Stability of lightly salted fillets during frozen storage

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Geymsluþol léttsaltaðra flaka í frosti

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Abstract

The aim of the project was to study the effect of frozen storage on stability of lightly salted fillets. Atlantic cod (Gadus morhua), was used as model fish, representing lean fish species. An experimental design was set up with two different storage temperatures (-18 and -25°C) and three storage periods (1 week, 3 and 6 months). Two different bleeding methods were used to test the effect of bleeding on product quality (traditional bleeding and insufficient bleeding). The effect of different fillet sizes on product quality after frozen storage was also tested (small fillets and large fillets). Additionally the age of the fish before processing (1 day and 4 days) was also tested. Quality-related changes during frozen storage were measured using the following analysis: water content, water holding capacity (WHC), glazing content, drip loss, color measurement, cooking yield, phosphate content, salt content, total lipid content, free fatty acid content and phospholipid content. A total of 180 fillets of cod were used in this study and a part of those fillets were control fillets (fillets from untreated fish). All the samples were received from two processors. Fish processor A collected the samples in July and fish processor collected the samples in October.

Fillets from traditional bled fish had less drip loss and more whiteness compared with fillets from insufficient bled fish. The age of the fish before processing was also an important quality parameter, were 1 day fillets had a much lower yield and also more yellowness on the fillets surface compared to 4 days old fillets. The results showed that large fillet size is more suitable for processing compared to small fillets, where the large fillets had higher lightness value and better cooking yield as well as total yield. Glazing was a conventional method for preventing changes in fillet color. Lightly salted fillets in October had a whiter appearance, better water holding capacity, higher lipid content, higher phospholipid content and lower drip loss, better cooking and total yield compared to fillets collected in July. However, the water content was similar between the season groups. Salt injection did not have any effect on drip loss in any of the lightly salted fillets compared to the untreated control fillets. Salting did, however, have an effect on water content, as more salt content in the fillets resulted in more water content. The WHC and the water content of lightly salted cod in all groups stored at -18°C and -25°C did not change during the storage period. The lightness of lightly salted cod fillets had the tendency to increase with frozen storage time, especially when stored at -25°C.

Keywords: Frozen storage, cod, lightly salting, fish, quality indicators

Ágrip

Markmið verkefnisins var að rannsaka áhrifin af frystingu á stöðugleika léttsaltaðra þorskflaka (Gadus morhua). Flökin voru geymd við tvö mismunandi hitastig (-18°C og -25°C) í viku, þrjá og sex mánuði. Tvær mismunandi blóðgunaraðferðir voru notaðar til að meta áhrifin af blóðgun (hefðbundin blóðgun og dauðblóðgun). Áhrifin af tveim mismunandi flakastærðum á hráefnisgæði eftir geymslu í frosti voru einnig skoðuð (lítil flök sem voru 500-1000 grömm á þyngd og stór flök sem voru meira en 1500 grömm á þyngd). Aldur hráefnis fyrir vinnslu var einnig þáttur sem var skoðaður (1 daga gömul flök annarsvegar og 4 daga gömul flök hinsvegar). Breytingar á hráefnisgæðum í frosti voru mældar með því að skoða: vatnsinnihald, vatnsheldni, íshúðun, drip við þíðingu, lit, eldunarnýtingu, magn fosfats, salts, fitu, frírra fitusýra og fosfólípíða. Í heildina voru 180 flök notuð við rannsóknina. Sýnin voru fengin frá tveimur framleiðendum. Framleiðandi A safnaði sýnum í júlí og framleiðandi B safnaði sýnum í október.

