicting shelf life in fish and other fish products in different storage conditions (Papadopoulos , et al., 2003; Chytiri, et al., 2004; Babadije & Oladipo, 2004; Caparro, et al., 2015).

In fresh iced fish, fat oxidation usually occurs after autolysis and bacterial spoilage. The first step of the oxidation process leads to formation of hydro-peroxides, which are tasteless but can cause brown and yellow discolouration of the fish tissue (Huss, 1994).

The lipid concentration in fish can contribute to the spoilage process in fish. The fats in fish are mainly unsaturated fatty acids that are easily oxidized by oxygen from the atmosphere. For fatty fish preserved in ice, spoilage due to rancidity is mainly caused by oxidation, with rancid odour and rancid flavour (Huss, 1995).

The pH is close to neutrality of muscle tissue when fish is alive. In post mortem process, pH varies between species, catching ground, and season. Due to post-mortem anaerobic formation of lactic acid, pH decreases usually within the first day of death (Shewan, 1977; Huss, 1988).

The pH for most fish is 7 or slightly lower than 7 immediately after catch (Huss, 1995). This is the most significant factor influencing the texture of the meat and the degree of “gaping”, i.e. the rupture of the connective tissue (Huss, 1988). The low pH is an indicator of stress which the fish might have encountered during catching or harvesting (Mohan, et al., 2012).

Viji et al., (2014) used pH, total volatile base nitrogen, thiobarbituric acid value, peroxide value and free fatty acid like chemical analysis when studying the quality characteristics and shelf

life of sutchi cat fish (*Pangasianodon hypophthalmus*) steaks during refrigerated storage. The result reveals that pH is a poor-quality indicator of freshness of catfish under refrigerated storage. But it can be considered as a quality indicator together with other quality indices.

## 2.5 Sensory evaluation of fish

Sensory evaluation can be used to evaluate sensory characteristics of raw materials, finished products in storage tests, quality control analyses, for new product development and in consumer tests (Jellinek, 1985).

In sensory evaluation, a panel is established, and panelists or inspectors trained to perform sensory analysis with clear and descriptive guidelines. The panel leader selects and trains the panelists in difference and descriptive tests. The panel leader also prepares the product samples and references/standards, maintains the technical skills and motivation of the panelists and compiles the sensory data (ISO/CD 13300-1, 2002).

Analysis and interpretation of the sensory data requires understanding of the methods used and are performed by the panel leader or in cooperation by the panel leader/sensory staff and project leaders (Martinsdóttir, et al., 2009). According to ISO 6658 (2005) the panelists must be monitored for their ability to perform the analysis by the panel leader.

There are different sensory methods for evaluation of seafood quality. The most used are ranking, scaling methods and grading e.g. EU scheme, Quality Index Method (QIM), Torry scale, raw fillets grading method and Quantitative Descriptive Analysis (QDA) (Martinsdóttir, et al., 2009).

The European Union regulation No. 2406/96 establishes the freshness rating for fishery products according to the EU grading scheme. There are schemes for different groups of fish, including whitefish, bluefish, selachii, cephalopods and crustaceans. The categories of freshness are: Extra, A and B (below B, the product is rejected for human consumption) according to the sensory evaluation of skin, eyes, gills, smell, flesh and other characteristics. EU grading scheme has some limitations, such as not taking into account the difference between the species (Hyldig, et al., 2010). It does not measure the quality itself, or freshness but rather the degree, or rate of change in important criteria used to describe these qualities (Green, 2011). For evaluation of fish freshness, raw material and others, a Quality Index Method appears to be an easy, rapid and efficient tool (Bernardi, et al., 2013). It does not require extensive training, is robust, easy to understand, and capable of integrating the effects of time and temperature (Hyldig, et al., 2007).

## 2.6 Quality Index Method (QIM)

According to Ólafsdóttir et al. (1997) and Sveinsdóttir et al. (2003) QIM can be defined as a grading system for freshness estimation of fishery products in which the descriptions of the individual grades are precise, objective and independent. It is usually based on quality attributes of raw fish, which are given scores according to descriptions of sensory attributes, and the method needs to be developed or adjusted to each fish product.

Hyldig and Green (2004) suggested QIM as a practical and objective tool for evaluation of fresh fish after catch and other parts of the production chain, also in official seafood inspection.

The main advantages in using this method are that it is non-destructive, and it takes into account the differences between the species (Green, 2010). According to Nielsen (2005), QIMs are

easier to use than some sensory methods and do not require any equipment other than the human senses.

QIM can be used for different marine species, including crustaceans, both wild and farmed, in different seafood, steaks, fillets, whole fresh and frozen fish. Also, in different storage conditions, ice, temperature ambient, modified atmosphere and different condition on chilled ( Bernardi, et al., 2013).

In the QIM reference manual ( Martinsdóttir, et al., 2001), an estimated shelf life for 12 fish species is given, assuming storage in ice at 0°C without fluctuations in temperature.

For raw fish, QIM defines characteristic changes that occur during storage in appearance of eyes, skin and gills, odour of gills and texture of flesh. A score from 0 to 3 is given for each quality parameter according to the specific parameter descriptions. The scores are summarised to give an overall sensory score referred to as the Quality Index (QI) ( Martinsdóttir, et al., 2001).

Bonilla et al (2005) defined a QIM for cod fillets (*Gadus morhua*). The attributes for skin side was brightness and slime but attributes for flesh side were regarding changes in texture, colour of blood, odour, colour of flesh, brightness and gaping. In another QIM for farmed tilapia fillets (*Oreochromis niloticus*), the descriptors for the skin side were colour and for the flesh side mucus, colour of flesh, texture, gaping, colour blood and odour (Cyprian, et al., 2014).

Bernardi et al (2013) summarizes a Quality Index (QI) range for 49 different fish species, between 2000 and 2011 available in the scientific literature, with the respective storage conditions and estimated shelf life.

After 2012, the QIM schemes have been developed for other species such as wild and fish farmed (*Boops boops*, L.) (Bogdanovic, et al., 2012), pacu (*Piaractus mesopotamicus*) (Borges, et al., 2013), whole fresh Lake Malawi tilapia (*Oreochromis spp.*) (Kapute, et al., 2013), arctic charr fillets (*Salvelinus alpinus*) (Tran Thi , et al., 2013), common carp (*Cyprinus carpio*) (Agueria, et al., 2016), hybrid tambacu (*Colossoma macropomum/Piaractus mesopotamicus*) (Borges, et al., 2014), acoupa weakfish (*Cynoscion acoupa*) (Billar dos Santos, et al., 2014), farmed tilapia fillets (*Oreochromis niloticus*) (Cyprian, et al., 2014), thawed Greenland halibut (*Reinhardtius hippoglossoides*) ( López, et al., 2014), mullet (*Mugil platanus*) (Andrade, et al., 2015), spiny lobster (*Panulirus argus*, Latreille, 1804) (Gonzalves, et al., 2015), gutted ice-stored hybrid tambatinga (*Colossoma macropomum/Piaractus brachypomum*) (Ritter, et al., 2016), gutted amazonian pintado (*Pseudoplatystoma fasciatum/Leiarius marmoratus*) (Lanzarin, et al., 2016).

## 2.7 The Torry scheme

The Torry scale is the most used scale for evaluating the freshness of cooked fish (Martinsdóttir, et al., 2009) (Table 4). It is also used in the fish industries of some countries and by buyers of fish products. Scores are given from 10 (very fresh in odour and flavour) to 3 (spoiled). It is considered unnecessary to have descriptions below 3, as the fish is then not fit for human consumption. The average score of 5.5 may be used as the limit for consumption. Then the members of the panel detect evident spoilage characteristics, such as sour taste and hints of “off” flavors ( Martinsdóttir, et al., 2001).

**Table 4: Torry scoresheet for freshness evaluation of cooked lean fish such as cod, haddock and pollock (Martinsdóttir, et al., 2001).**

Odour	Flavour	Score
Initially weak odour of sweet, boiled milk, starchy, followed by strengthening of these odours	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop	10
Shell fish, seaweed, boiled meat	Sweet, meaty, characteristic	9
Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity	8
Wood shavings, wood sap, vanillin	Neutral	7
Condensed milk, boiled potato	Insipid	6
Milk jug odours, reminiscent of boiled clothes	Slight sourness, trace of “off”-flavours	5
Lactic acid, sour milk, TMA	Slight bitterness, sour, “off”-flavours, TMA	4
Lower fatty acids (for example acetic or butyric acids) decomposed grass, soapy, turnipy, tallowy	Strong bitterness, rubber, slight sulphide	3

## 2.8 Microbiological measurements of fish freshness

Microbiological examination of fish evaluates the possible presence of bacteria or organisms of public health significance and gives an impression of the hygiene and quality of the fish including temperature abuse and hygiene during handling (Howgate, 1982).

The number of specific spoilage bacteria will give information on the remaining shelf life which can be estimated from such numbers (Huss, 1995).

When such microbiological measurements are needed it is recommended to use the numbers of specific spoilage organisms (SSO) or total viable counts (TVC) measurements (Olafsdottir et al, 1997).

Different peptone-rich substrates containing ferric citrate have been used for detection of H<sub>2</sub>S-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<sup>8</sup>-10<sup>9</sup> cfu/g of specific spoilage bacteria in the flesh are required to cause spoilage in iced fish (Gram & Huss, 1996).

## 2.9 Chemical measurements of fish freshness

Chemical methods involve analysing a sample to determine the concentration of a specific chemical (Howgate, 1982). A range of methods are used to measure total volatile bases (TVB), but in all of them the fish or an extract of the fish is made alkaline, the bases are distilled off, then 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).

According to the European Commission regulation No. 2074 (2005) unprocessed fishery products shall be regarded as unfit for human consumption where sensory assessment has raised doubts regarding freshness and chemical checks reveal that TVB-N has exceeded certain limits for example, is 25 MGN / 100 g maximum for perch species, 30 MGN / 100 g for flatfish and 35 MGN / 100 g for white lean fish such as cod and haddock.

### 3 METHODS

#### 3.1 Raw material

Pollock (*Pollachius virens*) was used in this study. The fish was captured and processed on board and cooled for preservation (Appendix 1). The fillets without skin were obtained in processing plant and packed as for flight: in polystyrene boxes, 10kg in each box with dry ice (Appendix 2).

All fish used for pre-observation was caught on December 7 and filleted on December 9, 2015. In order to train the panel on the new QIM scheme and at the same time evaluate the scheme itself, pollock fillets from different storage days were evaluated using the QIM scheme developed in the pre-observation. The information about dates of catch, time of arrival at Matis laboratories, temperature, storage time and training sessions is shown in Table 5.

