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STORAGE QUALITY OF FRESH REDFISH (*Sebastesmarinus*) FILLETSAS AFFECTED BY DIFFERENT COOLING METHODS

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ABSTRACT

The effect of pre-cooling prior to packaging by using combinations of slurry ice immersion and combine blast and contact (CBC) cooling technologies on physicochemical properties of fresh redfish (*Sebastesmarinus*) fillets during processing and storage at -2^{o}C to 2^{o}C was investigated. The cooling treatments did not influence the water content and lipid content of the redfish fillets. Meanwhile, the increase in drip and the cooking yield during storage could be caused by decomposition of the fish muscle. CBC cooling increased the water holding capacity (WHC) of the fillets. Slurry ice immersion of the redfish fillets led to increased lipid oxidation, while CBC cooling slowed down lipid oxidation. According to sensory evaluation skin-on fillets which were processed by CBC cooling technology spoiled faster compared to the other cooling technologies where the skin had been removed.

Keywords: Redfish (*Sebastesmarinus*), super chilling, slurry ice, combine blast and contact (CBC) cooling

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1 INTRODUCTION

Fish is a very perishable food. The spoilage of fresh fish is dominated by microbial activity and chemical changes, such as auto-oxidation, enzymatic hydrolysis of the lipid fraction and tissue enzyme activity (Clucas 1981, Huss *et al.*, 1997). Spoilage of fish begins as soon as the fish dies or is caught. In the high ambient temperature of the tropics, fish will spoil within 12-20 hours depending on species, size, feeding, season, capturing method, killing method, bleeding and gutting, as well as handling and cooling on board (Clucas 1981, Hattula *et al.*, 2002). The important activities to delay spoilage of fish are chilling the fish as soon as possible after catching, to keep it chilled during storage, and to maintain a high standard of clean processing both on deck and during processing.

Fresh fish is the dominant fish product in Indonesia. In 2007, the volume of fresh fish products increased by 4.6% compared to 2006.About 60% of the volume of fresh fish products is exported to countries such as the United States, Japan and the countries within European Union (Marine and Fisheries Statistics 2007).

In Indonesia, on board handling and processing of fresh fish is based on keeping the fish on ice. Block ice is crushed into flakes and then used to preserve fresh fish. Chilling and cooling are important in order to deliver high quality fresh fish products to the consumers, but freshness and good quality products are in great demand. The ability to forecast the shelf life of these products is therefore of major interest.

Recently, new chilling and cooling methods have been developed to extend shelf life. Various types of cooling systems have been used to reach super chilling (–4°C to 0°C) of fish products, including using immersion into liquid ice or slurry ice (Losada *et al.* 2004, Zeng*etal.* 2005) or to reach sub-zero temperatures during storage (–2 °C) (Sivertsvik *et al.*, 2003). In the industry, chilling of fillets is traditionally done by immersing the fillets in ice/water or brine solutions (Lee and Toledo 1984). It has been noted that the brining step may cause bacterial contamination of the fillets, both on skin-on and skin-less fillets. Generally, bacteria can also be found in the water or environment, as well as in the skin itself (Adams and Maurice 2008). Subsequent chilling in a freezer after packaging has proven useful to increase the refrigerating capacity of the product (Magnusson *et al.*, 1998). However, if the fillets are left too long, slow freezing may cause undesirable ice crystal formation, inducing tissue damage.

In Iceland, super chilling by combined blast and contact (CBC) cooling has recently been developed and is commonly used in fish processing factories. CBC is based on lowering the temperature of the fillets quickly to –1 °C by using both the advantages of contact cooling between the fish and the metal surface of the conveyor and cooling by blasting chilled air over the fillets. Olafsdottir *et al.,* (2006) studied the effect of CBC super chilling on shelf life of fillets and cod loins. The shelf life of the fillets stored at temperature of -1.5°C was at least 15 days, while fillets stored at 0.5° Chad a shelf life of 12.5 to 14 days. The rate of freezing and the size of the ice crystals formed are important factors influencing the quality of fillets.

The aim of this study was to investigate the effect of pre-cooling prior to packaging by using combinations of slurry ice immersion and CBC cooling technologies on physicochemical properties of fresh redfish fillets during processing and storage. The spoilage characteristics of fresh redfish fillets under these various cooling conditions were compared with traditional

processing cooling as a control. Spoilage changes were assessed by sensory, yield and physicochemical analysis.

The optimization of cooling methods to prolong the shelf life of redfish fillets during processing and storage is the ultimate aim of the study. In addition to this, the results of the study will be used to train students at Jakarta Fisheries University in the effects of super chilling, which may lead to implementation of the technology in Indonesia.

2 BACKGROUND

2.1 Redfish

The redfish (*Sebastesmarinus*)is a deep-waterfish species. They are commonly collected by bottom trawl nets and found at a depth range from 100 to 1000 m, most common at350-700 m depth. The species can be found in the Eastern Atlantic, southwest of Iceland, Kattegat and North Sea, northward to Spitsbergen, in the southern part of the Barents Sea eastward to the Kanin Banks and Novaya Zemlya, but is rare in the White Sea. It can also be found in the Western Atlantic, from Greenland and south-eastern Labrador in Canada to New Jersey in the USA (Pikanowski *et al*., 1999).

Generally, the redfish adult can grow and exist in deep-water at temperatures below 13^{o}C and salinities about 31-34%. The female redfish spawns from April to August. An adult redfish can live to be up to 100 cm long, 15 kg of weight and up to 60 years old (New England Fishery Management Council 1998).

In Iceland, redfish is commonly processed as fillets. Main mean nutritional value per 100 g of fillets are 17.1 g of protein (min 15.7 g and max 18.8 g), 3.9 g of fat (min 1.4 g and max 6.6 g), respectively (Matis 2011).

2.2 Fish spoilage

When fish die, certain irreversible changes begin to take place. Within a few hours the muscle gradually changes due to chemical processes, physicochemical, and biological activity. The processes that occur include the activity of the actomyosin enzyme during the rigor mortis process (Church 1998), autolyticdegradation, oxidation off at (Clucas1981, Huss *et al.*, 1997) and microbial growth (Huss *et al.*,1997). All processes runs imultaneously during storage. The spoilage of fish can be detected and assessed with sensory analysis (appearance, texture, flavour, odour and taste), as well as by microbial and physicochemical analysis.

Fish and shell fish are highly perishable, because of their high water activity (Aw), neutral pH and presence of autolytic enzymes (Clucas 1981). The rate of deterioration is highly temperature dependent and can be inhibited by decreasing storage temperature (e.g. fish stored in ice).

Different harvesting procedures e.g. gillnet, poundnet and trawling are also found to have similar effects on the sensory quality of fish (Hattula *et al.*, 2002). The stress and mechanical damage caused during capture, the structure and composition of the fish, pH and storage temperature prior to landing all influence the spoilage rate of the fish (Church 1998, Adams

and Maurice 2008). Hence it is difficult to control the initial quality of the raw material with any degree of repeatability. Fish quality is, therefore, a very complex concept (Bremner 2000, Nilsen *et al.,* 2002), which includes nutritional, microbiological, biochemical and physio chemical attributes related to this term.

2.3 Autolytic spoilage

At death, the supply of food ceases and the energy resources soon become depleted. However, the enzymes do not die. They continue to operate, but since energy is required to build the larger units of the body, the function of the enzymes post mortem is to break compounds into smaller units.

The flavour of fish flesh changes during the spoilage process. The characteristic sweet, meaty flavour of fish flesh is due at least in part to a compound called inosinic acid (Clucas 1981). The breakdown of inosinic acid through autolysis results in the loss of this sweet meaty flavour. Another compound, hypoxanthine, which is produced from the breakdown of inosinic acid, contributes to the bitter flavour of spoiled fish. Autolysis also contributes indirectly to fish flavours by providing a supply of compounds which bacteria in turn convert to compounds having unpleasant flavour and odours.

The colour of the fillets may also change during the spoilage process. Some of the discolorations commonly found in fish are likely caused by autolytic actions, in which sugars produced by enzymatic action interact with amino compounds already present in the flesh to produce brownish or yellowish coloured compounds (Adam and Maurice 2008).

2.4 Bacterial spoilage

The flesh of healthy live fish is sterile. When the fish dies, bacteria present on the skin and in the guts multiply rapidly and invade the flesh, which provides an ideal medium for growth and multiplication (Liston 1980). The bacteria can break down the muscle itself and will also feed on smaller units produced by autolytic action. The increase in number of bacteria results in a heavy slime on the skin and gills and an unpleasant ammonia and sour odour. Eventually they cause the flesh to soften.

The bacterial load present in the fish when caught will continue to multiply until the fish is consumed. However, during handling, the fish is likely to pick up more bacteria, from being washed in polluted water, careless gutting and dirty boxes. However, if one is careful when handling the fish, the numbers can be controlled.

The growth and activities of bacteria in fish muscle is effected by various environmental changes and the condition of the fish itself, such as the temperature, pH, salinity, water activity (A_w), oxygen and toxic substances (Hobbs 1985). The bacteria grow on a wide range of temperatures. The psychrophilic or psychotropic bacteria grow very slowly in chilling and frozen temperatures, but grow faster when the temperature is between 15^oC to 20^oC (Hobbs 1985). The most important bacteria present on fish muscle are *Shewanellaputrefaciens, Photobacteriumphosphoreum, Pseudomonas* spp. and *Vibrionaceae* (Huss 1995). Bacteria can also grow better in fish muscle between pH 6 and pH 8 and on the other hand show less growth at extremes of pH (Hobbs 1985).

2.5 Oxidation of fat

Fish has a high number of unsaturated lipids (Ackman 1989). During refrigerated storage, lipids can easily react with other compounds, such as proteins (Mackie 1993, Verma, Srikar, Sudhakara and Farma 1995, Adams and Maurice 2008). Lipid damage can be detected by the primary- and secondary lipid oxidation products, interaction between compounds and by lipid hydrolysis (Aubourg 1999b). The primary products are measured as peroxide values (PV). Thesecondary products (e.g. aldehydes) are measured with as thiobarbituric acid reactive substances (TBARS) and the tertiary products are polymers which can be measured with fluorescence, colorimetric and sensory analysis (Jónsdóttir *et al.*, 2008) (Figure 1).