Flök af fiski sem var blóðgaður á hefðbundinn hátt sýndu minna drip við þíðingu og þau voru einnig hvítari miðað við flök af dauðblóðguðum fiski. Aldurinn á fisknum fyrir vinnslu skipti miklu máli hvað varðar hráefnisgæði, 1 daga gömul flök höfðu minni nýtingu og voru gulari heldur en 4 daga gömul flök. Niðurstöðurnar sýndu að stór flök eru betri samanborið við lítil flök, þar sem að stóru flökin voru ljósari, höfðu betri eldunarnýtingu og þar af leiðandi einnig betri heildarnýtingu. Íshúðun er hentug aðferð til að koma í veg fyrir litabreytingar á flökunum. Léttsöltuð flök í október voru hvítari, höfðu meiri vatnsheldni, meiri fitu, meira magn fosfólípíða, minna drip við þíðingu og betri eldunarnýtingu samanborið við flök í júlí. Hinsvegar var vatnsinnihaldið svipað á milli flaka sem veidd voru í júlí og október. Léttsöltun hafði engin áhrif á drip við þíðingu. Hinsvegar hafði saltmagn áhrif á vatnsinnihald, því meira sem saltinnihaldið var í flökunum því meira vatnsinnihald var einnig í flökunum. Niðurstöðurnar sýndu einnig fram á að fosfat er bætt í flökin í framleiðslunni en fosfatið hafði engin áhrif á nýtni flakanna, frekar en drip við þíðingu. Auk þess, því meira sem var af fosfati í flökunum, því lægra vatnsinnihald og vatnsheldni var einnig í flökunum. Vatnsheldni og vatnsinnihald í léttsöltuðu þorskflökunum breyttist ekki á geymslutímanum við -18°C og -25°C. Ljósi liturinn í léttsöltuðu þorskflökunum hafði tilhneigingu til að aukast í frosti á geymslutímanum, sérstaklegar við geymslu við -25°C.

Lykilorð: Geymsla í frosti, þorskur, léttsöltun, fiskur, gæðavísar

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Abbreviations

a_w	Water activity
CMVS	Color machine vision system
CY	Cooking yield
FFA	Free fatty acids
PUFA	Polyunsaturated fatty acids
PL	Phospholipids
SR	Sarcoplasmic reticulum
T	Temperature (°C)
t	Time
TL	Total lipid
TMAO	Trimethylamine N-oxide
TY	Total yield
WHC	Water holding capacity

1 Introduction

Lightly salted cod fillets entered the market about 20 years ago, with only ≈ 2% salt content, as substitute for dehydrated heavily salted cod. This product has been growing in popularity, especially in Spain (Figure 1) (Lindkvist, 2009). It is therefore important for the Icelandic producers to meet this increasing demand with high quality products.

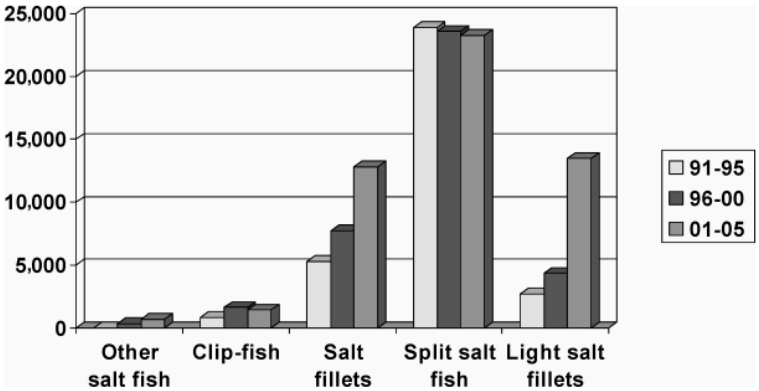

Figure 1. Structural changes in the Spanish importation of salted fish 1991-2005 (Lindkvist, 2009).

Preservation of the quality characteristics of raw materials and high utilization, are two of the main prerequisites so that the Icelandic fishing industry can maximize its value as possible from the limited natural resource of fish stocks around Iceland.