**Table 5: Fillets for training sessions. Dates of catch, time of arrival at lab, temperature, storage time and training sessions.**

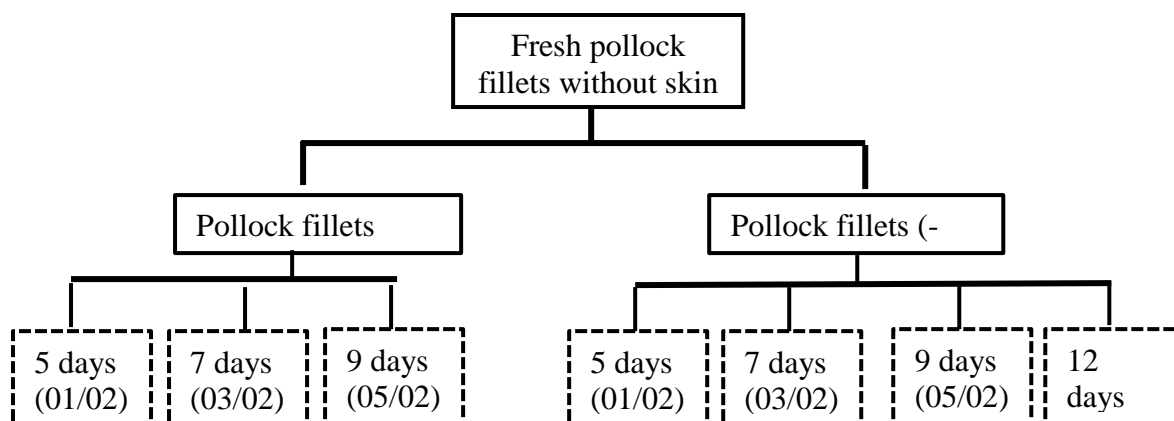
Captured Pollock (day)	Filleted (day)-received in Matis Lab	Temperature/days in storage	Training sessions	
			Days	Number
04/01/2016	06/01/2016	-1°C/9 days	15/01	First
06/01/2016	11/01/2016	-1°C/ 4 days	15/01	First
		-1°C/ 7 days	18/01	Second
		-1°C/ 8 days	19/01	Third
13/01/2016	15/01/2016	-1°C/3 days	18/01	Second
		-1°C/ 4 days	19/01	Third
17/01/2016	18/01/2016	-1°C/ 0 days (fresh)	18/01	Second
		-1°C/ 1 day	19/01	Third

For the shelf life study, pollock was captured on January 25 and processed January 27. The fillets were separated in two groups for the storage, one group was stored in a chilling chamber at 2°C and the other in a super- cooling chamber at -1°C (Appendix 3).

#### 3.2 Experimental design

The experiments were conducted at MATIS laboratories in Reykjavik, Iceland, 15 December 2015 to 15 February 2016.

For the shelf life study of fresh pollock fillets, stored at -1 and 2 ° C, it was necessary to follow a schedule for sampling (Figure 2). Five boxes were prepared for each storage temperature, one of each group per sampling day. Each box contained 8 fillets, 4 fillets for QIM and 4 fillets for microbiological, chemical and Torry analyses. The microbial division collected their samples before anyone else touched the insides of the boxes.



**Figure 2: Flow work for the shelf life study of fresh pollock fillets without skin (-1 and 2°C)**

### 3.3 Monitoring of temperature

The temperature was monitored during shelf life study using temperature loggers. For fish stored at 2°C, the loggers were put in the boxes that was analysed on day 9, on the top of the box and inside it. The same procedure was carried out for fish stored at -1°C, but in this case, the boxes for sampling days 9 and 12 were monitored. Furthermore, in order to monitor the temperature in the chambers, two loggers were put in each chamber, one near the door and another further inside the chamber.

### 3.4 Development of QIM scheme for pollock fillets

#### 3.4.1 Preliminary observation (pre-observation)

The experiment was conducted in the processing facilities at Matis, on white table under white light. The fillets were observed by the panel leader and an expert, using a QIM scheme for cod fillets described by Bonilla, 2007 as a basis for the evaluation. The scheme was modified in line with the attributes of the pollock fillets as needed during the pre-observation. Pollock fillets were evaluated 0, 2, 7 and 9 days from filleting. The attributes analysed were colour of fat line and flesh, and odour of skin side and colour of flesh, odour, and texture, colour of blood, brightness and gapping in the flesh side.

#### 3.4.2 Training of panellists

In the first training session, the storage day was given for two samples (three fillets per storage time, four and nine days). The remaining fillets were coded with a three-digit number. The panel leader explained the QIM scheme for pollock fillets and demonstrated the evaluation, using the fillets with the shown storage time. Then the panel evaluated the remaining fillets, supervised by the panel leader. At the end of the session, the panel was informed about the storage time. After the training session, the panel leader and the panellists discussed the scheme and the panel leader made changes in the scheme, according to suggestions by the panellists.

During the second training session, the panellists proposed and discussed many changes in the scheme for pollock fillets, after the evaluation. With this information, the panel leader made corrections in QIM scheme and got the final version.



In the third session, the sensory panel leader presented pollock fillets of different storage time (new fillets, fillets stored several days at 2°C and fillets at the end of shelf life) and discussed with the panellist the final version of the QIM scheme. The new QIM scheme was used to evaluate 12 pollock fillets, coded with three-digit numbers.

### 3.5 Shelf life study of fresh pollock fillets (-1 and 2°C)

#### 3.5.1 Sensory evaluation by QIM scheme

The new QIM scheme, developed during pre-observation and panel training sessions, was applied for the sensory analysis of pollock fillets. The panellists evaluated four fillets of each group each sampling day individually and registered their evaluation for each quality parameter in the scheme.

#### 3.5.2 Sensory evaluation of cooked pollock fillets

For training of the sensory panel in sensory evaluation of cooked pollock fillets, Torry scale for lean white fish was used to evaluate samples of different storage time (Table 4). Each panellist received samples weighing about 40–50 g. The samples were taken from the loin part of the fillets and placed in aluminium boxes coded with A (fresh), B (fillets stored several days at 2°C) and C (end of shelf life). Comparable samples were served in big containers.

The samples were cooked at 95 to 100°C for seven minutes in a pre-warmed oven with air circulation and steam. In the sensory room, the panellists individually evaluated the samples using the Torry scale (Table 4). Afterwards, the panellists discussed the results with the panel leader, and were asked to repeat the evaluation of samples in the larger container. The panel leader explained the Torry scale and how odour and flavour of pollock changes with storage time.

The same procedure was used for evaluation of the fillets in the shelf life study. Four fillets per group per storage day were coded with three-digit numbers without information about the storage time.

#### 3.5.3 Microbial evaluation

Minced flesh (20 g) was mixed with 180 g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done with cooled MRD as required. Total viable psychotropic counts (TVC) and counts of H<sub>2</sub>S-producing bacteria were evaluated on iron agar (IA) as described Gram (1987). Plates were spread-plated and incubated at 17 °C for 4-5 days. Counts were reported as logarithmic average values (log<sub>10</sub> colony-forming unit's cfu/g).

#### 3.5.4 Chemical evaluation

TVB-N was determined in the remaining minced pollock fillets after sampling for microbial analysis. Analysis was carried out according to the method described Malle (1989) used at Matis ohf.

200 ml of 7.5% aqueous trichloroacetic acid (TCA) solution was added to 100 g of minced fish meat and homogenized in Waring blender. Then the mixture was filtered through a Whatman No.3 filter paper. Total volatile basic nitrogen (TVBN) was determined by distillation after the addition of 6ml of 10% NaOH to 25ml of filtrate. The distillate was collected in a beaker containing 10 ml of 4% aqueous solution of boric acid and a mixed indicator produced from

dissolution of 0.04 ml of methyl red and methylene blue. Finally, the boric acid solution was titrated with a 0.0347 N H<sub>2</sub>SO<sub>4</sub> solution.

### 3.6 Data analysis

Microsoft Excel 2013 was used to calculate the means, analysis of variance, linear regression and to generate graphs. Significance level was set at 95% ( $p < 0.05$ ).

## 4 RESULTS

### 4.1 QIM scheme for pollock fillets (*Pollachius virens*)

A QIM scheme for pollock fillets was developed through some changes during the pre-observation and training (Table 6). The preliminary QIM scheme developed during the pre-observation of pollock fillets is shown in Appendix 4.

During the training sessions, several modifications were made in the QIM scheme, and the final scheme described four parameters for skin side and seven for flesh side (Table 6). Quality parameters regarding skin side in colour and odour were changed. On the skin side, the most important changes were evidenced in colour and odour of fat lines. The colour changed between dark brown, transparent and reddish to yellowish. Meanwhile, the odour changed from being fresh and marine to rancid, sour milk and spoilage odour.

Moreover, the characteristic translucent was added with consensus among the panellists.

In addition, all attributes in the flesh side were modified during training, based on how the sensory attributes changed with the storage time. The panellists found colour and odour in the flesh side difficult to describe. In the pre-observation, the colour changed gradually, but during training, several new descriptions were included for the colour of flesh; pale, grey, bluish, pinkish, pale brown, beige, yellowish and discoloured. For odour, the description was described as cucumber and melon on both sides, but fresh, marine, neutral, rancid, spoilage and sour milk, were the same for the bout side.

To make a preliminary estimate of the QIM scheme, a linear regression was made between the average QI scores and the storage time (Figure 3).

The mean QI was calculated for three storage days, at 2°C (1, 3 and 7 days) (Figure 3). The results showed a high correlation ( $R^2=0.9856$ ) between QI and the storage time.

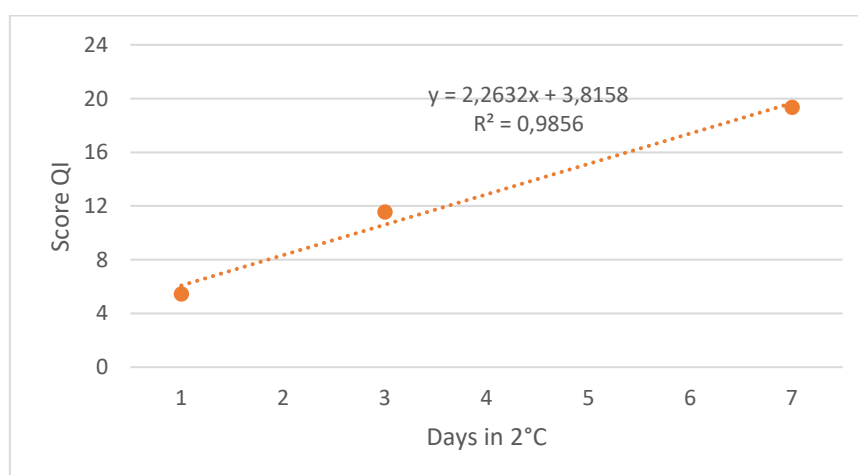
The QI scores for quality parameters increased with storage time at 2°C in the skin side (Appendix 3, Figure 19). The scores for all evaluated parameters were around 0 in the beginning of the storage for both skin and flesh side. Gaping was already evident in the beginning of storage (slight gaping, less than 25% of the fillet). Progressively, the gaping increased with storage time, between 25 – 75% (Appendix 5, Figure 20).

Although the rest of attributes for flesh side followed the same quality changes as the skin side during training, the evaluation of blood in the fillets depends on another factor. The handling and bleeding on board can have an influence. If blood was present, it should be bright red in very fresh fillets and change during storage to dull red and shadowy, brown. However, if blood is not present, the punctuation will be zero while other parameters change the quality level.