In the case of fatty fish, lipid oxidation has a strong influence on the spoilage process of the muscle. The lipid oxidation happens simultaneously as autolysis and bacterial spoilage. High temperature or exposure to light can increase the oxidation rate. For fatty fish preserved in ice, spoilage due to rancidity is mainly caused by oxidation. This produces a bad and unpleasant odour as well as rancid taste. Fatty fish species like redfish, herring, mackerel and salmon are most affected by rancidity. The lipid content of lean fish is about 0.1-0.9%, while the lipid content of fatty fish species is higher than 0.9% (Liston 1992).

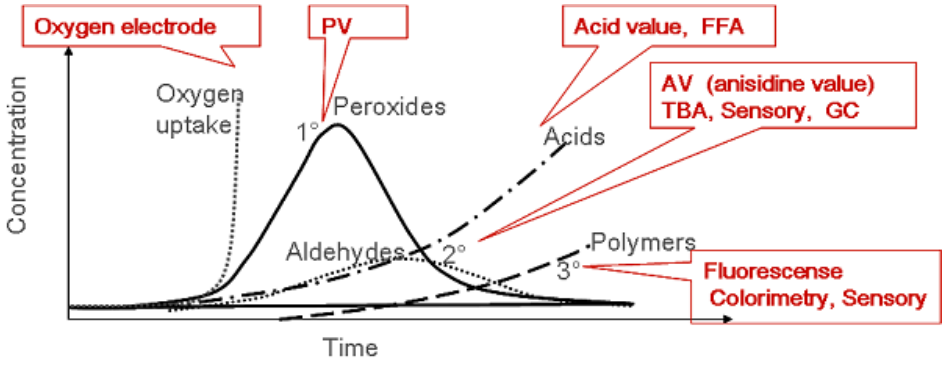

Figure 1. An example of the progress of lipid oxidation and breakdown of lipid-oxidation products as assessed by different methods (Jónsdóttir *et al*., 2008).

2.6 Water content of fish

The water content of most common fish species is in the range of 70 to 80% of the muscle weight, although some deep water species may have a water content of about of 90% (Love 1970). With increasing crude fat content, water content decreases and dry matter content increases (Schreckenbach *et al.*, 2008).

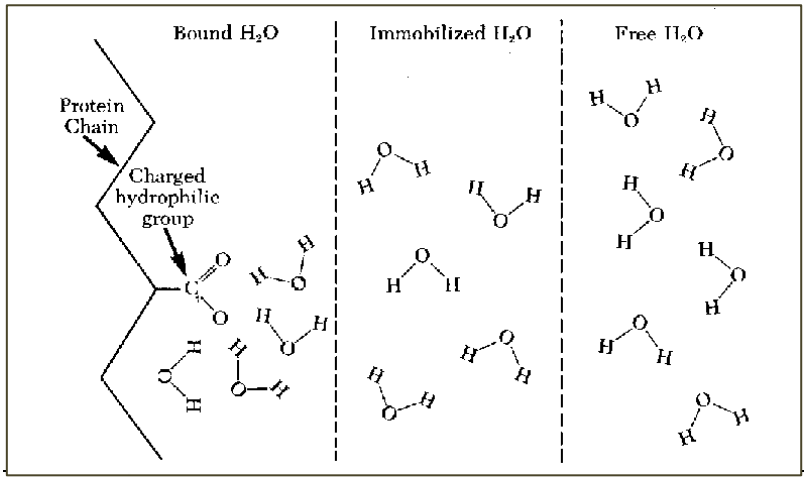

Figure 2. The water distribution in the muscle.
(http://labs.ansci.illinois.edu/meatscience/Library/free%20water%20fig.gif.)
Retrieved on February 21st, 2011

The water in the muscle can be divided into three different categories. The first category is *bound water* (Fennema *et al.*,1973). Approximately 5% of the water found in muscle is strongly bound to the charged hydrophilic amino acid groups on the protein surface or is trapped within the protein structure (Sikorski 2001).This water portion remains unfrozen in the muscle at temperatures below -20°C (Fennema *et al.*,1973).It is not disrupted during operations such as freezing, thawing, heating and drying and it is tightly bound to protein chains.

The second group is *immobilized water*, also called *entrapped* or *restricted water*, which is mechanically immobilized within the protein filaments and cellular membranes of the fish muscle. This water can be irreversibly disrupted with many forces from common processing environments as compared to *bound water* (Ofstad and Hermansson 1997).

The last category is *free water*, also called *bulk water* (Ofstad and Hermansson 1997). This water can be found between the muscle cells in small amounts (approximately 5%), and is easily lost with environmental changes during processing.

2.7 Water holding capacity (WHC)

A considerable amount of water is exuded from fish muscle tissue after death due to muscle contraction during the rigor process. Water holding capacity (WHC) may be defined as the ability of a muscle to hold water under external influences such as compression or centrifugation. The WHC can be decreased as an effect of the pH drop during glycolysis towards the isoelectric point (pI) of the muscle proteins. Normally the WHC of proteins is lowest at their pI (Sen 2005).

Others factors which may affect the WHC are the salt content of fish muscle, the environmental temperature during processing due to ice formation, the freezing method and bad handling during processing (Sen 2005, Gao 2007). Gao (2007) observed a WHC of about 80% and a pH value between 6.4 and 6.8 in cod fish (*Gadusmorhua*) fillets, which were processed by a super chilling method and stored at -1.5°C. The WHC of meat can be lowered as an effect of an increased temperature of muscle due to protein denaturation (Zhang *et al.*,1995). The size of the ice crystals that form during a freezing process is

influenced by the freezing rate, the initial temperature of the product, initial freezing point and the surface area of the product. Slow freezing results in a low number of large crystals (extra-cellular), generally decreasing the product quality through extensive mechanical damage, and accelerated enzyme activity. More rapid freezing will produce a large number of small ice crystals, but may still result in intracellular ice crystallization and/or mechanical cracking in the tissues (Bello *et al.,* 1982, Martino *et al.,* 1998 and Bahuaud *et al.,* 2008). Bad handling during processing can also affect the WHC of the product (see chapter 2.4).

2.8 Pre-cooling and cooling techniques

Different preservative methods, such as cooling with traditional flake ice (Nunes *et al*., 1992), refrigerated sea water (Kraus 1992) or by using chemical additives (Hwang and Regenstein 1995) have been employed with the aim of reducing loss in freshness of fresh fish.

In recent years, an additional storage technique, i.e. super chilling, has attracted considerable interest. Super chilling, as used for preserving seafood, has been defined as a process by which the temperature of a food product is lowered to 1-2^{o}C below the initial freezing point of the product for several minutes. Storing food at super chilled temperatures can be advantageous in terms of maintaining food freshness and suppressing harmful microorganisms (Bohnert and Jensen 1996).

Several studies have been published on the subject of super chilling and super chilled storage; most of these are concerned with the storage of fish and other seafood (Ando *et al.,* 2004, Bao, 2004, Wang *et al.,* 2006, Olafsdottir *et al.,* 2006, Duun and Rustad 2007, Gallart-Jornet *et al.,* 2007, Bahuaud *et al.,* 2008, Duun *et al.,* 2008, Magnussen *et al.,* 2008, Beaufort *et al.,* 2009, Fernandez *et al.,* 2009, Fernandez *et al.,* 2010, Hansen *et al.,* 2009). During super chilling, which was originally used on board trawlers in the 1960s (Pearson 1980), some ice crystals are formed inside the product (Haard 1992, Sikorski and Kolakowska 1994). The amount of frozen water is highly temperature dependent (Huss 1995). The initial freezing point for a fishery product varies from about –2.5°C to –1°C (Rahmanand Driscoll 1994) and is usually dependent on the water content of the product (Chang and Tao 1981). Super chilling can either be used prior to traditional chilled distribution to improve the cooling capacity of the product (Magnusson *et al*., 1998) or that the super chilling temperature is maintained throughout the storage and distribution. Storage at super chilled conditions may enhance phospholipid hydrolysis and protein denaturation (Ashie *et al.,* 1996). Super chilling also inhibits most autolytic and microbial reactions, and thereby increases shelf life (Huss 1995, Chang *et al.,* 1998). During super chilling temperatures low enough to substantially reduce bacterial activity are obtained but still kept high enough to avoid significant levels of ice crystal growth, which may cause structural damage, but the ice fraction is one of the most important parameters during super chilling (Magnussen *et al.,* 2008).

2.8.1 Slurry ice

Slurry ice, also known as fluid ice, slush ice or liquid ice, can be used to preserve fish products. The temperature of fish products can be decreased below zero temperature or below the initial freeze point of fish products by immersion into slurry ice (Chapman 1990, Harada 1991). Slurry ice can prevent physical damage by decreasing the temperature below the initial freeze point of the products and has a better heat exchange rate than flake ice. The physical characteristic of slurry ice is consisting of microscopic ice crystals. It can flow and

covers the surface of the product completely leading to rapid energy transfer from the medium to the product. Losada *et al.,* (2004a) studied inhibition of chemical changes related to freshness loss during storage of horse mackerel (*Trachurustrachurus*) in slurry ice. This study showed that the slurry ice immersion can increase shelf life and give better quality of the product than immersion into flake ice. The use of flake and slurry ice on warm water fish species, such as sea bass, *Deicentrarchuslabrax*, were shown to have no significant effect on the spoilage rate (Martinsdottir *et al.,* 2002). However, cooling of sea bream (*Sparusaurata*) with ice slurry was shown to be a good cold storage method (Huidobro *et al.*,2001). Similar results were obtained for Australian prawn sand shrimps (Chinivasagam *et al.,* 1998, Huidobro *et al.,* 2002). Gao (2007) and Magnusson *et al.,* (2008) showed that pre-cooled product scan with stand thermal load, both during transport and storage better than products stored at stable temperature conditions.