Lightly salting is a process that can be used to control utilization and nutritional status of the fillets thereby achieving more uniform quality and increasing the stability (Thorarinsdottir, Arason, & Thorkelsson, 2001a). It can possibly reduce fluctuations that are caused by seasonal changes in condition and characteristics of the fish muscle and therefore, have a positive effect on the processing of the fish. Lightly brining alone, is thought to have an effect on taste, texture, water holding capacity, color and shelf life of fish.

In recent years, systematic research has provided very important information, which has led to a dramatic increase in value for the processing of lightly salted fillets. Earlier researchers have shown that brine concentration, brining time, brine injection and use of salt with regard to utilization and chemical content have an effect on the fish muscle (Gudmundsdottir, Thorarinsdottir, Arason, & Thorkelsson, 2003; Thorarinsdottir, Gudmundsdottir, Arason, Thorkelsson, & Kristbergsson, 2004; Thorarinsdottir, Arason, & Thorkelsson, 2001b). The fillets are brined and/or brine injected and then frozen (Lindkvist, Gallart-Jornet, & Stabell, 2008). It is necessary to control the salting process so that the salt content is within the limits of the purchaser´s demands and to control the weight changes. As mentioned before the purchaser's demand is to have the salt content around 2%. The most important variables are brining content, pressure of injection and brining time (Lindkvist, 2009).

Many studies have investigated the relation between storage temperatures, time and quality-related changes in fish muscle, but systematic research has not yet been done on long-term stability

of lightly salted fillets during frozen storage. That is why manufacturers have not been able to secure a standard product to the buyer. Storage life of untreated fish fillet in frozen storage is nearly 10 months at -18°C and 18 months at -25°C (Johnston, Nicholson, Roger, & Stroud, 1994). However, lightly salted fillets contain eight times more salt than untreated fillets and that is why it is important to evaluate the effects of this supplement on the stability of the fish muscle. Studies have shown that fish muscle containing 2.2% salt content has a lower freezing point (-4 to -5°C) compared with untreated fish muscle (-2 to -3°C) (Gudjonsdottir, 2006). Furthermore, the addition of salt slows down chemical and physical changes during freezing, but it does not stop the process. Freezing itself also causes certain changes, such as decreasing the water binding properties of the fish flesh (Bello, Luft, & Pigott, 1981; Hurling & Mcarthur, 1996).

In present study, an experimental design was set up with two different storage temperatures (-18 and -25°C) and 3 storage periods (1 week, 3 and 6 months). Cod fillets were used as model fish representing lean fish species. The samples were received from processor A that uses a long line fishing technique and processor B that uses a bottom trawl fishing technique. Quality-related changes during frozen storage were measured using the following analysis: water content, water holding capacity (WHC), glazing content, drip loss, color measurement, cooking yield, phosphate content, total lipid content, salt content, free fatty acid content and phospholipid content. The main focus of this study was the effect of various processing and physical variables on quality-related changes in the fish muscle during frozen storage. The processing variables that were selected for this study were: bleeding method and brining time. Physical variables were: weight of fillets, seasonal changes and age of the fillets. The research presented here is a part of a larger project aiming at increasing the knowledge of long-term stability of lightly salted fillets during frozen storage.

2 Review of the literature

2.1 The fish

2.1.1 Atlantic cod

Gadus morhua (Linnaeus 1758) (Figure 2) is commonly known as Atlantic cod. Its habitats are on both sides of the North Atlantic. In the Northeast Atlantic, the Atlantic cod may be found from Novaya Zemlya and the Barents Sea to the White Sea and along the Norwegian coast south of the Baltic Sea and the North Sea. The Atlantic cod can be found all around Britain and the Bay of Biscay, around Iceland and East Greenland. In the Northwest Atlantic, the Atlantic cod can be found Southwest and West of Greenland and mainland America from the southern side of Baffin Island and Labrador, south to Newfoundland and Cape Hatteras in North Carolina (Jonsson & Palsson, 2013).