**Table 6: Quality Index Methods (QIM) scheme for pollock fillets (*Pollachius viren*)**

Quality parameters		Description	Points
<b>Skin side</b>	Colour of Fat lines	Dark brown, transparent, reddish	0
		Beige, pinkish	1
		Yellowish	2
	Colour of Flesh	Grey, bluish, reddish	0
		Paler grey, pink	1
		Pale, yellowish	2
	Translucent	Translucent	0
		Less translucent	1
		Opaque, milky	2
	Odour	Fresh, marine	0
Capelin, trace of sour milk		1	
Rancid, spoilage, sour milk		2	
<b>Flesh side</b>	Colour of Fat lines	Dark, brownish	0
		Slightly yellowish	1
	Colour of flesh	Pale, yellowish	2
		Pale grey, bluish, pinkish	0
		Pale brown, beige	1
	Translucent	Yellowish, discoloured	2
		Transparent, bluish	0
		Less translucent	1
	Odour	Opaque, milky	2
		Fresh, marine, neutral	0
		Cucumber, melon	1
	Texture	Overage melon, trace of sour milk	2
		Rancid, spoilage, sour milk	3
		Firm: recover quickly after pressure	0
	Blood	Rather soft: recover slowly after pressure	1
		Very soft: does not recover after pressure	2
		Bright red, not present	0
	Gaping	Dull red	1
		Shadowy, brown	2
		No gaping, one longitudinal gaping at the neck part of the fillet	0
	Slight gaping, less than 25% of the fillet	1	
	Gaping, between 25 - 75% of the fillet	2	
	Extensive gaping, over 75% of the fillet	3	

**Quality index (0–24)**



**Figure 3: Changes of average Quality Index with storage time for pollock fillets (*Pollachius viren*) stored at 2°C.**

## 4.2 Shelf life study of fresh pollock fillets (-1 and 2°C)

### 4.2.1 Temperature profile

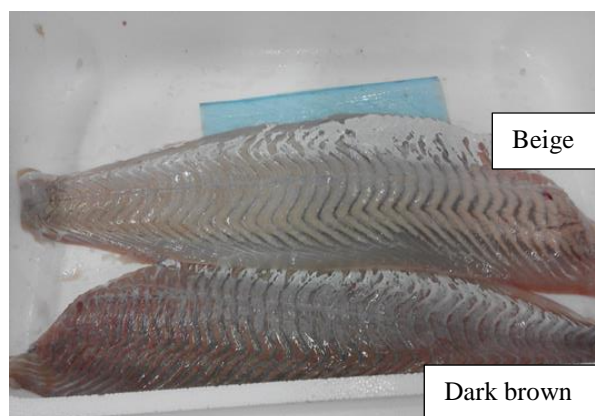
The temperature profile by loggers are shown in Appendix 5. On average, the temperature in the chamber set at -1°C was -0.96°C during the 12 days (Appendix 6). The temperature was constant and without fluctuations on the top and inside the boxes. The temperature close to the door and further inside the chamber fluctuated around 0°C. The temperature inside the chamber set at 2°C, was on average 2.7° C during the study. In the door and inside the chamber, the temperature was around 2°C (Appendix 6).

### 4.2.2 Quality Index Method

In the QIM scheme, the sum of the Quality Index = 0 should indicate fresh fish, but the maximum sum of scores (24) represents very spoiled fish. A score of about 12 coincided with the level at which the fish were considered unacceptable by the members of the panel according to sensory evaluation of cooked fillets.

During the shelf life study, a great difference in colour of fillets in the same box were observed. These differences were evident in both analysed temperatures.

At the beginning in the shelf life study, the pollock fillets showed differences in the colour on the skin side. On day zero, four fillets were analysed, two presented the beige colour in fat lines and two dark brown (Figure 4). According to QIM, when the sample is fresh, the fat lines should be dark brown, transparent or reddish but become yellowish with deterioration. One important thing to note, is that beige coloration did not translate into off-odours, like rancid or sour. Moreover, the rest of attributes for the skin side, colour of flesh, translucent and odour, the characteristics were grey, bluish, reddish, translucent and fresh, marine, respectively.



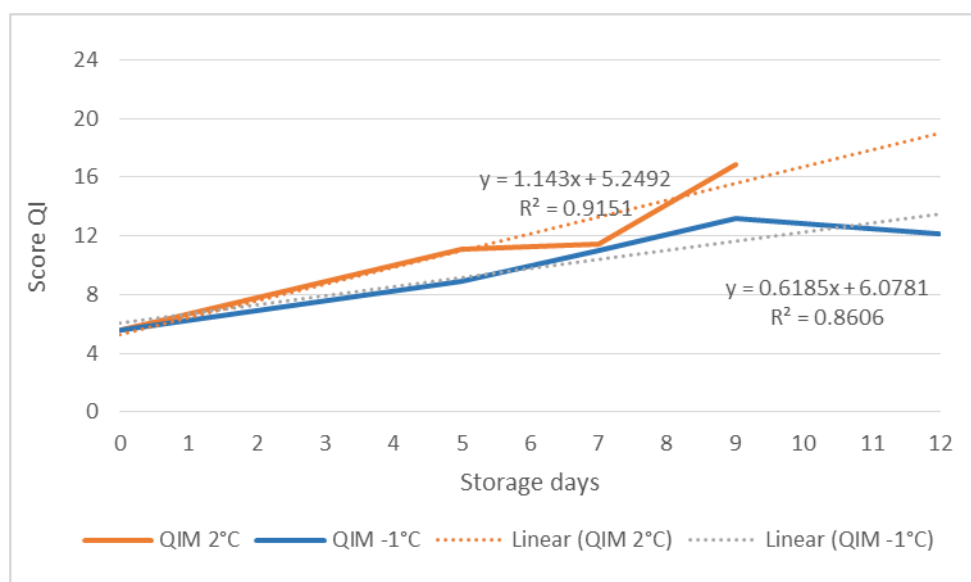
**Figure 4: Appearance of skin side fillets of pollock at the beginning of storage time, colour fat lines dark brown and beige.**

On the flesh side, the colour of fat lines was dark or brownish, colour of flesh was pale grey, bluish, pinkish, transparent and bluish, with fresh, marine odour, firm texture, and blood generally not present. If blood was present, the colour was bright red. Slight gaping, less than 25% of the fillet was present at the beginning of the study (day 0). The evaluation for colour and gaping at day zero, influenced the initial QI score, and was approximately 6.0.

At day 9 of storage it was decided to stop the study for 2°C treatment and at day 12 for treatment at -1°C, based on the sensory evaluation of cooked fillets. At that time the colour of fat lines and flesh was yellowish, pale and the brightness was opaque. The flesh texture was very soft. If blood was present, it was shadowy brown, and an extensive gaping was observed. The spoilage odour of the skin side of fillets stored at -1°C was rancid and therefore reflected spoilage due to oxidation rather than microbial spoilage. Spoilage odour of fillets stored at 2°C reminded of sour milk, both for skin and flesh side, which indicates microbial spoilage.

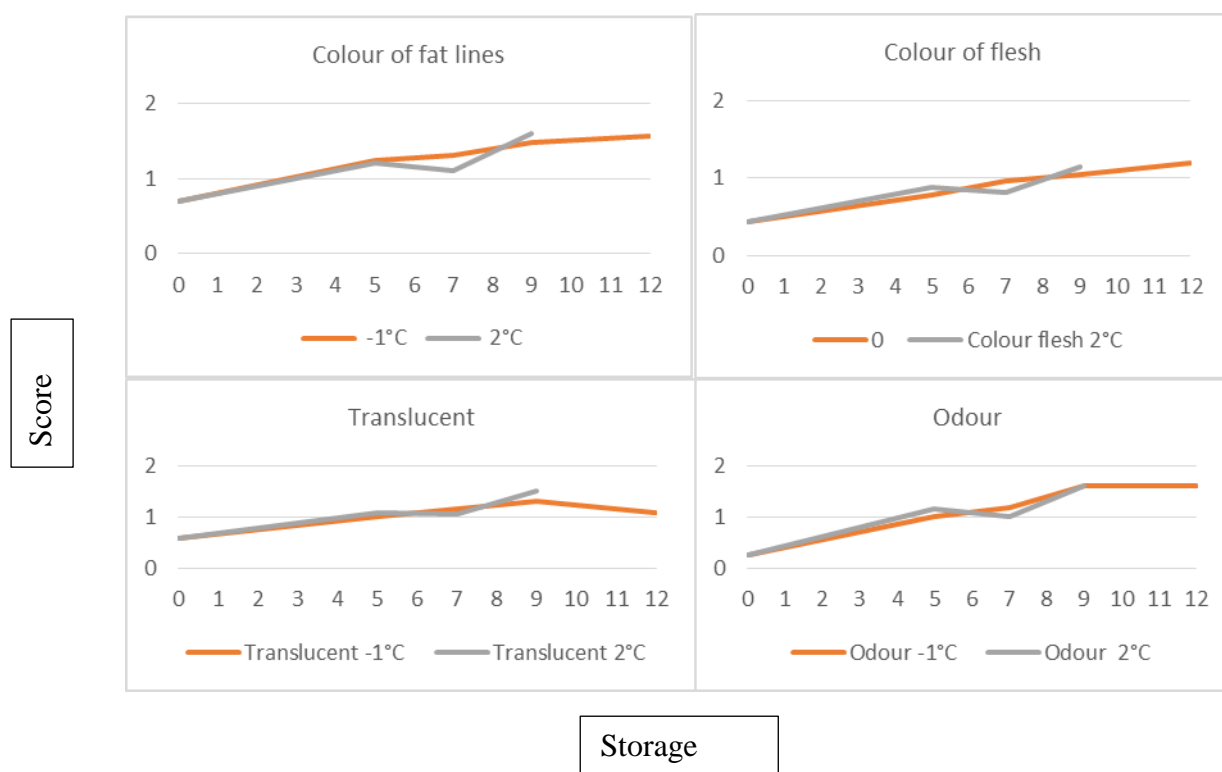
The average scores of parameters according to the QIM scheme was presented as QI, and was based on average score of four fillets per trial. Figure 5 shows the QI changes with time for both temperatures.

The correlation between the average QI and days of storage at 2 and -1°C was different. The linear relationship was strong for 2°C ( $R^2 = 0.915$ ). This result shows that the parameters were gradually deteriorating over time under these storage conditions, translating into a high QI. However, the correlation between the average QI and days of storage at -1°C was weaker or  $R^2 = 0.829$ .



**Figure 5: QI of pollock fillets. Averages scores for each day of storage analysed against days at -1 and 2°C.**

The QI scores for the quality parameters increased with storage time in the skin side for both temperature, as shown in Figure 6. Although, the colour for the fat lines started the study with high score compared with colour of flesh, odour and translucent. Nevertheless, in 5 days all attributes increased the score and quality deteriorated, until the end. The characteristics showed similar conduct for both treatments.