2.8.2 CBC Technology

The application of the super chilling technique has been developed particularly for the processing of fish fillets. In the Combined Blast and Contact (CBC) method, cooling by air blast and contact cooling are combined. The fish fillets are passed through a tunnel freezer at a temperature of around -8°C for 8-10 minutes. The skin side of fillets is put on a Teflon-coated aluminium conveyor belt and simultaneously cold air is blasted over the fillets. This causes the temperature of the fillets to drop rapidly to -0.5°C to -1°C (Arnþórsdóttir *et al.,* 2008). According to Rha (1975), approximately 10 to 15% of the water in the muscle is frozen at this temperature.

The freezing of the fillets in the CBC tunnel can be avoided by increasing the salt content of the fillets by immersion in liquid or slurry ice with a salt concentration from 1.0 to 2.5%. The de-skinning process then also becomes easier and more effective cooling before packaging is achieved (Martinsdóttir *et al.,* 2005). Olafsdottir *et al.,* (2006) showed that the CBC process can preserve the quality of fillets and extend the freshness and shelf life. The concentration of salt used in this study was about 2.5% and the cooling time affected the absorption of salt in fish muscle as well as its initial freezing point. However, brining of skin-on fillets may cause cross-contamination of bacteria to other products, which can contribute to rapid growth of fish spoilage bacteria at favourable conditions, which may develop in poorly controlled chill chains. To reduce the number of spoilage bacteria in the salt water, fillets may be immersed in fresh ice-water or running water before entering the brine. However, renewal of the water (running water) in the immersion tank is crucial so that bacteria from fillets are removed so fillets arriving in the tank later are not contaminated. It is therefore recommended to use a well temperature-controlled cooling medium with a restricted salt concentration and frequent renewal of the medium to avoid the proliferation of fish spoilage bacteria.

In this study combinations of slurry ice and CBC cooling were used, in comparison to traditional non-cooling prior to packaging to assess the effect of cooling on physicochemical properties and shelf life of redfish fillets.

3 MATERIAL AND METHODS

3.1 Redfish fillets

In this study, the processing of redfish involved a number of steps: pre-treatment, fish filleting, various cooling, packaging and storage. The fish fillets were made from fresh redfish (*Sebastesmarinus*), which was caught in December 2010 in Icelandic territorial waters by trawling. The fish was stored on flake ice for 3 days in insulated tubs on a fishing vessel of HB Grandi fishing company before being delivered to the processing plant. Pre-treatment of the redfish included removal of ice, washing, be-heading and filleting. Filleting was done by mechanical filleting machines. The fish fillets were prepared and processed at HB Grandi hf. and Eskja hf, fish processing companies, in Reykjavik and Hafnarfjörður, Iceland, respectively. Prior to transfer to the laboratory at Matis in Reykjavik, Iceland, the fish fillets were packed in EPS (*expanded polystyrene*) boxes (with 3 kg box and inside dimensions 35.6 x 21.6 x. 6.5 cm).

3.2 Experiment design

Fillets were randomly divided into 5 groups which received different cooling treatments before being packed into EPS boxes. Approximately 3 kg of fillets were packed in each box, including about20 pieces of fillets with one ice pack on top. The weight of each fillet was about 120 g. Each EPS box was marked with a numbered sticker and weighted before it was put into cold storage. The groups are presented in Table 1.

Table 1. Description of experimental groups with regards to the different cooling methods used during processing prior to packaging, their packaging solutions, the cooling medium and the raw material used.

Group	Raw material	Cooling Prior to Packing	Packaging	Cooling medium in box
No further cooling (NC)/Control	Skin less fillets	No cooling	Closed 3 kg EPS	Ice pack
Slurry Ice (SIC)	Skin less fillets	Slurry Ice	Closed 3 kg EPS	Ice pack
Slurry and CBC (SIC-CBC)	Skin on fillets	Slurry Ice + CBC	Closed 3 kg EPS	Ice pack
CBC cooling (CBC)	Skin on fillets	CBC	Closed 3 kg EPS	Ice pack
Sea freight-EPS (SF-SIC)	Skin less fillets	Slurry Ice	EPS with holes	Flake ice

In this study, the NC group, where no cooling was applied prior to packaging, was kept as a control group. Groups NC, SIC and SF-SIC, were processed with *skin-less* fillets and were transferred directly to the cold storage at Matis after processing at HB Grandi. The CBC treated groups (SIC-CBC and CBC) were pre-processed with *skin-on* fillets at HB Grandi hf. while the cooling process (slurry ice immersion and CBC cooling) was performed at Eskja hf. These groups were then transferred to the cold storage at Matis. The cold storage temperature was kept at -2^{o}C for the first 6 days and at around 2^{o}C for the rest of the experiment. The main reason for this is that this describes the conditions often occurring during land transport in Iceland and sea transport to the most common destination countries in Europe. This can be related to the result of Mai *et al.*,(2010) and Margeirsson *et.al.*, (2010), but a common time of transport from Icelandic processors to retailer or consumers in Europe is about 6-7 days for sea freight and around 1 to 2 days in the case of air transport. In this study, pre-cooling by slurry ice immersion was used in the SIC, SIC-CBC and SF-SIC groups. The groups were immersed in the slurry ice for 15 minutes. The slurry ice was produced by mixing flake ice with salt and fresh water.

3.3 Sampling

Samples were taken from the cold storage on days 1, 6, 10 and 13 of the study. On each sampling day, about 20 fillets from each sampling group were collected. The fillets from the top layer were used. Approximately 5 cm were cut of the tail, which was then discarded. The loin and middle parts of the fillets were used to measure the water content, water holding capacity (WHC), cooking yield, as well as lipid contents, free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS) and the fluorescence value of the fillets. Before each measurement, the skin of the redfish fillets on the super chilling groups (SIC-CBC, CBC) was removed. The samples were kept chilled in EPS boxes and directly transferred from storage to the laboratory where analysis was done.

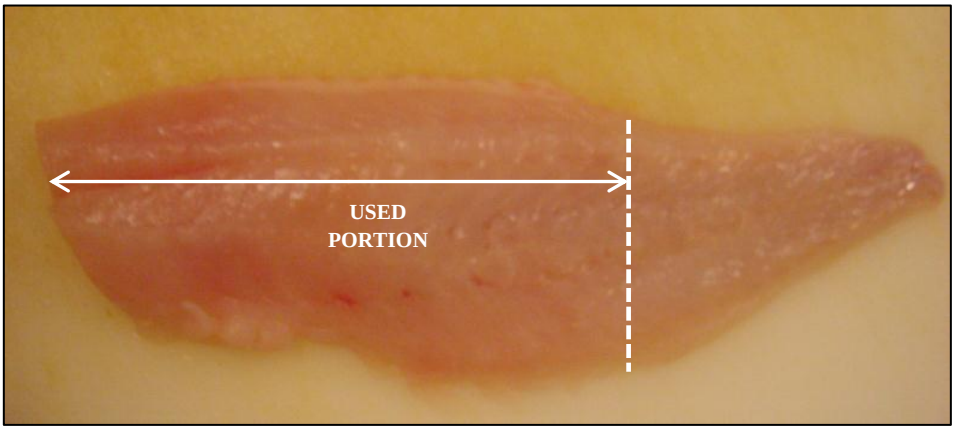

Figure 3. The figure shows where the tail of the fillet was cut-of and discarded. The loin and middle parts of the fillet were used to measure water content, WHC, drip, cooking yield, TBARS and PV.

3.4 Sensory analyses

Quantitative Descriptive Analysis (QDA), as introduced by Stone (1992), was used to evaluate cooked samples of redfish fillets. Fillets were cooked in a steam oven (6 minutes at 98^{o}C) and an unstructured scale (0-100%) was used on a list of words describing odour and flavour, such as characteristic redfish, metallic and oily. Eight to eleven panellists of the Matis Laboratories' sensory panel participated in the QDA of the cooked redfish fillets. They were all trained according to international standards (ISO, 1993), including detection and recognition of tastes and odours, in use of scales, and in development and use of the descriptor. The members of the panel were familiar with the QDA method and experienced in sensory analysis of redfish fillets. Each panellist evaluated the samples in two sessions each day of the sensory evaluation.

The Torry scheme was also used to assess cooked samples of redfish fillets by the same panellists. The scheme is a detailed description of the characteristic flavour changes (odour and taste) and ranges from 10 = very fresh to 3 = very spoiled, with a rejection level at 5.5.

3.5 Colour

The colour changes of the muscle samples were measured by using a Minolta type CR-300 calorimeter unit (Konika Minolta, Tokyo, Japan) with a D65 light source. The instrument records the intensity of the L- (lightness, scale from black to white), a- (scale from green to

red) and b- (scale from blue to yellow) values of the fillets. The colour changes of redfish fillets were measured on three different positions on each fillet (Figure 4).

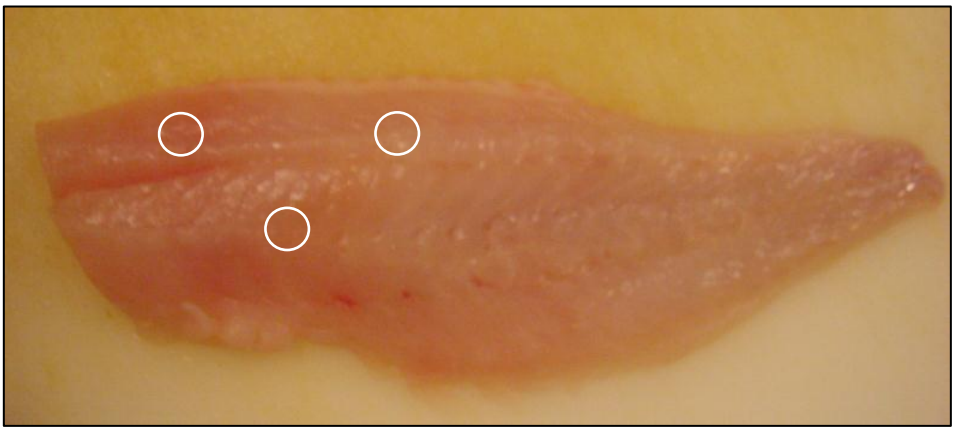

Figure 4. The circles show where colour measurements were performed on the fillets.