The Atlantic cod is a well-known demersal fish that lives at various depths in the ocean, ranging from a few meters down to 600 m or deeper. Usually, the cod lives at 100-400 m around Iceland. The cod prefers sea temperature of 4-7°C but can be found in sea temperature of 0 to 16°C and even 20°C (Jonsson & Palsson, 2013).

The Atlantic cod is omnivorous, feeding at dawn or dusk on a variety of invertebrates and fish, but the main feed of the cod is capelin. The cod can grow up to two meters in length and weight up to 96 kg but on average, cod weigh 40 kg and 50-90 cm in length. It can live for 24 years and sexual maturity is generally attained between ages 2 to 4, but can be as late as 8 years in the northeast Arctic (Jonsson & Palsson, 2013). The color of Atlantic cod varies with respect to age and the environment in which the fish lives. Iceland cod are generally dark in color because Icelandic grounds are often covered with dark volcanic debris (Love, 2001a). The most common color of the dorsal (upper) area of the fish and towards the ventral side is yellow-gray with small dark spots, with a paler ventral (under) side. A number of variations exist. Cod in water with a large volume of algae will yield a red to greenish skin color.

The Atlantic cod is caught mainly with bottom trawls and is fished throughout the year. The major fishing grounds are mostly around Iceland and Norway. In 2012, the cod was about 14% of the total catch in Icelandic waters, or nearly 205.000 metric tons (Hagstofa Íslands, 2013).

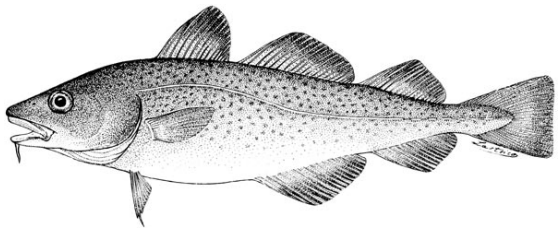

Figure 2. Graphical representation of the species *Gadus morhua* (Cohen, Inada, Iwamoto, & Scialabba, 1990).

2.1.2 Seasonal and other variation in the initial quality of cod

Differences in condition of cod are related to its spawning cycle. The cod's sex organs are small during the summer. Before spawning in the spring the sex organs in the cod usually start to grow rapidly around October. Instead of providing energy for building up reserves in the liver or energy for swimming, the fish is progressively diverting energy to the sex organs. In the wintertime the cod starts to break down components of its body to supply the growing sex organs because the food supply diminishes during this time of year. The fish muscle is made from hair-like cells, which shrinks and the spaces between them increases and fill up with fluid when the proteins are drawn from all parts of the flesh that causes the fish texture to become moist. The flesh becomes even softer after spawning due to the low food supply in the ocean. The cod does not repair the damages until early summer when surface plankton and the organisms that feeds in it has grown in early summer (Love, 2001a).

The cod in the North Sea usually begins to spawn early in March and nearly into May off Iceland. Larger cods draw more deeply on their body reserves to make sperm or eggs than smaller cods and therefore the larger cods become more watery during spawning. That means that large cod are therefore softer than small cod during and just after spawning, but the larger cods are firmer during the rest of the year. When large cod is frozen at sea during and just after spawning, the drip on thawing becomes even greater which makes them less suitable to be frozen. During other parts of the year the tough and firm texture of larger cods makes them less suitable then smaller cods to be frozen. Small cod tend to gape more than large ones. Gaping occurs when slits or holes start to appear on the fillet due to the separation of the flakes. In general the large cod are less suitable to be frozen at sea, but they nearly always keep better when stored on ice during summer and autumn (Love, 2001a, 2001).