**Figure 6: Average scores for each quality attribute for skin side of pollock fillets; colour of fat lines, colour of flesh, translucency and odour, evaluated with QIM scheme for pollock fillets during the shelf life study.**

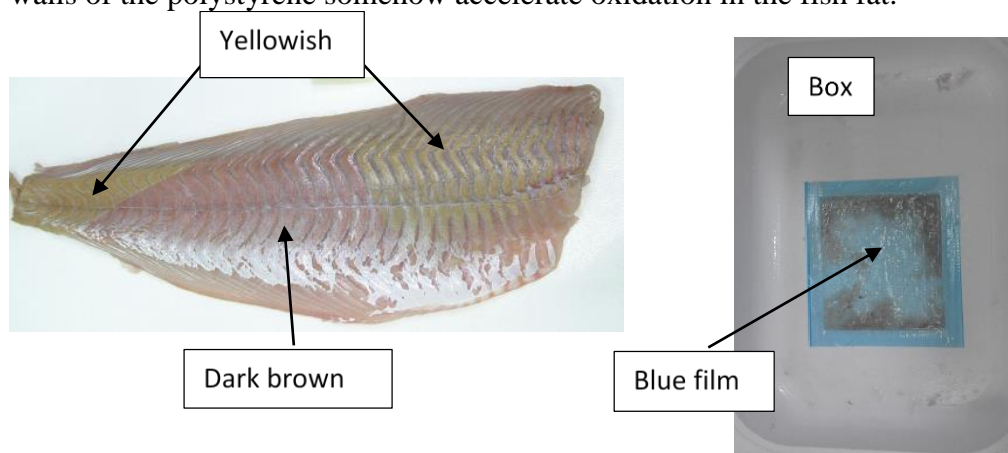
The scores for all evaluated parameters for flesh side were close to 1 at storage day zero (Figure 7) except for gaping which scored higher than 1. The scores increased days five and the end of shelf life. The changes in the blood attribute, depended on if it was present or not.



**Figure 7: Average scores for each quality attribute for skin side of pollock fillets; colour of fat lines, colour of flesh, translucency, odour, texture, colour of blood and gaping, evaluated with QIM scheme for pollock fillets during the shelf life study.**

During the experiment, a change in colour was seen both in the fillets and in the box, change of dark brown to yellowish (Figure 8), thus, the change in colour and odour for the skin side. Two different colours appeared in the same fillet at the same time. This seem to coincide with if the fillet lied at the bottom of the box with the skin side partly attached to the blue film. The colour was dark brown, and the odour was marine and fresh in the parts of the fillets attached to the

blue film. The parts of the fillets which lay against the box (not the blue film) were yellowish and had a rancid odour, indicating oxidation in those parts of the fillets. This indicates that the walls of the polystyrene somehow accelerate oxidation in the fish fat.

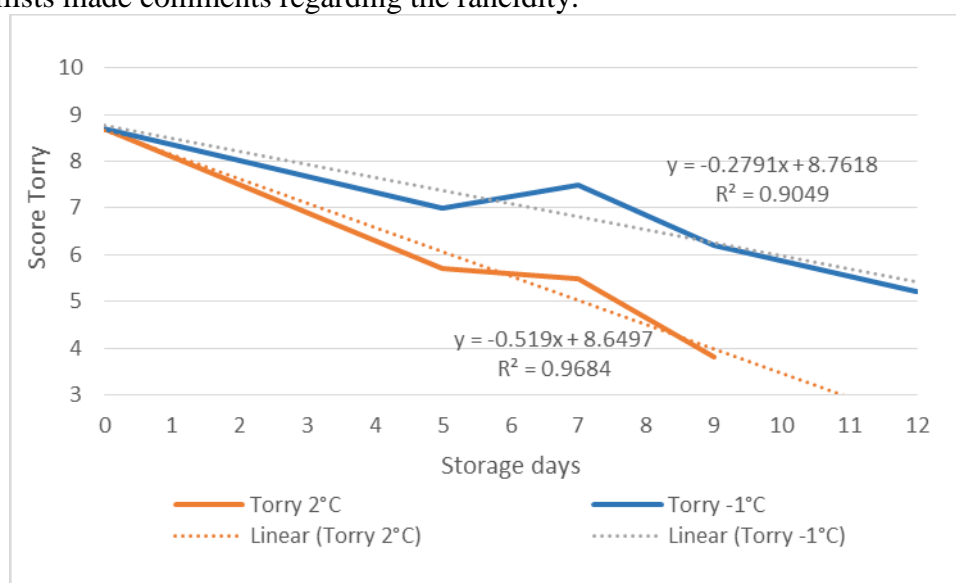


**Figure 8: Reaction between fillet and box. Changes in colour for the skin side (yellowish and dark brown).**

#### 4.2.3 Torry scheme

The Torry results for cooked pollock fillets is shown in Figure 9. On day zero the Pollock received an average score of 8.7, reflecting an odour of shellfish, seaweed and boiled meat. The flavour was sweet, meaty and characteristic for fresh pollock. Progressively, the freshness deteriorated at temperature 2°C, and after 9 days the pollock had a TMA odour and slightly bitter flavour, sour flavour, off-flavour and TMA flavour.

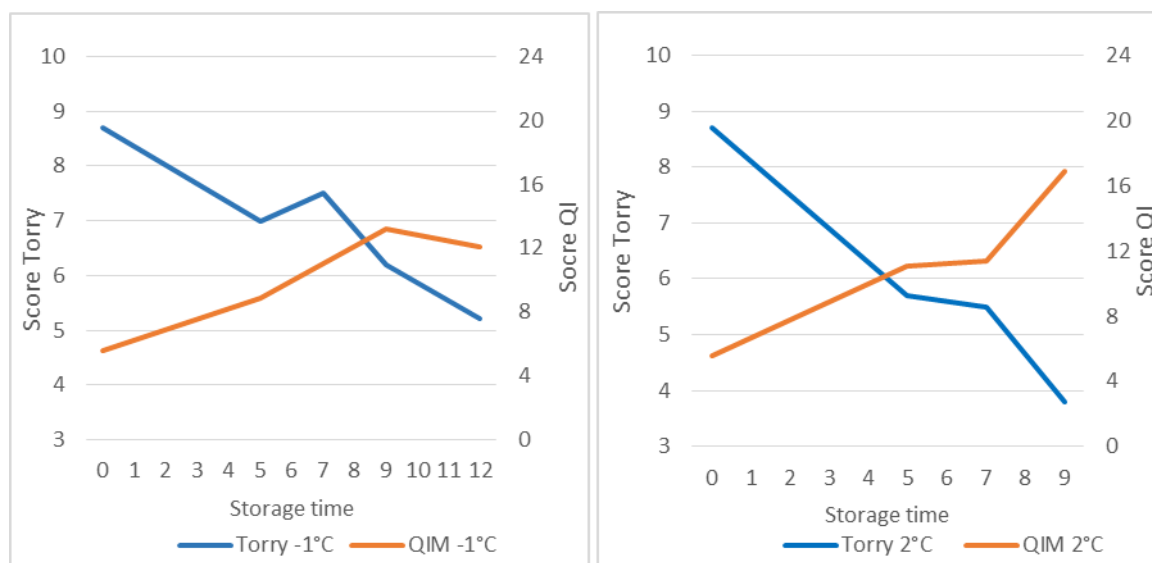
The fillets stored at -1°C had a rancid flavour after 9 days of storage and rancid odour and flavour were the dominating attributes after 12 days of storage, more than TMA and spoilage sour odours and flavours. Rancid flavour is not mentioned in Torry scheme, however, some of the panellists made comments regarding the rancidity.



**Figure 9: Torry scores for cooked pollock fillets. Averages over each day of storage analysed against days at -1 and 2°C.**



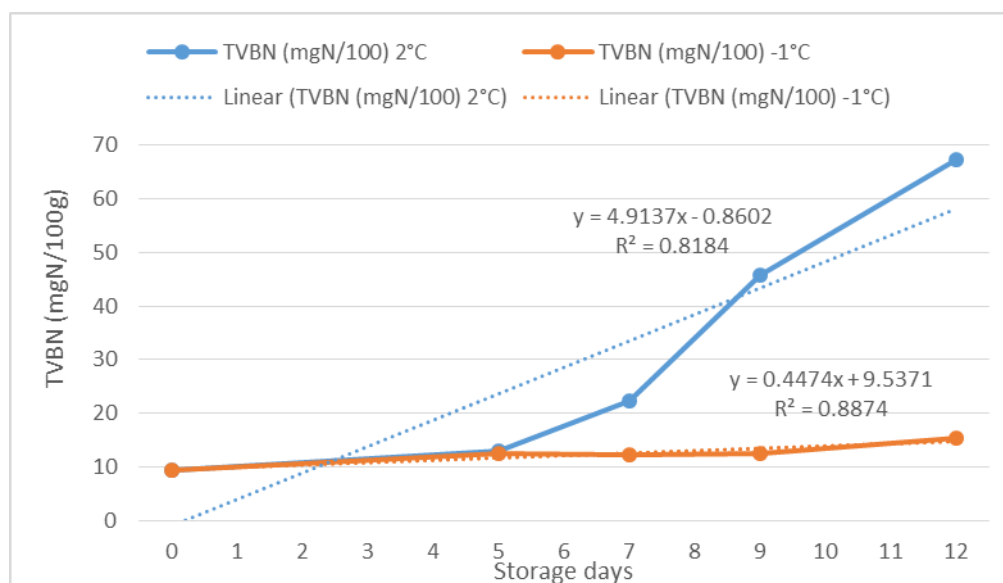
Figure 10 shows the Quality Index score and Torry for pollock fillets stored at -1 and 2°C. Results for fillets stored at 2°C showed a high correlation with storage time,  $R^2 = 0.9151$  for QI and  $R^2 = 0.9684$  for Torry ( $p < 0.05$ ). For fillets stored at -1°C, the correlation was lower than for 2°C and not significant ( $p > 0.05$ ),  $R^2 = 0.8606$  for QI and  $R^2 = 0.9049$  for Torry.



**Figure 10: Mean scores for QI and Torry during storage time for pollock fillets stored at -1 and 2°C.**

#### 4.2.4 TVBN contents

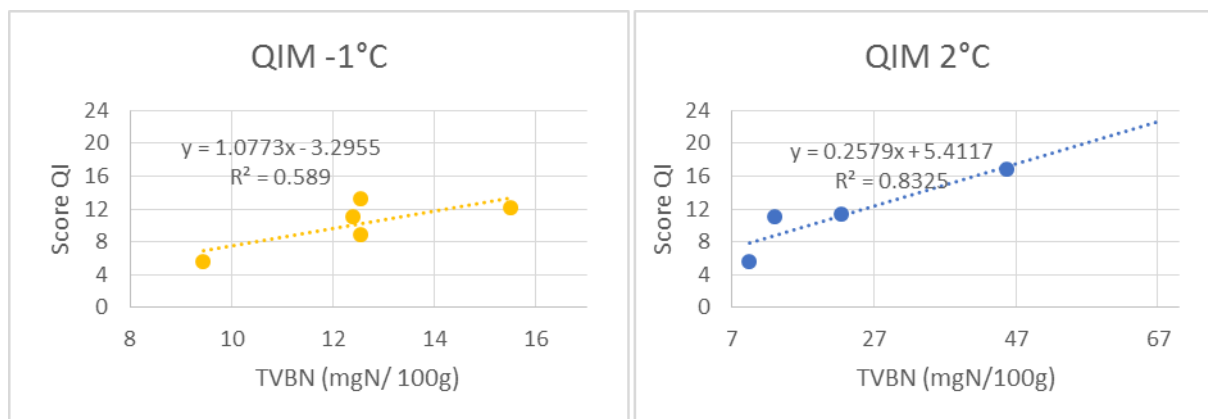
TVBN contents of pollock fillets showed slow increase during storage at -1°C (Figure 11). After 5 days, the level increased more rapidly during 2°C storage, as shown in Figure 11. TVBN value at the beginning of the storage time was 9.45 mg/100g, it increased from on day 0 to an acceptable value of 15.5 mg/100g in 11 days at -1°C (EC No. 2074, 2005). Whereas, at 2°C to a rejection value of 67.3 mg/100g at the end of the same period.