3.6 Temperature measurements

The temperature of each EPS box was recorded in 6 min intervals, by temperature data loggers (iButton® type DS1922L, Maxim Integrated Products, Sunnyvale, CA, USA), which were placed at the bottom corner, in the middle, and in the top layer of the redfish fillets inside each box as well as on the outside of the EPS boxes. These loggers were used to measure the temperature during the experiment. The mean time-temperature series for each box was found. Furthermore, the ambient air temperature inside the air climate chamber was measured at three positions with the same type of temperature data loggers.

3.7 Water content

The water content of the fillets was measured with two replicates per group. The fillet was minced in a grinder for 10-15 seconds at low speed. Approximately 5 g of the minced sample was weighed and spread in a thin layer on a porcelain dish and then left to dry for 4 hours in an oven at 103±2 °C. The dishes were then removed from the oven and cooled down in a desiccator at ambient temperature for 30 minutes before weighed again. The water content was calculated according to the ISO 6496:1999 standard, as follows:

$$W = \frac{m_1 - m_2}{m_1} \times 100(\%)$$

Where:

W is water content of sample (%).
m_1 is the mass of the sample (g).
m_2 is the mass of the sample after dried (g).

3.8 Water holding capacity (WHC)

The water holding capacity (WHC) was determined according to a method described by Eide *et al.*, (1982) for 6 replicates per group. The samples were prepared and minced in a grinder for 10-15 seconds at low speed and about 2 grams of the sample was weighed. The samples

were then centrifuged (Heraeus Biofuge Stratos, Kendro Laboratory products, USA) at 1350 x g for 5 minutes at 4°C in special sample glasses made from Plexiglas. Water removed during centrifugation was drained through a nylon membrane in the bottom of the sample glasses. The WHC was calculated as the ratio of water remaining compared to the water content in the sample before centrifugation by using the formula:

$$\mathrm{WHC} = \frac{W - \Delta r}{W} \times 100\%$$

Where:

W is the water content of the sample before centrifugation (%).

Δr is the weight lost by centrifugation (%) and is calculated by the formula:

$$\Delta r = \frac{W_1 - W_2}{W_1} \times 100(\%)$$

Where:

W_1 is the weight of the original sample (g).

W_2 is the weight of the sample after centrifugation (g).

3.9 Drip loss and cooking yield

Drip loss in each group through was calculated during the storage period by measuring the weight of the fillets before packaging and again at sampling. The drip (%) was then calculated as the ratio of the weight of the water lost during storage to the original weight of the fillets, as follows:

$$Drip\ loss = \frac{W_1 - W_x}{W_1} \times 100(\%)$$

Where:

W_1 is the weight of the original fillets (g).

W_x is the weight of the fillets after x days of storage (g).

The loin parts from two fillets per group were weighed before and after cooking to determine the cooking yield of the samples. They were cooked on a grill pan by boiler for 8 minutes at 85-100°C and then left to thoroughly cool down in ambient temperature. The cooking yield (CY) was calculated as:

$$\mathrm{CY} = \frac{W_{\ cooked}}{W_{\ raw}} \times 100(\%)$$

Where:

W_{cooked} is the weight of cooked sample.

W_{raw} is the weight of raw sample before cooking.

3.10 Lipid content

The extraction method was used to measure the lipid content of the redfish muscle samples (Bligh and Dyer 1959). The samples were minced for several seconds at medium speed. Approximately 25 g (80±1% water) minced sample was weighed and homogenized by homogenizer (Ultra-turrax T25, IKA-Labor technik) with 25 ml of chloroform and 50 ml of methanol on cooling condition for 2 min to obtain a monophasic system. Then, 25 ml of chloroform was added and mixed to it in a homogenizer for 1 min. After that 25 ml of 0.88% (w/v) KCl solution was added and homogenized again for 1 min. The next step is centrifugation at 2500 rmp (1017 x g) for 20 min at 0-5°C by a Sorwall, RC-5B, GSA rotor, to separate the mixture solution. The chloroform layer was separated, collected and filtered through glass filter paper (Watman GH/D) under suction. The filtrate was collected and the volume of the chloroform layer measured. To determine the lipid content, 2 ml of aliquot chloroform was evaporated at 60^{o}C in a sand bath for 15 min followed by 10 min drying at 105^{o}C in an oven. The residual was weighted and expressed as grams of lipids per 100 g wet muscle.

3.11 Free fatty acid (FFA)

To determine the free fatty acids (FFA), the method created by Lowry and Tinsley (1976), with modifications from Bernardez *et al.*,(2005) was used. This method is based on a complex formation between the acid group of FFA and cupric acetate in the presence of pyridine at pH = 6.1. The rotary evaporated was used to evaporate the previous solvent resulted by lipid extraction. To this solvent, 3 ml of cyclohexane and 1 ml of cupric acetate/pyridine reagent were added and agitated for 1 to 2 min. Then, the solvent was centrifuged at 2000g for 10 min and the upper layer of the centrifuged solution was collected and read by spectrometer at 715 nm. The FFA concentration was determined as micromolar oleic acid based on the standard curve spanning in the 2-22 µmol range. Results are expressed as grams of FFA per 100 g of lipids.

3.12 Thiobarbituric acid reactive substance (TBARS)

The redfish fillets samples were homogenized by grinder before analysis. Exactly 5 g of the minced samples were weighed and then put into 50 ml tubes for TBARS determination. All tubes were stored in a *deep freezer* at -80^{o}C until analysis. All TBARS measurements were performed in duplicate.

To determine the TBARS, a modified version of the method created by Lemon (1996) was used. The 5 g samples were thawed and homogenized with 5 ml of 7.5% trichloroacetic acid (TCA) extraction solution containing 0.1% of propyl gallate and 0.1% of ethylene diaminetetraacetic (EDTA) by homogenizer Ultra-Turrax T-10 basic, IKA, Germany for 10 sec, followed by adding 5 ml of TCA extraction solution. After centrifugation at 94.000 *x g* for 15 min by centrifuge (Model Z323K, Hermle laboratories, Germany) the supernatant was collected in a plastic tube and mixed with 0.5 ml of 0.02 M of thiobarbituric acid, whereby it was agitated and heated in a water bath at 95 ^{o}C. After 40 min, all samples were directly cooled down in ice and transferred to 96-wells micro plates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for reading by a fluorescence spectrometer (POLAR star OPTIMA, BMG Lab tech, Offenburg, Germany) at 530 nm. The tetraethoxypropane was used to generate a standard curve and the results were expressed as µmol of malomalde hydediethylacetal per kg of samples.

3.13 Tertiary oxidation compounds

Tertiary lipid oxidation events were investigated by measuring the formation of interaction compounds between primary and secondary lipid oxidation products and nucleophilic molecules (protein-like) present in the fish muscle. To evaluate tertiary oxidation compounds, the fluorescence measurement were performed at 393/463 and 327/415 nm excitation/emission maxima on a Perkin Elmer instrument equipment. The slit of excitation and emission was set to 2.5 nm (Aubourg 1999a, Aubourg 2001, Aubourg *et al.*, 1997, Aubourg *et al.*, 1998, Aubour*g* 1999b). The relative fluorescence (RF) was calculated as follows:

$$\mathrm{RF} = \frac{F}{F_{st}}$$

Where:

F is the sample fluorescence intensity at each excitation/emission maximum.

F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/mL in 0.05M H_2SO_4) at the corresponding wavelength.

The fluorescence shift (δF) was calculated as the ratio between the two RF values, i.e. $\delta F = RF_{393/463nm} / RF_{327/415nm}$, and was analysed on the aqueous (δF_{aq}) and organic (δF_{or}) phase resulting from the lipid extraction (Blight and Dyer 1959).

3.14 Data Analysis

Statistical analyses were perform during Microsoft Excel 2010 (Microsoft Inc, Redmond, Wash., U.S.A.). The program was used to calculate mean values and standard deviations and significant differences were determined with ANOVA single factor test with a significance level of 95%. All graphs were generated in the program as well.

Comparison of data with respect to treatments was done using the Duncan's multiple comparison test. The threshold level for significance was 95%. Multivariate analysis was conducted in the statistical program Unscrambler (Version 9.7, CAMO Software AS, Oslo, Norway) with principal component analysis (PCA), assessing the QDA data determined from 30 attributes in 4 groups. Full cross validation was used.

4 RESULTS

4.1 Temperature results

4.1.1 Temperature of slurry ice

The measured temperature of the slurry ice during processing of the SIC-CBC group in Eskja hf. fishing company are showed in Figure 5.The salt content of the slurry ice used was 3.1% (Semwanga 2011) had a temperature about -2^{o}C, see Figure 5.