For the processer, the pH of cod is an important factor. In the case of cod with a low pH, the texture will already be firm, therefore it will cross the boundary of unacceptability after even a little cold storage, and the effect of freezing and immediate thawing alone toughens the texture appreciably (Love, 1962, 2001a). The degree of acidity (more acid is present when pH is lower) goes in hand with nutritional state of the cod. Little glycogen is in the muscle of starved cod and if the cod dies, hardly any lactic is formed and the pH is high. After spawning, while the cod is feeding lightly, the pH remains high. In the North Sea in late May the cod begins to gorge itself, which drops the pH rapidly (Figure 3). The pH remains low for about a month, until it begins to rise again, perhaps because the cod adjusts to the greater amounts of food, and therefore deposits less glycogen in the muscle. It is also possible that the rise in pH is due to the change in diet, when the cod begins to gorge itself. Fortunately for the processor, the pH levels in cods stays low for a short time (Love, 2001a).

During the short period of heavy feeding and low pH the flesh of the still spent cod is too firm for freezing, the fillets will gape badly, and the flesh will become opaque, white and watery. Heavy feeding is thought to be the main cause to the soggy white flesh, but that is not the case, the reason for that is that they have not been eating long enough to recover their, elastic condition.

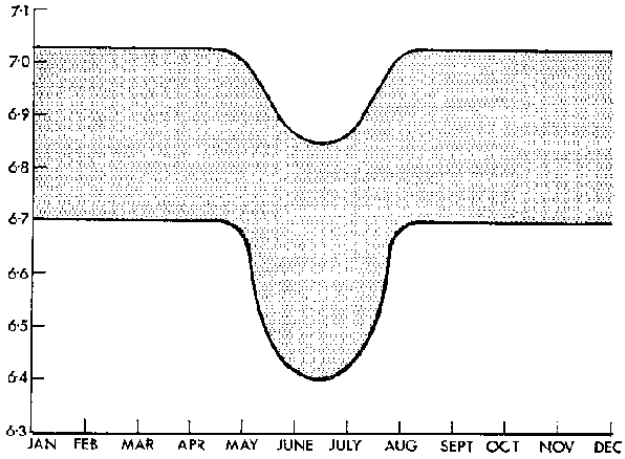

Figure 3. Average pH values of groups of cod taken from grounds all over the north Atlantic at different time of year. Fish with a pH below about 6.6 are too tough for freezing, and gape after thawing and filleting (R. M. Love, 2001a).

First of all, cod are least suitable for freezing during the period of heavy feeding following the return of usual food supplies. The flesh is tough, gapes badly and is opaque, especially after cold storage (Love, 2001a). Furthermore, cod are also not suitable for processing during the period of heavy feeding.

The effect of fish size on texture is “small”, because it is well known that large fish can be considerably tougher than smaller fish. The principle reason for this is that the muscle of larger fish, although intrinsically tougher, also tends to equilibrate after death at a lower pH than that of smaller fish (Hall, 1997).

2.2 Fish muscle

2.2.1 Composition

Water is the main constituent of fish flesh, which usually accounts for about 80% of the weight of a fresh white fish fillet. Another large constituent of fish flesh is protein, the amount of protein is usually somewhere between 15% and 20%. The fat content of fish can vary much more if all species are taken into account. The lipid content differs from approximately 0.2 to 25%. Depending on their lipid content, fish are classified as lean or fatty. Fatty species deposit lipid in fat cells in flesh and lean species in the liver. In white fish, such as the cod, the fat content of the muscle is low or usually below 1%, and seasonal fluctuations in fat content are noticeable mainly in the liver, where the majority of the fat is stored (Murray & Burt, 2001).