**Figure 11: Changes in TVBN levels in pollock fillets stored at -1 and 2°C.**

The correlation between mean scores for Quality Index and TVBN levels (mgN/100) for -1 and 2°C during shelf life study for pollock fillets is shown in Figure 12.

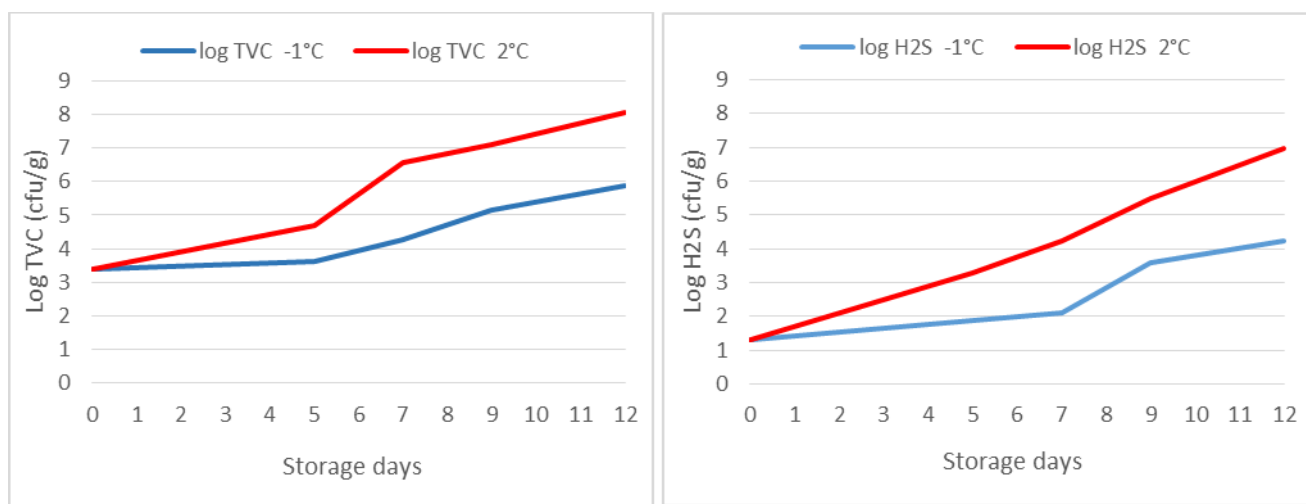
The correlation was much lower for fillets stored at -1°C ( $R^2 = 0.589$ ) than for fillets stored at 2°C ( $R^2 = 0.8325$ ) the correlations in neither temperature was found significant ( $p > 0.05$ ).



**Figure 12: Correlation between mean Quality Index scores and TVBN levels of pollock fillets stored at -1 and 2°C.**

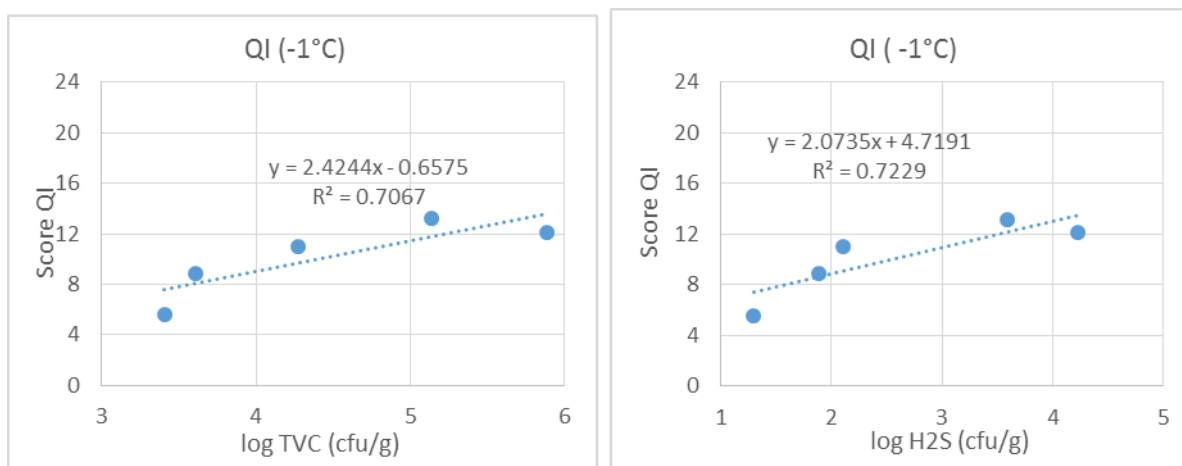
#### 4.2.5 TVC and H<sub>2</sub>S count

The microbial counts increased with storage time, as shown in Figure 13. At the beginning of storage, the TVC was around  $3.3 \times 10^2$  cfu/g and H<sub>2</sub>S-producing bacteria were 20 cfu/g. However, after 12 days of storage at -1°C (time of sensory rejection of samples), TVC was approximately  $7.5 \times 10^5$  cfu/g and H<sub>2</sub>S-producing bacteria  $1.9 \times 10^4$  cfu/g. For fillets stored at 2°C, TVC was  $1.2 \times 10^8$  and H<sub>2</sub>S-producing bacteria  $9.5 \times 10^6$  cfu/g after 12 days of storage.



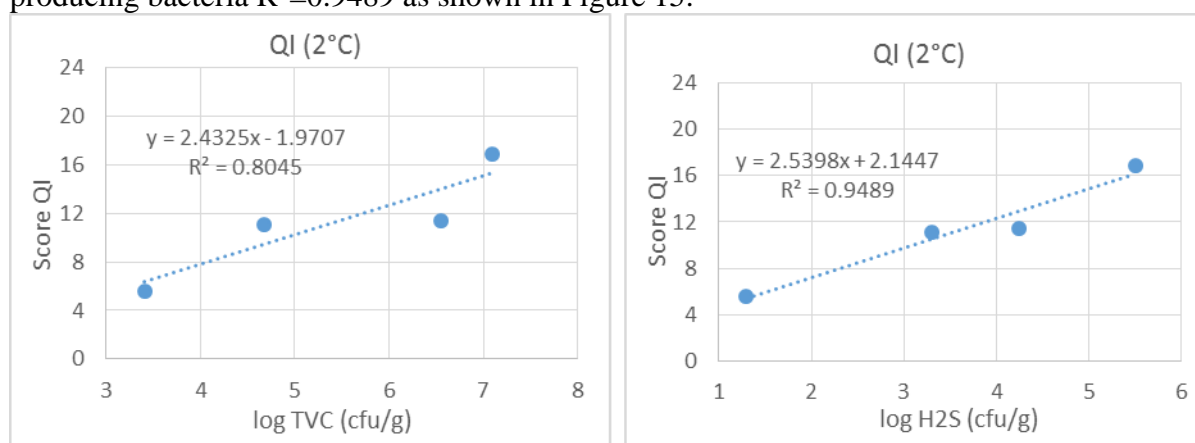
**Figure 13: Total viable counts and selective counts of H<sub>2</sub>S -producing bacteria in pollock fillets stored at -1 and 2°C during shelf life study.**

The correlation at -1°C was weaker than at 2°C, between mean Quality Index and TVC (cfu/g) ( $R^2 = 0.7067$ ) and selective counts of H<sub>2</sub>S (cfu/g) producing bacteria ( $R^2 = 0.7229$ ) as shown in Figure 14.



**Figure 14: Correlation between mean Quality Index and TVC and selective counts of H<sub>2</sub>S producing bacteria of pollock fillets stored at -1°C.**

The correlation between QI and TVC at 2°C was  $R^2=0.8045$  and selective counts of H<sub>2</sub>S producing bacteria  $R^2=0.9489$  as shown in Figure 15.



**Figure 15: Correlation between mean Quality Index and TVC and selective counts of H<sub>2</sub>S producing bacteria of pollock fillets stored at 2°C.**

## 5 DISCUSSION

### 5.1 Development and evaluation of QIM scheme for pollock fillets (*Pollachius viren*)

The deteriorative changes occurring in pollock fillets were observed during the pre-observation sessions. Changes occurred on the skin side (fat lines, flesh and odour) and flesh side (fat lines, colour, transparent, odour, texture, blood and gaping) of the fillets. These attributes were included in the pre-observation scheme at 2°C.

Although the cod QIM was used as a reference in the pre-observation, some changes had to be made during the training sessions. Some of the words used to describe the colour and the odour had to be changed. To better describe these attributes, the scores were increased from 2 to 3 during the training sessions. The QIM takes into account specific aspects of each species, or product, assessing the quality and freshness of the fish by sensory analysis of a set of attributes considered relevant (Esteves and Anibal, 2007). Moreover, attributes like “transparent” was included to describe the skin side. The first sensory changes of fish during storage were related to appearance and texture (Huss, 1995).

Detailed guidelines were needed for the panellists to describe the flesh texture for every score (0- firm: recover quickly after pressure, 1- rather soft: recover slowly after pressure and 2- very soft: does not recover after pressure). It is important to always evaluate the texture at the same place on the fillet to make the evaluation constant and reliable. The tail of the fillet is very soft but near to the neck the flesh is firm. These guidelines include recommendations to evaluate the texture in the middle of the spine muscle by pressing a finger and observing if and how fast the flesh recovers (Martinsdottir, et al., 2001). The same behaviour for texture in flesh was described by Bonilla (2007) for cod fillets with skin in the training session.

Pollock fillets stored at 2°C were used for the Torry training session and therefore rancid odour and rancid flavour was not detected in the samples before the start of the shelf life study.

## 5.2 Shelf life study of fresh pollock fillets (-1 and 2°C)

The temperature loggers showed the true (real) conditions in the experiment. For the -1°C treatment, the temperature did not change inside the box. For the 2°C treatment, the temperature increased slightly by 0.7°C after the third storage day. This narrow increase in temperature probably influenced the shortened shelf life. Any small increase in temperature during storage affects microbial growth and consequently decreases the shelf life (Ryder et al. 2014). Changes in the freshness of fish can be accelerated or retarded by physical conditions like temperature, physical damage to fish, pollution and contamination by bacterial flora. Of all the physical and chemical factors influencing spoilage, temperature is the most significant. This is because chemical reactions, enzymatic activity and bacterial multiplication require an optimal temperature range (Doyle, 1995; Cyprian, 2006) and by keeping the fish temperature below this range, the impact of those factors can be reduced.

Many factors may affect the freshness of fish. These include the method of catching, overall handling and the processing of the fish (Massaquoi, 2011). Also, it depends on the species, quality of fishing ground, season, sexual and nutrition status (Jones and Disney 1996). At the beginning of the shelf life study, a difference was seen in the appearance between the pollock fillets regarding colour of fat lines, gaping and blood. The status of the starting material affected the initial conditions of the Quality Index, and consequently, the shelf life.

The gaping and the blood in fresh pollock fillets, can possibly be explained through the on-board processing methods. Mechanical strain due to fishing gear, drops or handling points can cause gaping, bruising, bloodstains and increased stress (capture induced). Moreover, if the bleeding is not efficient during handling process in the industry, the fillets may have presence of blood (Huss, 1995; Matis, 2016).

The differences in the colour on the skin side at the beginning of the experiment may be attributable to biological difference between Pollock individuals. This explanation is likely since the fish for this experiment was all caught at the same time in the same fisheries areas and processed at the same time.