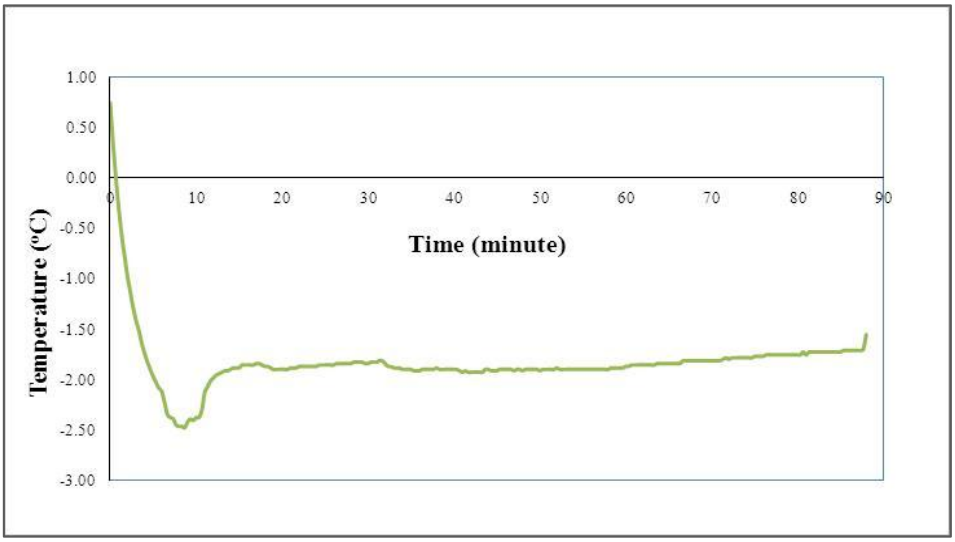

Figure 5. The temperature of the slurry ice during processing of the SIC-CBC group at Eskja hf.

4.1.2 Temperature during storage

The temperature inside the cooling chamber was maintained at approximately -2^{o}C from day 0 to day 6 (Figure 6). This temperature profile was supposed to represent well controlled containerised land- and sea transport from Iceland to Europe.

At the 6th day the temperature inside the chamber was raised from -2°C to 1-2° C until the end of the study. This is a simulation of the temperature which commonly occurs during marketing of fresh fillets in many destination countries in Europe including transport from the retailers to the consumers. The temperature fluctuated due to opening of the chamber door when samples were taken.

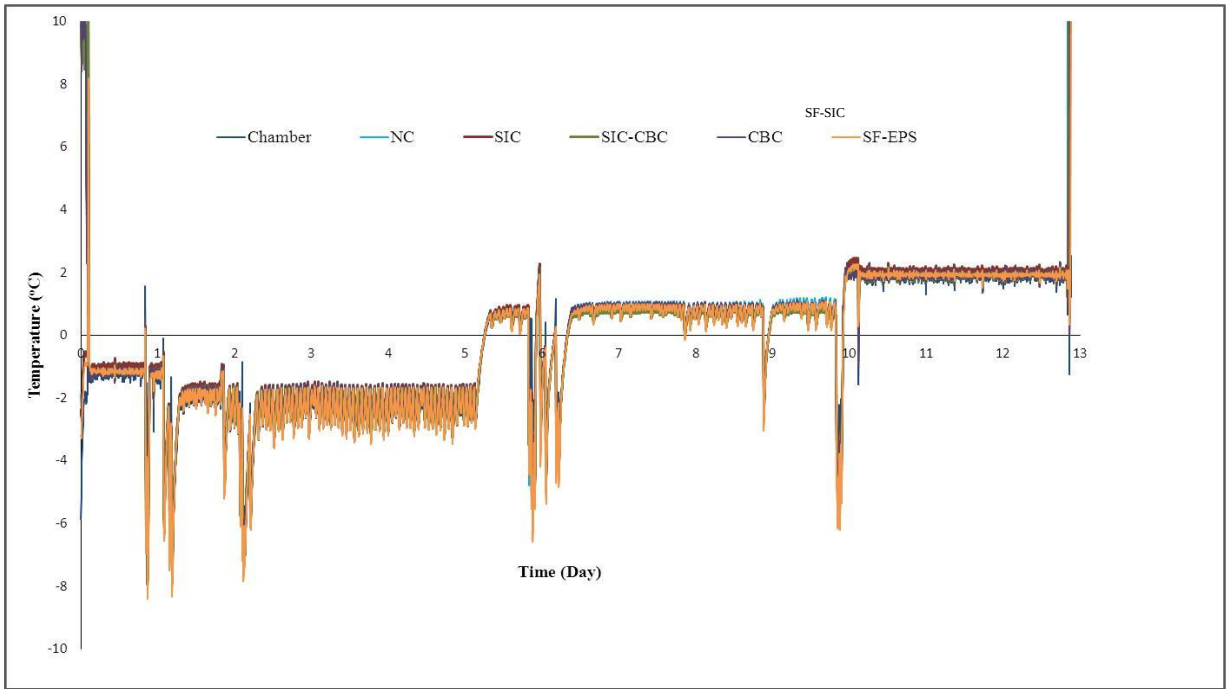

Figure 6. The average ambient air temperature inside the air climate chamber and outside surface temperatures

In the beginning, while the temperature of the chamber was kept at -2^{o}C, the CBC and SIC-CBC reached the ambient storage temperature already on the first day of storage. The SF-SIC and the NC groups needed approximately 2 days to reach the ambient storage temperature at -1°C, while the NC group required a longer time, up to 4days (Figure 7). The temperature in the NC and SIC groups increased faster in the second half of the storage period compare to the CBC groups and the SF-SIC group.

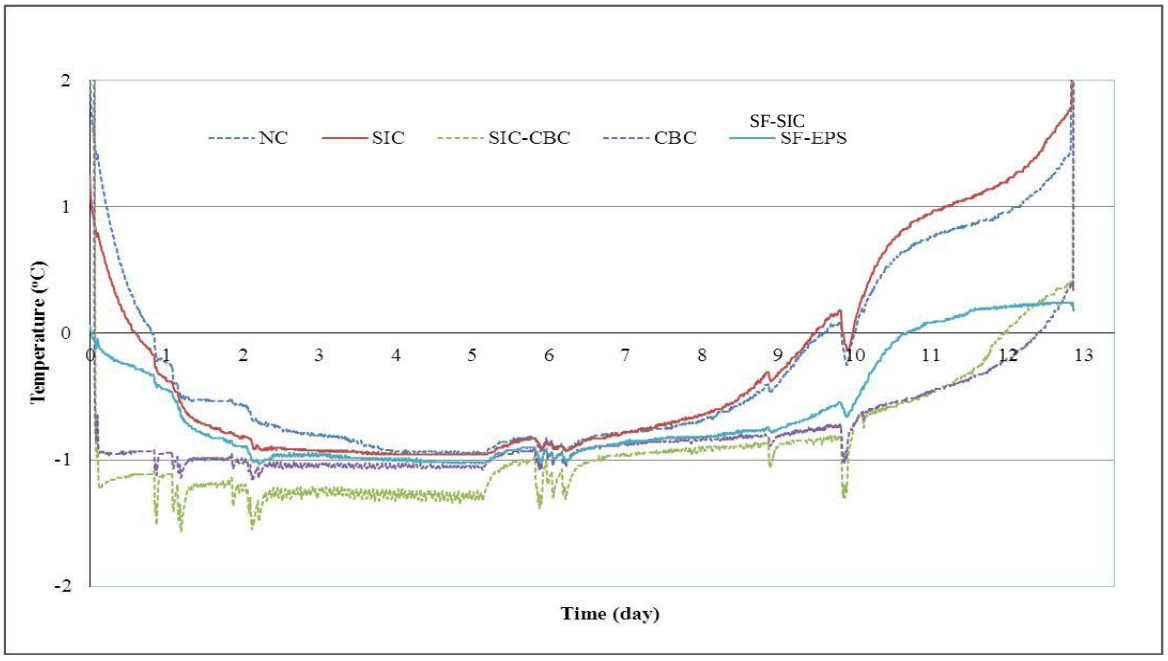

Figure 7. The average temperature of each group was measured at the upper, middle and bottom part.

4.2 Water Content

On day 1 there was no significant difference in the water content between the groups (Table 2). A significant decrease in water content was only observed in the SIC group during storage. However, a significant increase in water content was observed in the SF-SIC group due to melting of the flake ice.

Table 2. Water content of redfish fillets samples with different cooling methods during storage of 13 days.

Group	Water Content (%)			
	Day 1	Day 6	Day 10	Day 13
NC	77.70±1.32 [a]	79.20±0.96 [a]	79.23±0.26 [ac]	75.02±3.74 [ab]
SIC	79.54±0.05 [a]	78.92±1.14 [ab]	77.53±0.61 [bc]	77.67±0.01 [b]
SIC-CBC	80.36±0.87 [ad]	80.79±0.54 [acd]	78.29±0.43 [ac]	80.26±0.37 [d]
CBC	78.14±2.47 [a]	79.49±0.57 [a]	78.96±1.20 [ab]	78.26±0.28 [ab]
SF-SIC	-	76.15±0.14 [bc]	77.98±0.22 [ad]	78.01±0.74 [bd]

Values are mean ± standard deviation of 6 determinations. Data in each column and each row with different superscripts are significantly different at $p<0.05$.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.3 Water holding capacity (WHC)

The measured WHC of all groups in this study are described in Table 3. On day 1, the SIC-CBC had the highest WHC. No significant difference was observed between the other treatments. The WHC of the SIC did not change significantly until at day 10 of storage where a slight decrease was observed. The others groups: NC, SIC-CBC and CBC, decreased about 5% between days 1 and 6. After the 6th day, the storage temperature was changed to about 2^{o}C and then the WHC of the groups was generally stable except in the CBC group, in which it increased again by about 5% between the 10th day of storage and until the end of experiment, and for the SF-SIC group it significantly decreased.

Table 3. WHC of redfish fillets samples with different cooling methods during storage.

Group	Water Holding Capacity (%)			
	Day 1	Day 6	Day 10	Day 13
NC	92.41±2.53 [ac]	87.93±2.38 [bd]	89.00±1.47 [b]	87.41±1.95 [b]
SIC	90.84±0.75 [a]	90.86±1.89 [a]	90.61±1.80 [ab]	90.94±1.13 [ae]
SIC-CBC	94.65±1.15 [c]	90.90±1.82 [a]	92.09±0.85 [a]	91.64±1.06 [a]
CBC	92.46±1.39 [abe]	87.55±1.76 [c]	88.08±3.16 [bce]	92.56±4.82 [ae]
SF-SIC	-	92.12±1.99 [a]	90.83±1.63 [abe]	89.70±1.06 [e]

Values are mean ± standard deviation of 6 determinations.Data in each column and each row with different superscripts are significantly different at $p<0.05$.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.4 Drip loss

Generally, percentage of drip of all groups increased during the storage period (Table 4). On day 1, the CBC groups had the lowest drip while the SIC treated fillets had the highest drip. Between day 10 and 13, the drip of the CBC group sharply increased from 0.93% to 4.82%.