A high correlation was found between the total QI score and the storage time at 2°C ( $R^2 = 0.915$ ), which shows that the attributes gradually deteriorated with time. The individual attributes were independent of each other, but all changed and received higher scores through increased storage time. However, for -1°C ( $R^2 = 0.829$ ), the correlation was lower than for 2°C. The most important attributes influencing that difference were colour of fat lines and odour, both on skin side.

The most probable cause is an oxidation reaction between fat lines and the inner surface of the box. The chemical composition for pollock fillets include total fat of 1.9%, with saturated fat 0.3%, polyunsaturated fat 0.9%, monounsaturated fat 0.2% and cholesterol 135mg.

Whereas, the large amount of polyunsaturated fatty acids moieties found in fish lipids makes them highly susceptible to oxidation by an autocatalytic mechanism. They result in production of a range of substances among which some have an unpleasant (rancid) flavour and odour. The reaction mainly depends on fish species and storage temperature (Ryder, 2014).

Lipid oxidation of dark muscle has been shown to be closely related to meat darkening and development of the rancid off-odour during the early stage of ice storage of cultured yellowtail, *Seriola quinqueradiata* (Sohn et al., 2005).

Bonilla (2007), who created the QIM for cod fillets, did not report any changes in the attributes related to oxidation reaction. For fish classification by fat content, pollock and cod belong to the group of whitefish (maximum content 2%) (Ryder, 2014). However, pollock has a higher quantity of fat (1.9%) compared with cod (1.5%). Furthermore, the pollock fillets were without skin but the skin prevents access of oxygen (Huss, 1995). During evaluation of pollock fillets it is important to note that when a fillet is heterogeneous regarding the colour of fat lines on the skin side, the parts of the fillet that show signs of deterioration, that is yellowish and rancid odour, should be evaluated.

Due to individual variations between fillets of the same storage day (and conditions), four samples per group were analysed. According to Martinsdóttir et al. (2001), in the manual 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 differences.

Using the QIM system, the linear relationship between the quality index (QI) and the storage time on the ice makes it easy to calculate the remaining shelf-life of fish (Hyldig, et al., 2007). Pollock fillets reached the limits of acceptance between nine and eleven days at  $-1^{\circ}\text{C}$  and six to nine days for  $2^{\circ}\text{C}$ , according to the sensory evaluation of cooked pollock (Torry scale 5.5).

The pollock fillets were fresh at the beginning of the shelf life study, with only two days between catch and processing, but the Torry score was 8.6 at day zero. The same methods for evaluate different species, the characteristics in flavour and odour between pollock and cod, using the same commission, are the most important factors. The panellists have expertise evaluate cod, the flavour and odour are sweeter for it, but in pollock case, the attributes are more neutral while being fresh. In Torry scale, neutral belong to 7 point for flavour and 8 point for odour, furthermore, the panellists have not enough experience in evaluate this species, therefore is necessary increase the Torry training sessions.

For the pollock stored at  $-1^{\circ}\text{C}$ , rancid odour and flavour during storage, and was the main reason for sensory rejection according to comments made by the sensory panellists. While at the time of sensory rejection, the spoilage characteristics of pollock stored at  $2^{\circ}\text{C}$  could be identified in the Torry scale. Rancid odour and flavour characteristics were not included in the Torry scale, which was developed for white lean fish, and might therefore have affected somewhat the estimation of maximum shelf life for pollock stored at  $-1^{\circ}\text{C}$ .

Literature was reviewed to inform a modified the Torry scale before used in their experiments, e.g. in cultured sea bream (*Sparus aurata*) stored in ice, included the attributes texture too in the scale (Alasalvar et al, 2001), Mediterranean anchovies (*Engraulis encrasicolus*) storage in ice (Pons et al. 2006), whole Mediterranean *Sea common sole* (*Solea solea*) in ice (Özoğul, et al., 2011).

For the  $2^{\circ}\text{C}$  storage group, TVBN showed a considerable increase after seven days. Nonetheless, for  $-1^{\circ}\text{C}$ , TVBN was not a good indicator of evaluate grade of freshness as such analyses only reflect advanced spoilage, and are not considered very reliable for measuring the deterioration of certain species (Castro et al., 2006; Tejada et al., 2007; Özogul et al., 2007).

Thus, other indicators should be chosen with good correlation with the degree of freshness. Alasalvar et al. (2001) found a high correlation between QI and K, Ki and G values in cultured sea bream (*Sparus aurata*) stored in ice. Hernandez et al. (2009) found very strong correlation between TBA, sensory and microbiological analyses with storage time, and they may be considered suitable indicators for evaluating meagre (*Argyrosomus regius*) fillet spoilage during refrigerated storage, while TVBN and TMA failed to demonstrate a clear tendency to increase over the storage period.

The initial (day 0) total viable count was 3.41 log cfu/g which indicated that the pollock fillets were of a good quality, in addition, it showed the hygiene in the industry and the safety fish handling. Paleologos et al. (2004) and Taliadourou et al. (2003) found similar initial mesophilic counts for whole sea bass (3.5 log cfu/g in both studies), and higher counts for fillets (4.9 and 5.2 log cfu/g, respectively) stored under the same conditions, attributing the relatively lower quality of the fillets to contamination during the filleting process.

The low total counts reported at the beginning of storage time were due to the flesh of newly caught fish being sterile, since the immune system of the fish prevents the bacteria from growing (Cyprian et al., 2008). Furthermore, the bacterial flora on newly-caught fish depends on the environment in which it is caught rather than on the fish species. After catch, 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. Then, they invade the flesh, during storage (Ryder et al. 2014).

Importantly, different pollock species have been reported to contain histidine, such as Alaska pollock (*Theragra chalcogramma*) contained 316 mg/100g (Bechtel and Johnson 2004) and Atlantic pollock (*Pollachius virens*) had 390 mg/77g of flesh (self-nutrition data, 2016). Several studies of the natural microbial population of marine fish revealed their ability to produce high amounts of histamine at low temperatures (Baranowski et al., 1985; Halasz et al, 1994; Valeiro and Pilas, 1994).

According to Joshi and Bhoir (2011) it is necessary to analyse the fish belonging to non-scombroid group may also cause histamine poisoning. Although, in European Regulation 2073 (2005), established the species with control for histamine and its limits (*Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae* and *Scombrosidae*).

The QI had a high correlation with H<sub>2</sub>S producing bacteria (cfu/g) than TVC (cfu/g) at 2°C. This type of bacteria is an important spoilage organism of chilled and aerobically stored fresh fish (Hozbor et al. 2006). Then, these affected the sensory attributes like odour and flavours, detected by Torry methods. Different behaviour was presented for -1°C, the growth was lower compared with the same time and Torry results.

The ICMSF (1986) has established an aerobic mesophilic count limit of 7 log cfu/g for fish that is fit for human consumption. These limits were reached on day 9 of storage for fillets stored at 2°C (7.1 log cfu/g). With these limits were reached the Quality Index was 15.

In the -1°C storage group, the bacterial counts were lower than 7.1 log cfu/g, 6.8 log cfu/g, after 12 days it was impossible to calculate the Quality Index for it during shelf life study using TVC count. Then for predicting the shelf life for pollock fillets at -1°C was used the Torry rejection, being 10 days.

When using a Torry value of 5.5, the Quality Index were 12.1 and 12.3 for 2°C and -1°C, respectively. Although TVNB was only possible used for the 2°C, reaching the quality index average 14.4.

Pollock fillets seem to have different shelf life than fillets from most temperate species: cod fillets at 8 days in ice (Bonilla et al., 2007), salmon fillets at 6.5 days at 4°C (Rasmussen et al., 2002) and cultured Thai-Pangas fillet 18 days in ice (Islamil, et al., 2015).

## 6 CONCLUSION

The QIM scheme for pollock fillets developed in this study consisted of 11 parameters which gave a total of 24 demerit points. At the beginning of the storage time, the differences between fillets in colour and gaping affected the QI, resulting in an inaccurate evaluation of freshness. As the colour and gaping observed at the beginning of storage were not related to storage time, changes need to be made in the QIM scheme for pollock before further use, including a repeated shelf life study to evaluate the quality of the QIM scheme. Moreover, it is necessary to include more training of the panellist in using QIM scheme and Torry scale for pollock fillets, in the specific problems like high biological variation in colour, and flavour and odours that get affected at low temperature. Due to the high biological variation between fish, it is necessary to increase the number of samples. The shelf life at 2°C, according to Torry, TVC count and TVBN, was approximately 6 days, while for -1°C, according to Torry was 10 days. Low correlation was observed between QI at -1°C with TVC and TVBN. This was mainly due to rancidity being the main cause of sensory rejection as low temperature preventing and microbial growth. The shelf life study showed the importance of keeping good control of the temperature since small abuse can shorten the shelf life by 4 days. In addition to these tests, another test for quality control in pollock fillet when importing to Cuba, will be histamine analysis.

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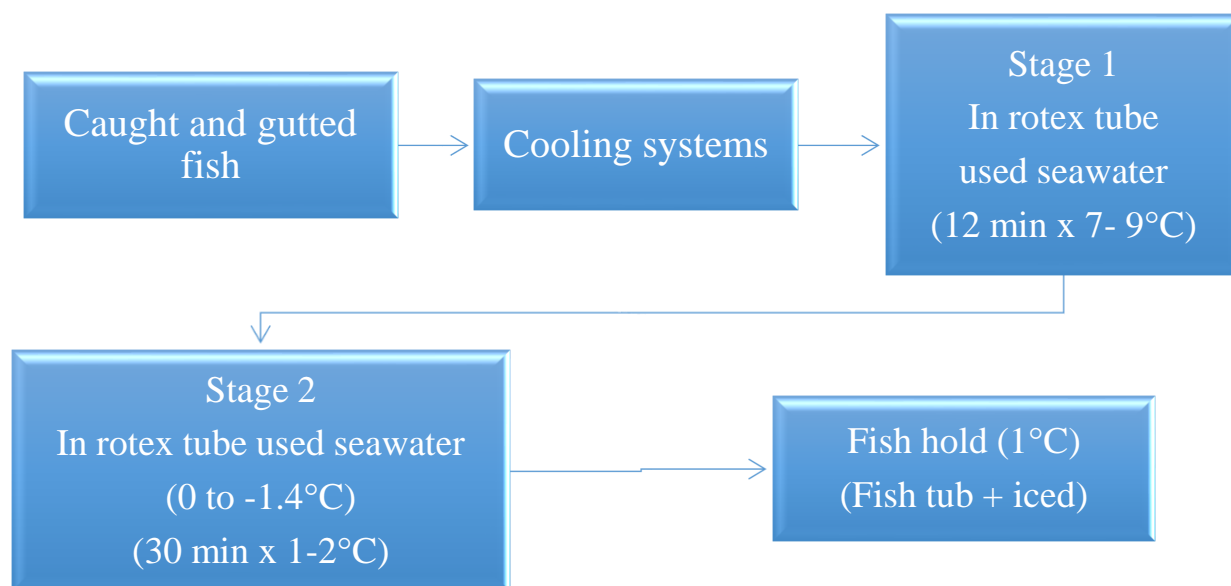
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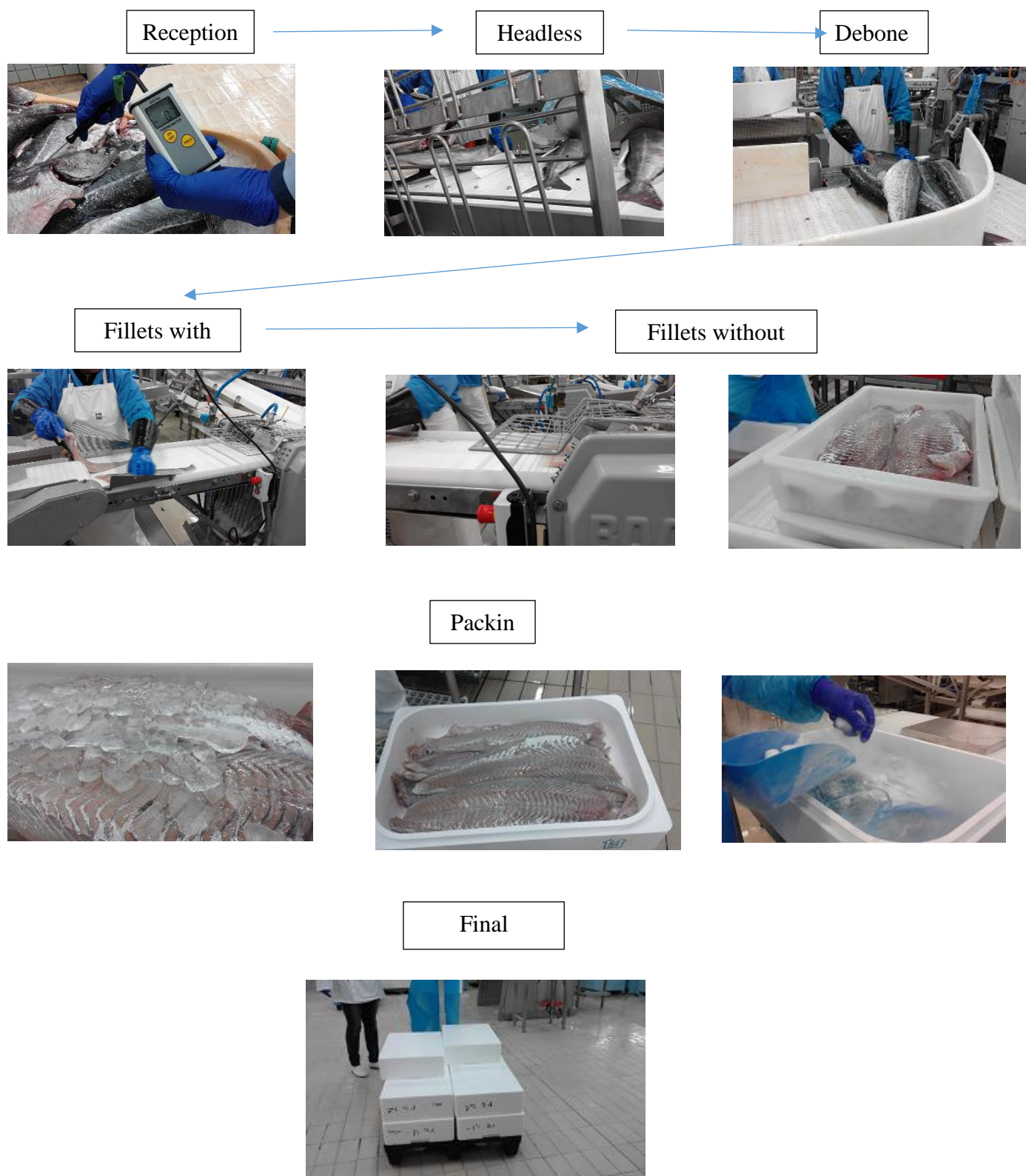
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**APPENDICES****Appendix 1. The cooling systems used on board in caught pollock****Figure 16: The cooling systems used on board in caught pollock**

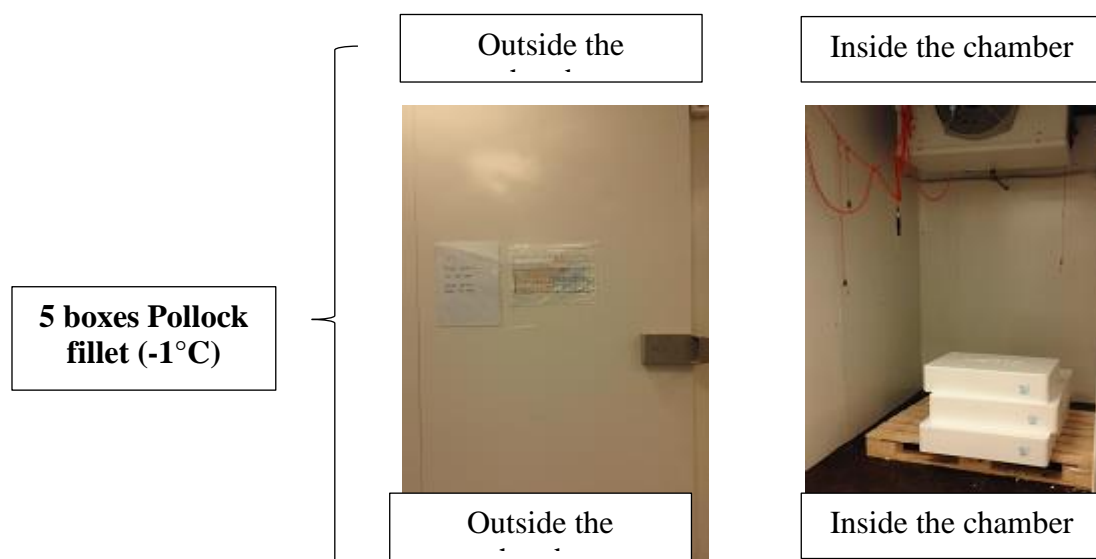
**Appendix 2. Process of obtaining of pollock fillets without skin**



**Figure 17: Process of obtaining of pollock fillets without skin**



**Appendix 3. The pollock fillets stored at -1°C and 2°C (Shelf life study)**



**Figure 18: The pollock fillets stored at -1°C (Shelf life study).**

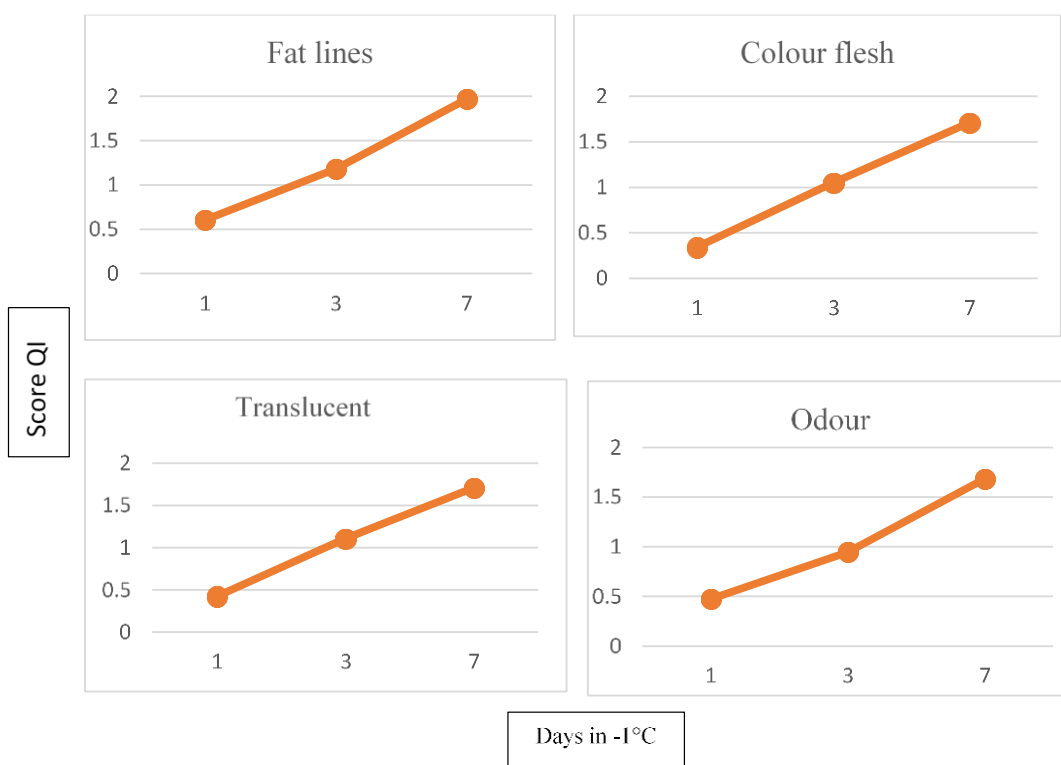


**Figure 19: The pollock fillets stored at 2°C (Shelf life study)**

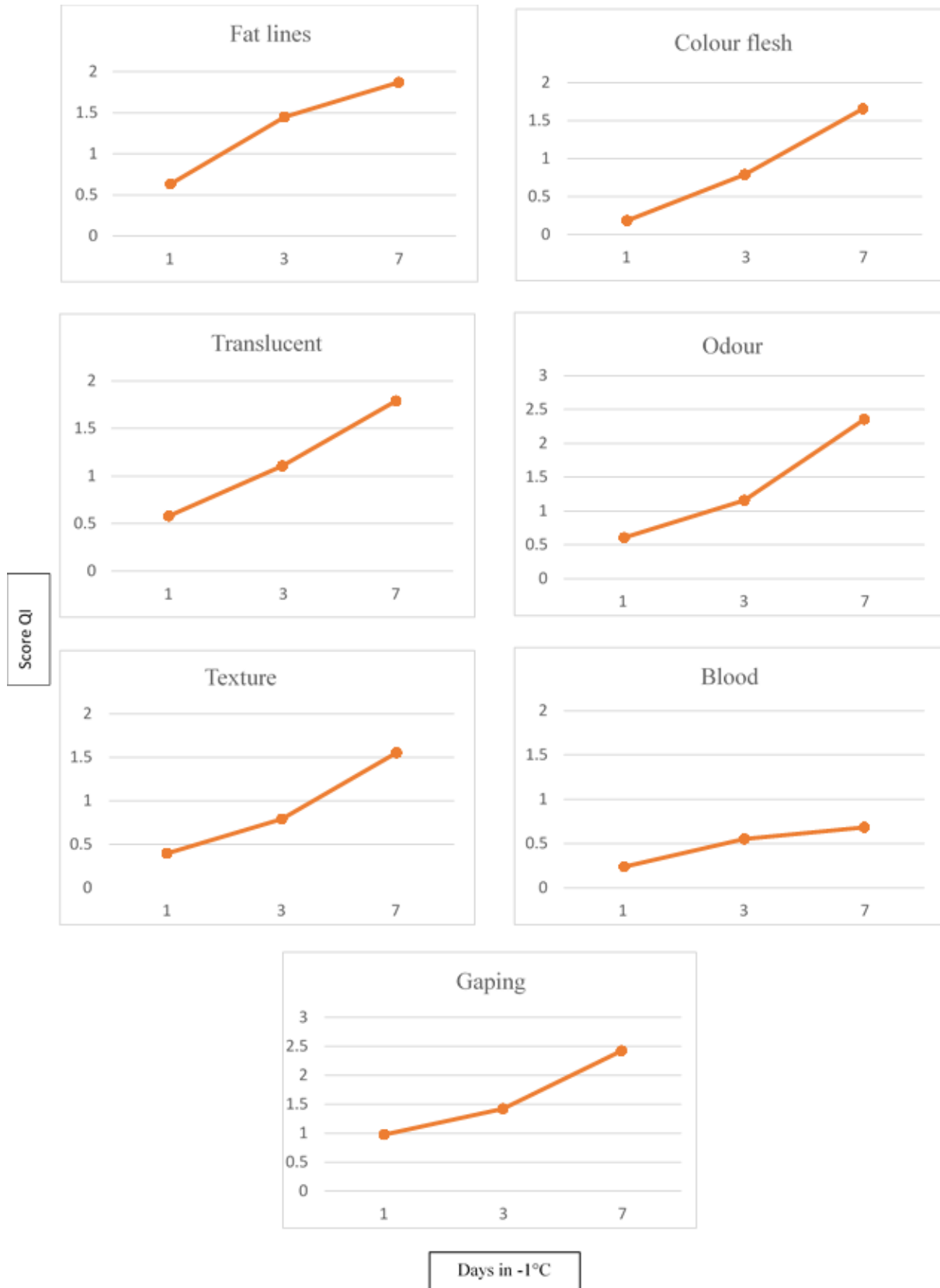
**Appendix 4. Quality Index Methods (QIM) scheme during pre- observation for pollock fillets (*Pollachius viren*)**