Table 4. Percentage drip loss of redfish fillets samples with different cooling methods during storage.

Group	Drip loss (%)			
	Day 1	Day 6	Day 10	Day 13
NC	1.06±0.24 [a]	2.51±0.31 [b]	5.19±0.51 [e]	5.99±0.80 [e]
SIC	3.24±0.24[b]	4.25±0.63[b]	7.40±0.65[ef]	8.08±0.79[f]
SIC-CBC	0.29±0.05[c]	0.48±0.16[c]	1.26±0.54[ac]	3.01±1.03[ce]
CBC	0.03±0.00[d]	0.10±0.00 [ac]	0.93±0.33[ad]	4.82±0.23[be]

Values are mean ± standard deviation of 2 determinations.Data in each column and each row with different superscripts are significantly different at p<0.05.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC.

4.5 Cooking yield

The cooking yield of the redfish fillets of each group in the experiment are shown in Table 5. During the first day of storage, the cooking yield of all groups did not significantly differ. After 6 days of storage, the cooking yield of the SIC group gradually increased from 74% to 80% at the 13th day of storage. Only the CBC group was fairly stable from the first day of storage until the 13th day of storage. The cooking yield of the SF-SIC group and the CBC treated was significantly higher compared to the control group (NC) on day 10.

Table 5. Percentage cooking yield of redfish fillets samples with different cooling methods during storage.

Group	Cooking Yield (%)			
	Day 1	Day 6	Day 10	Day 13
NC	76.42±1.44[ae]	75.51±2.14[ae]	75.44±0.95 [a]	78.43±1.72[cef]
SIC	75.13±2.54 [a]	74.58±2.66 [a]	76.00±2.07[ac]	80.47±1.53[be]
SIC-CBC	79.40±3.05[adf]	81.41±0.95[b]	79.32±1.19[d]	84.74±1.27[f]
CBC	78.29±3.45 [ac]	78.11±2.25[ac]	79.12±2.62[cd]	79.99±2.20[abc]
SF-SIC	-	79.31±6.75[ab]	76.67±3.86[ad]	79.08±2.32[abc]

Values are mean ± standard deviation of 4 determinations.Data in each column and each row with different superscripts are significantly different at p<0.05.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.6 Colour

The colour intensity values for the surface of redfish fillets of all groups in this study are shown in Tables 6 to 8.

At day 1, no significant difference was observed in the L-value intensity between the groups. During storage at -1^{o}C (day 1 to day 6), the L-values of both SIC-CBC and CBC groups showed a slight decrease, followed by an increase after 6 days of storage until end of experiment. However, the L-value of the SF-SIC fillets started to decrease between days 10 and 13.

Table 6. Measured colour intensity of the L-value (black to white) of redfish fillets samples with different cooling methods during storage.

Group	Intensity (L-) %			
	Day 1	Day 6	Day 10	Day 13
NC	49.33±3.83[a]	50.63±2.96[a]	50.22±1.98[a]	50.28±1.82[a]
SIC	47.91±2.84[a]	50.15±2.82[a]	50.61±1.59[a]	49.64±0.95[a]
SIC-CBC	49.02±1.83[a]	42.90±1.77[b]	50.88±2.90[a]	48.41±1.88[a]
CBC	48.01±2.07[a]	44.86±1.93[b]	48.49±2.32[a]	47.96±1.91[a]
SF-SIC	-	49.65±2.13[a]	49.36±1.17[a]	45.92±1.99[b]

Values are mean ± standard deviation of 6 determinations. Data in each column and each row with different superscripts are significantly different at $p<0.05$.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

The intensity of green to red colour (a-value) of the redfish fillets varied among sampling days in all groups, but was generally not significantly different between the groups, with the exception of the SF-SIC. The a-value intensity of this group was significantly higher at day 10 compared to day 6.

Table 7. Measured colour intensity of the a-value (green to red colour) of redfish fillets sample with different cooling methods during storage.

Group	Intensity (a-) %			
	Day 1	Day 6	Day 10	Day 13
NC	0.91±1.01[a]	0.54±0.93[a]	1.61±1.29[ac]	0.79±1.15[a]
SIC	1.41±1.27[a]	1.67±1.20[a]	1.77±1.09[ac]	1.14±0.85[a]
SIC-CBC	1.07±0.65[ab]	0.67±0.58[ab]	0.56±0.60[b]	0.03±1.31[ab]
CBC	0.51±0.48[a]	0.89±0.74[a]	0.89±0.56[ac]	0.43±1.91[a]
SF-SIC	-	1.04±1.02[ac]	1.87±0.53[c]	1.51±0.71[ac]

Values are mean ± standard deviation of 6 determinations. Data in each column and each row with different superscripts are significantly different at $p<0.05$.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

The intensity of the b-value (blue to yellow colour) was not significantly changed for the CBC treated (SIC-CBC and CBC) and the SF-SIC group during storage. However a significant increase was observed in the control (NC) and the SIC groups. The b-value of the SIC group was significantly lower from the 6th day of storage until the end of the experiment. The lowest b-values were observed in the SIC-CBC group during the storage period.

Table 8. Measured colour intensity of the b-value (blue to yellow colour) of redfish fillets samples with different cooling methods during storage.

Group	Intensity (b-) %			
	Day 1	Day 6	Day 10	Day 13
NC	-2.45±1.74 [a]	-2.04±1.48 [ac]	-0.04±1.55 [b]	-1.31±1.68 [ab]
SIC	-3.41±0.67 [a]	-1.90±1.45 [c]	-0.37±1.70 [bc]	-1.44±1.23 [bc]
SIC-CBC	-3.78±1.81 [ad]	-4.42±0.48 [d]	-3.88±0.87 [d]	-2.92±0.78 [bde]
CBC	-3.47±1.41 [a]	-1.93±1.71 [ac]	-1.57±1.70 [ab]	-1.04±2.71 [a]
SF-SIC	-	-2.49±0.90 [ac]	-1.48±0.90 [ab]	-0.09±2.41 [a]

Values are mean ± standard deviation of 6 determinations. Data in each column and each row with different superscripts are significantly different at $p<0.05$.NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.7 Lipid content

Lipid content varied on day 1 between 1.6 g and 3.6 g per 100 g wet weight of sample. During storage the lipid content fluctuated for all groups, but the variation was the greatest for the SIC-CBC group (Figure 8).

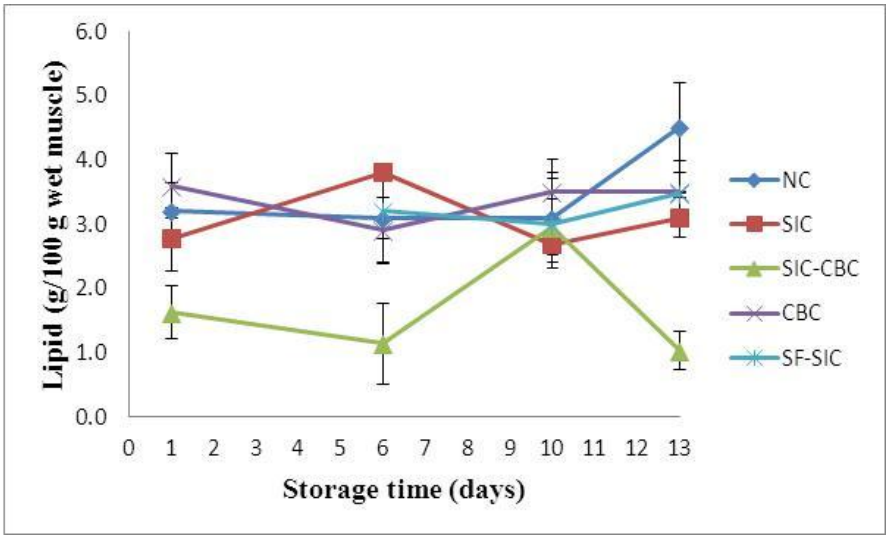

Figure 8. Lipid content of the redfish fillets.

Results are expressed as g lipids per 100 g of wet muscle. Vertical bars show the standard deviation of the measurements. NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.8 Free fatty acid (FFA)

The evaluation of lipid hydrolysis is presented in Figure 9. On the first day, the amount of the formatted FFA in each group as a result of the hydrolysis process of redfish fillet lipid varied from 0.6 g to 1.2 g per 100 g lipid. During the first 6 days of storage at -1°C, FFA of all groups slightly decreased. After day 6 when the storage temperature was changed from -1°C to 2°C, the FFA of the SIC-CBC group increased gradually until end of experiment followed by the SIC group as well as the others.

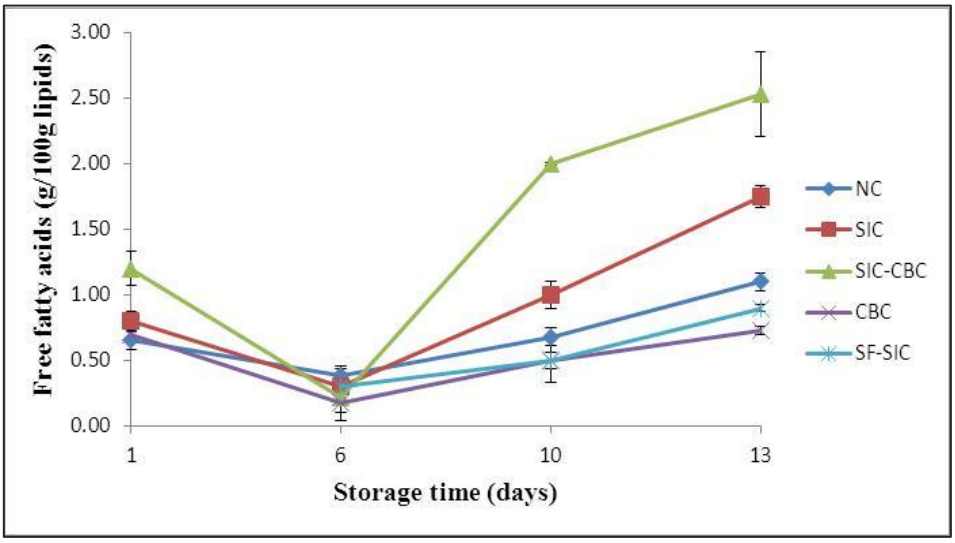

Figure 9. Free fatty acids (FFA) content.