**Table 7. Quality Index Methods (QIM) scheme during pre- observation for pollock fillets (*Pollachius viren*)**

Quality parameter		Description	Score	
Skin side	Fat lines	Bright red	0	
		Red- brown, some yellowish	1	
		Yellowish	2	
	Flesh	Bright brown	0	
		Dark- beige, a little pinkish	1	
		Beige, greyish, light pink	2	
	Odour	Fresh, marine	0	
		Fresh capelin	1	
		Rancid, spoilage, sour milk	2	
Flesh side	Fat lines	Dark, brownish	0	
		Some yellowish	1	
		Pale, Yellowish	2	
	Colour	Bright brown, greyish	0	
		Dark beige, pinkish, dark- greyish	1	
	Transparent	Transparent, bluish	0	
		Opaque	1	
		Milky	2	
	Odour	Fresh, marine, neutral	0	
		Cucumber	1	
		Queasy sweet, trace of rancid, spoilage, sour milk	2	
		Rancid, spoilage, sour milk	3	
	Texture	Firm	0	
		Rather soft	1	
		Very soft	2	
	Blood	Bright red, not present	0	
		Dull red	1	
		Shadowy, brown	2	
	Gaping	No gaping, one longitudinal gaping at the neck part of the fillet	0	
		Slight gaping, less than 25% of the fillet	1	
		Gaping, between 25 - 75% of the fillet	2	
		Extensive gaping, over 75% of the fillet	3	
	Quality index (0–21)			

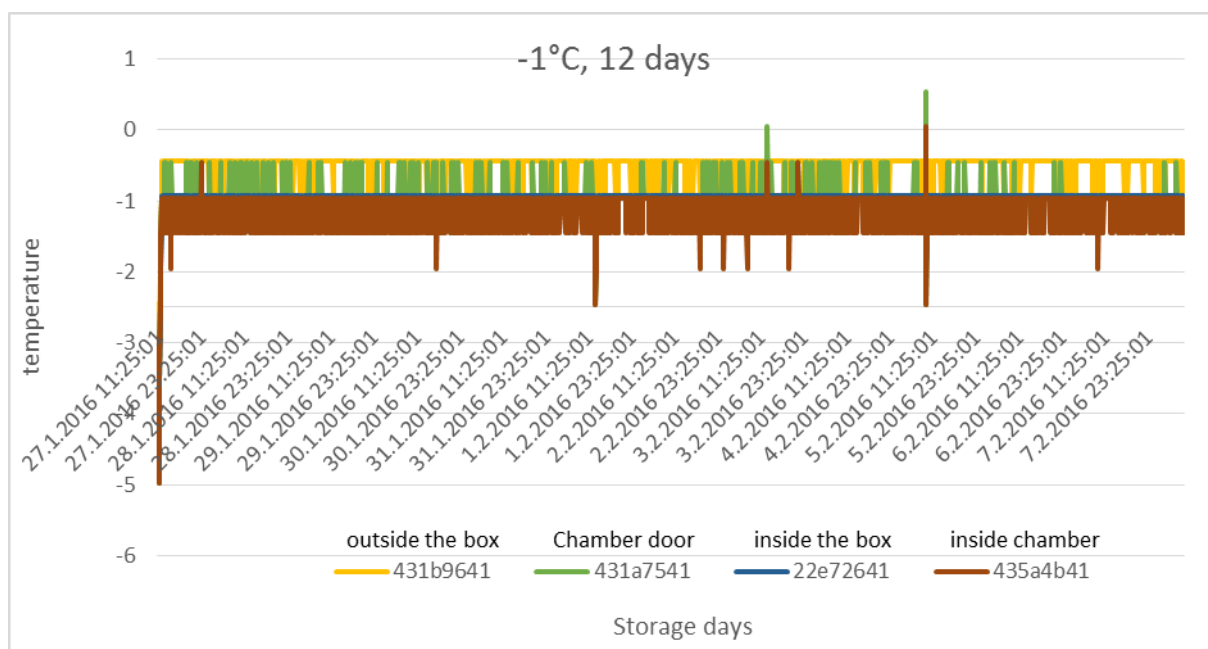
**Appendix 5. QI scores for quality parameters in training panellist session.**

**Figure 20: Average scores for each quality attribute evaluated with QIM scheme for pollock fillets (skin side).**

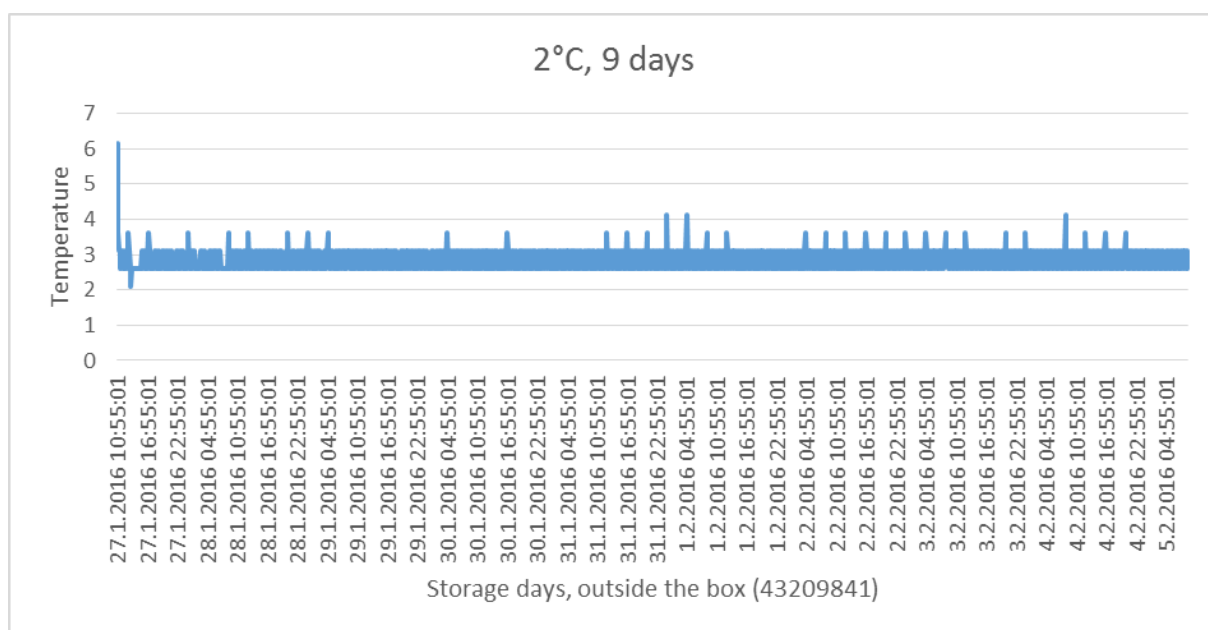


**Figure 21: Average scores for each quality attribute evaluated with QIM scheme for pollock fillets (flesh side).**

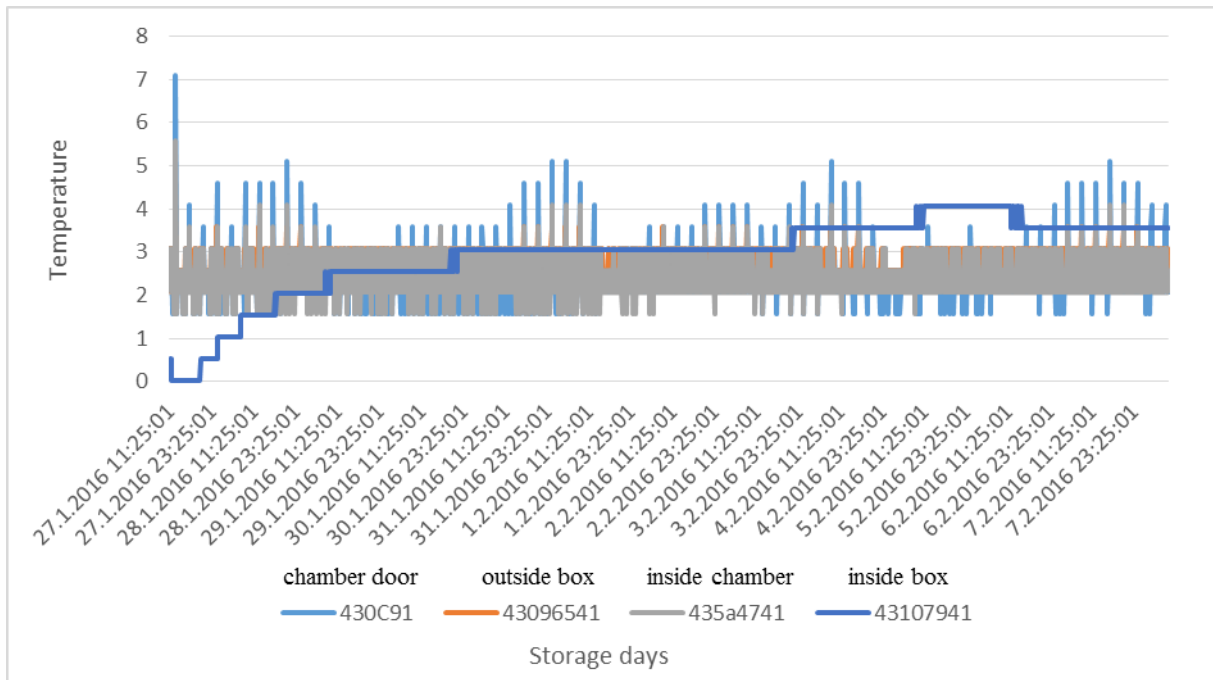
**Appendix 6. Temperature loggers during storage days for -1 and 2°C**



**Figure 22: Temperature loggers at -1°C, inside and outside the box, door and inside the chamber, at 12 days in stored condition.**



**Figure 23: Temperature loggers 43209841 at 2°C, outside the box, at 9 days in stored condition.**



**Figure 24: Temperature loggers at 2°C, inside and outside the box, door and inside the chamber, at 9 days in stored condition.**