Results are expressed as g FFA per 100 g lipids. Vertical bars show the standard deviation of the measurements. NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.9 Thiobarbituric acid reactive substance (TBARS)

The TBARS (secondary lipid oxidation compounds) of all samples which were formed during the storage period are presented in Figure 10 as µmol MDA/kg using malomalde hyde diethylacetate as a standard. On the first sampling day, the TBARS had values ranging from 4.69 and 6.62 µmol MDA/kg for the CBC treated (CBC and SIC-CBC) and 7.53 and 7.76 µmol MDA/kg for the SIC and control (NC) group, respectively. The SF-SIC group was not measured on day 1. At the 6th day of storage, the TBARS of the CBC groups slightly increased. However, after 6 days of storage the TBARS value of the SIC-CBC group decreased and had the lowest TBARS value at the end of storage. The SIC group reached its maximum value at day 6 and then decreased until end of the storage. The other groups were still rising at the end of storage.

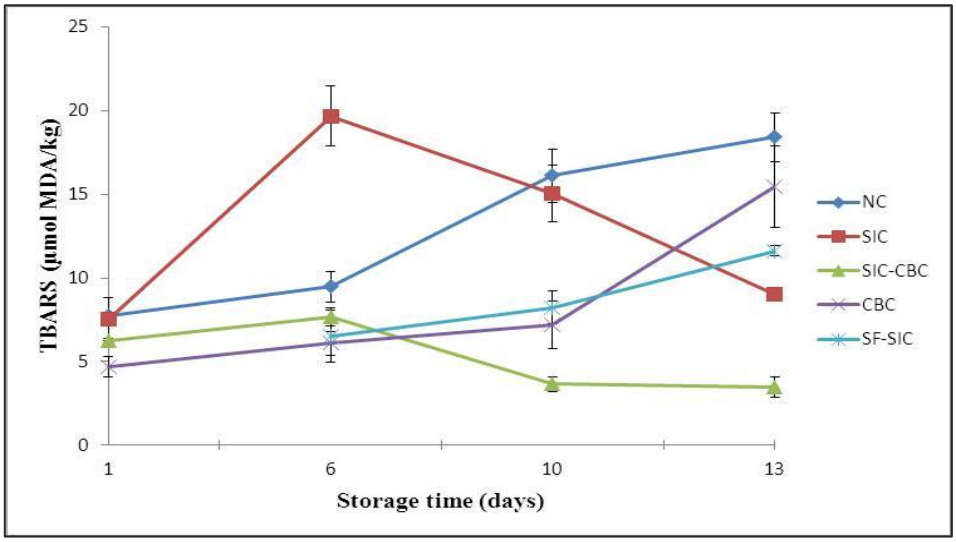

Figure 10. TBARS content (µmol MDA/kg).

Vertical bars show the standard deviation of the measurements. NC = no further cooling as control, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC, SF-SIC = cooling by slurry ice, stored in EPS boxes with holes cooled with flake ice.

4.10 Tertiary oxidation compounds

Figure 11 shows the formation of interaction compounds of all samples was assessed by the fluorescence ratio. On day 1, no significant difference was observed in the fluorescence ratio. However, it increased during storage, most in the SIC and the SIC-CBC groups.

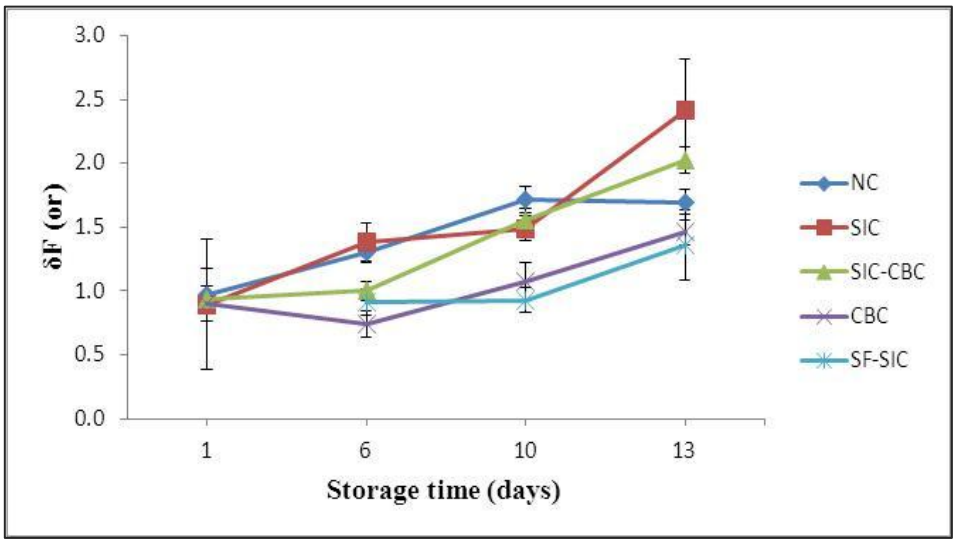

Figure 11. Fluorescence shift ratio of the organic phase resulting from Bligh and Dyer lipid extraction.

4.11 Sensory evaluation

The sensory results from the assessment of cooked redfish fillets (Figures 12) show that during the storage period, the Torry score of skin-on fillets (NC and SIC groups) and skin-less fillets (SIC-CBC and CBC groups) gradually decreased. Compared to the control (NC group), the score of the SIC group was high while the opposite was observed for the SIC-CBC and CBC groups.

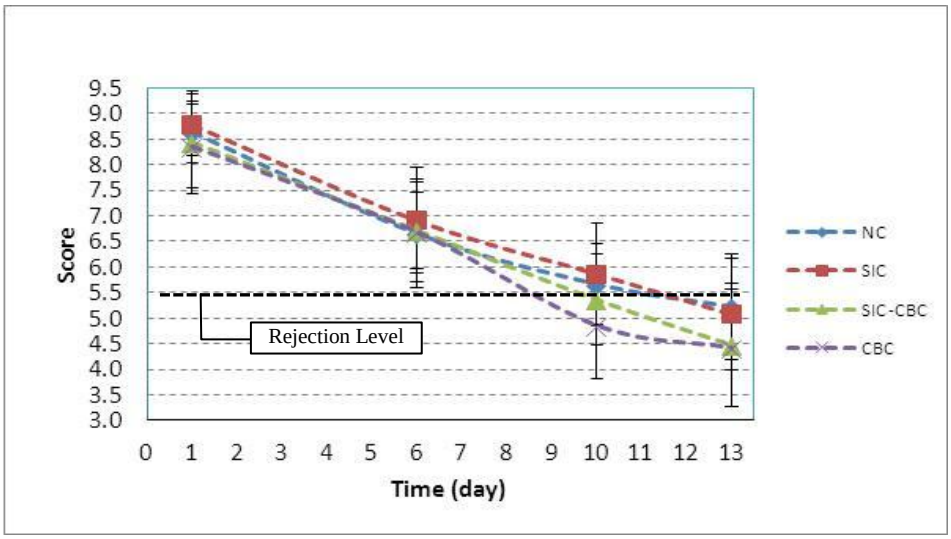

Figure 12. Torry score of the redfish fillets of each group.

Vertical bars show the standard deviation of nine measurements. NC = no cooling, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC.

The shelf life for fish products will vary considerably and is primarily dependent on the temperature of storage and initial condition of the fish products. The CBC and SIC-CBC groups reached the rejection Torry value of 5.5 approximately after 8 and 9-10 days, respectively, followed by the NC and SIC groups, which were assumed spoiled on the 11th day. Skin-on fillets cooled with the CBC technique and stored in a chilling storage at -1 to 2 ^{o}C therefore spoiled faster than skin-less fillets without cooling and cooled with slurry ice.

Figure 13 shows how the redfish fillet groups were characterized by sensory attributes. The SF-SIC group was not included in this analysis. The main variation (PC1, 86%) between groups was due to differences explained by storage time. During the first 6 days of storage, the groups differed with regards to flavour and odour; sweet, cod liver, shellfish, vanilla/warm milk and metallic. After 6 days, the groups were mainly described as soft, mushy and sticky and with boiled potatoes and TMA odours. At the end of the storage time, the colour changed, white precipitation increased and the muscle became more heterogeneous, especially in the CBC groups.

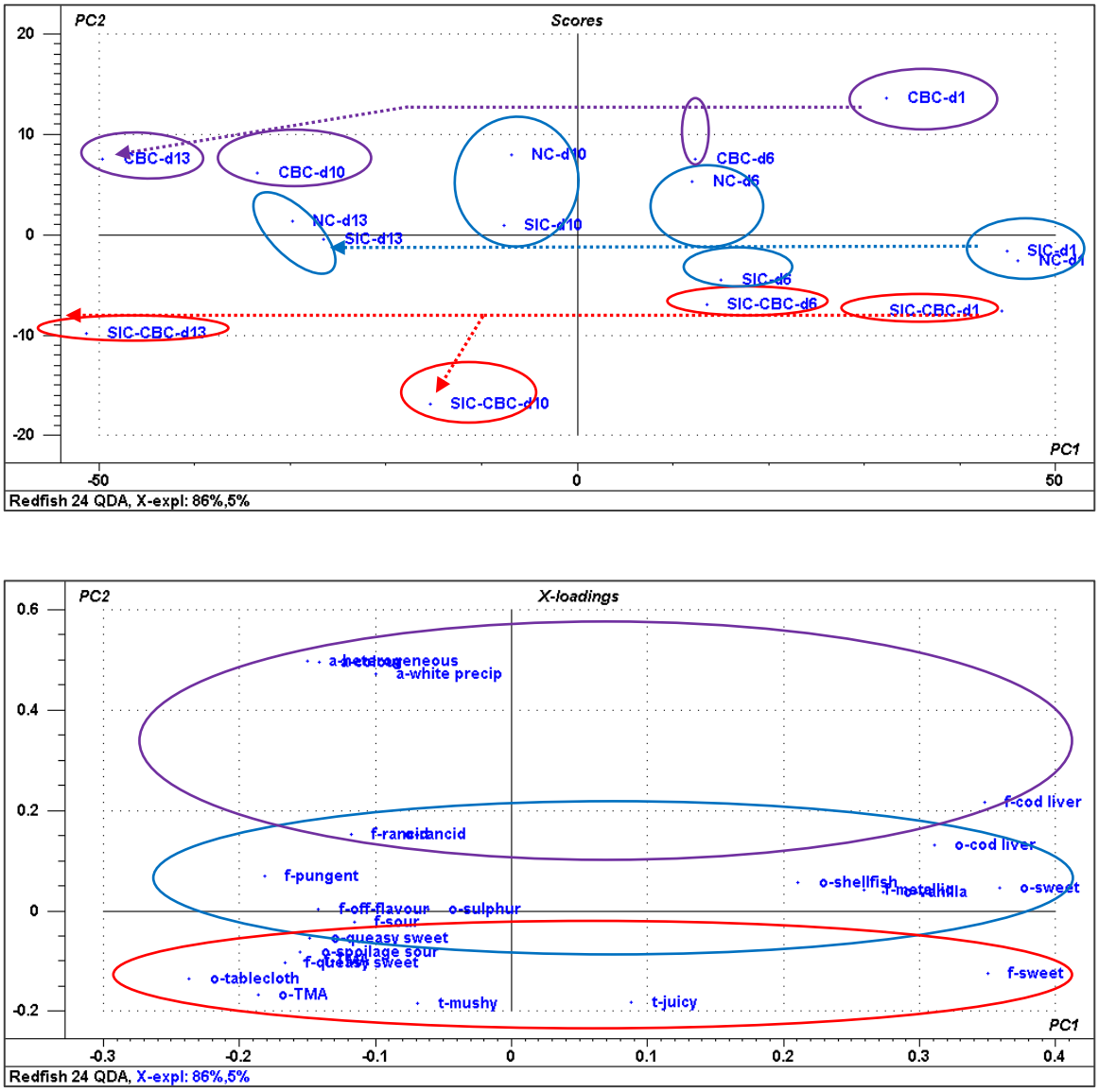

Figure 13. Principal component analysis (PCA) scheme of scores (top figure) and loadings (bottom figure) of the red fish fillets of the each group.

Odour (O-), appearance (a-), flavour (f-) and texture (t-). NC = no further cooling, SIC = slurry ice cooling, SIC-CBC = cooling by slurry ice and CBC, CBC = cooling by CBC. PC1 vs. PC2 (X-expl: 86%, 5%).Storage time of the all groups was from day 1 to day 13.Circles and lines with same colour between scores and loading figures indicate the changes of sensory attributes of each group. Purple circle colour indicates changes in the CBC group, blue changes in the NC and SIC groups and red indicates changes in the SIC-CBC group.

5 DISCUSSION

The temperature inside the chamber fluctuated between -8^{o}C and 2^{o}C (Figure 4) on days 1, 2, 6 and 10, but the temperature inside box of each experimental group (Figure 5) only showed slight fluctuation of approximately 0.5^{o}C. This shows that the heat conductivity of the EPS boxes is very low and that they maintain the temperature in the box well. This is in agreement with the observation of Margeirsson *et al.,* (2009, 2011).

In Figure 6, when looking at rapid cooling prior to storage it is clear that CBC cooling, either with or without prior slurry ice immersion, is a suitable cooling treatment. Magnusson *et al*., (1998) stated that the implementation of super chilling can be used prior to improve cooling capacity of the product and it would be maintained in the product throughout the storage and distribution. The slower temperature rise in the CBC treatments and SF-SIC group at the end of storage indicates that these treatments maintain the low storage temperature better than the NC and SIC treatments.

The water content of all groups was not significantly different on day 1 indicating that the cooling treatments did not influence the water content of the fillets significantly. However, the observed decrease in water content in the SIC group during storage indicated that water added by slurry ice immersion is more easily lost during storage than water already present in the muscle. The added water might add to the *restricted water* or *free water* populations and thus change the water distribution within the muscle. Ofstad and Hermansson (1997) stated that the *restricted water* and *free water* is easily lost with environmental changes during processing.

This study showed that the WHC was not significantly different between the treatments. Nevertheless, the WHC of the CBC group, between the 10th day of storage and until end of experiment, increased by about 5%. This could be explained that the temperature of the redfish muscle was better maintained during cold storage if rapidly cooled by the CBC method during processing (Magnússon *et al.,* 2009). Cracking of the muscle tissue can be avoided due to small size of the ice crystals formed during a rapid freezing (Bello *et al.,*1982, Martino *et al.,* 1998, and Bahuaud *et al.,*2008).

The changes in drip and the cooking yield during storage could be caused by decomposition of the muscle fish. Generally, the fish muscle is spoiled because of the combined effect of enzymatic activity and bacterial growth (Huss *et al.,* 1997, Church 1998). After 6 days of storage, the cooking yield of the CBC groups was significantly higher than in the control group. This might be due to muscle degradation caused by bacterial activity presence on the skin of the CBC groups. Although bacteria grow very slowly in chilling temperatures (Hobbs 1985) they can still cause muscle degradation and can break down the muscle (Liston 1980). The muscle degradation of redfish could also happen due to ice formed during the super chilling process and storage. According to Bello *et al.,*(1982), Martino *et al.,* (1998) and Bahuaud *et al.,*(2008), rapid freezing might crack the intracellular tissue of the product. However, slow freezing could result in a low number of large crystals (extra-cellular), generally decreasing the product quality through extensive mechanical damage, and accelerated enzyme activity.

Based on measurement on the FFA, TBARS and fluorescence ratio, immersion into slurry ice increased lipid oxidation. The lipid oxidation parameters indicated slower oxidation in the CBC group than in the SIC-CBC group. The CBC group also showed lower TBARS and

fluorescence ratio than the control group without cooling during processing (NC). This indicated that the CBC group is a good chilling method to prevent oxidation of lipids. Slower tertiary lipid oxidation was also observed in the CBC and the SF-SIC groups, compared to the other treatments. The rigor process and added water due to immersion in slurry ice might have an effect on the FFA decrease observed in the beginning of the storage period.

Chang *et al.*,(1998) stated that products which have been processed by super chilling would have an increased shelf life. Meanwhile, the Torry result of this study (Figure 6) showed that super chilling by CBC (SIC-CBC and CBC) had a shorter shelf life than the control (NC) or the slurry ice super chilled group. This can be explained by the fact that the CBC chilled products were processed with the skin on while the control (NC) and slurry ice chilled (SIC) groups were processed as skin-less products. Bacteria present on the skin might invade the muscle and cause cross-contamination. The bacteria can then multiply rapidly and grow on the ideal media which fish muscle forms (Liston 1980, Huss 1995, Adam and Maurice 2008). Reynisson *et al.*,(2010) showed that some bacteria, such as *pseudomonas*sp, can grow in cooling media with a salt content ranging from 0 to 2.9%, but the used salt concentration of the slurry ice is the mentioned range.

The sensory results showed that the flavour and odour of the boiled fillets changed during storage from sweet, cod liver, shellfish, vanilla/warm milk and metallic, to sticky and with boiled potatoes and TMA odours. The decrease of the sweet flavour may be caused by the breakdown of inosinic acid through autolysis (Clucas 1981). The spoilage mechanism is however a shared contribution of the autolysis processes as provided by a supply of compounds, due to bacteria activity, the produced sugar by enzymatic actions during interaction with amino compounds (FAO 1986), as well as hydrolysis and oxidation processes of the lipids of the redfish fillets.

6 CONCLUSION

The water content of the redfish muscle was not significantly affected by the different cooling methods during processing. Also this study showed no significant difference in the water holding capacity between the different cooling methods during storage period except the combine blast and contact (CBC) cooling methods, which was significantly higher than the other groups during storage. The CBC groups reached the stable storage temperature faster than the other treatments such as the control group which did not reach a stable storage temperature until on day 4, indicating that CBC is a good method to reach a low stable storage temperature in a rapid way. The low temperature of the redfish fillets was clearly maintained by the CBC cooling methods and SF-SIC compared to the control (NC) and SIC groups.

The lipid content of the redfish muscle was not affected by different cooling methods. However, the free fatty acid, secondary and tertiary lipid oxidation compounds were affected by the different cooling treatments during processing and the chilled temperature during storage. Slurry ice immersion of the redfish fillets led to increased lipid oxidation, while CBC slowed down lipid oxidation.

According to the sensory evaluation, the CBC groups were spoiled faster compared to the control (NC) and the SIC groups where the skin had been removed. This indicated that the skin should be removed to get longer shelf life. Based on this study as well as earlier results

on CBC cooling, this cooling method can be recommended, as long as skin-off fillets are processed. However, this is something that should be studied further.

6.1 Future perspectives

The study shows that it is possible get high quality fresh fish fillets by implementing super chilling pre-cooling methods during processing. This is something that the fish processing industry in Indonesia can benefit from. Some of the cooling treatment solutions studied in this experiment can also possibly be implemented for other fish species, increasing the possibilities of using these techniques in a wider range of processing lines. The information gained in this project will be transferred to the fish processing sectors in Indonesia by means of lectures for students at the Jakarta Fisheries University, by training, publishing of posters on exhibitions, as well as by presentations at seminars and workshop events.

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