The lipid content is divided into two main groups: triglycerides and phospholipids. The phospholipids are also called the structural lipids because they make up the structure of the cell membranes. In lean fish species with lipid contents of less than 1%, about 90% of the muscle lipids are phospholipids. Triglycerides are used as an energy source and are deposited in fat cells distributed in the whole body, but primarily located in the belly flap, in the muscles that move the fins and tail, in the liver and also in the abdominal cavity. Fish have a high content of polyunsaturated fatty acids (PUFA) and are, therefore, susceptible to lipid oxidation. During frozen storage, lipid oxidation

occurs in lean as well as fatty species and can cause quality deterioration such as yellow/brownish discoloration of the flesh surface (Mercedes Careche, Del Mazo, Torrejón, & Tejada, 1998; M. Careche, Herrero, Rodriguez-Casado, Del Mazo, & Carmona, 1999; Chichester & Stewart, 1981; Kim & Hearnsberger, 1995; Lauritzsen, Martinsen, & Olsen, 1999; Sikorski, Olley, Kostuch, & Olcott, 1976).

The proteins in fish muscle are divided into three groups: the myofibrillar proteins accounting for 70-80%, the sarcoplasmic proteins accounting for 25-30% and the stroma proteins (collagen) accounting for 3%-10%. The denaturation and aggregation of especially the myofibrillar proteins during frozen storage lead to textural changes such as loss of juiciness and a hard and fibrous product (Mercedes Careche et al., 1998; M. Careche et al., 1999; Chichester & Stewart, 1981; Sikorski et al., 1976).

2.2.2 Structure

Fish muscles main function is movement, while muscle in mammals gives the skeleton important support. The biggest difference between striated fish muscle and the muscle found in higher vertebrates is firstly the separation of fibre types into discrete layers in fish, where 90-95% of the muscle in most fish species is dominated by high glycolytic and anaerobic type. Secondly in the majority of fish species the muscles grows bigger throughout most of its lifetime, which is due to formation of more muscle cells (fibres) and the increased size of the fibres already existing (Dunajski, 1980).

Normally in vertebrates the muscles are bundled but muscles of fish are layered. The muscle fibres are separated by connective tissue, which divides the muscle fibres into ‘W’-formed segments called myotomes (Figure 4). The myotomes are not flat sheets of muscle but are folded into a three-dimensional shape. A myotome is composed of fibres arranged parallel to each other and is only one cell long. Each muscle cell contains sarcoplasm, nucleus, glycogen granules, mitochondria and a number of myofibrils, which run from one end of the fibre to the other. The myofibrils are primarily composed of the thin and thick contractile protein filaments actin and myosin, which are arranged in a way that gives the muscle its striated look (under a microscope) and divides the myofibrils into segments called sarcomeres (Figure 4). Myosin accounts for about 40-60% of the myofibrillar protein content and actin for 15-30%. Myosin is more sensitive towards frozen storage than actin.

A connective tissue, called septa is the name for a connective tissue that occurs along the vertical midline of the body and separates the myotomes of the left and right sides of the body. A horizontal septum separates the myotomes of the lower and upper halves of the body. The muscles of the lower half are called ‘hypaxial’ and those of the upper half of the body are called ‘epaxial’ (Badii & Howell, 2002; Mackie, 1993). The muscle fibres cross the sectional area are smaller at the head and tail than in between and the length of the muscle fibres decreases in length towards the tail end of the fillet (Love, 1988).

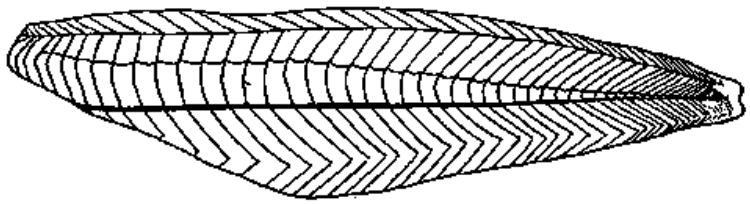

Figure 4. Mechanical construction, typical of all white fish like cod (Murray & Burt, 2001).

The connective tissue accounts for only a small percentage (2% to 5%) of the muscle in bony fish, smaller than for instance in beef muscle and that is one reason why fish is generally less tough to eat than meat (Dunajski, 1980).

There are two main types of locomotor muscle fibers in all fish. Those two types are red and white, specialized for either short bursts of maximum speed or low-speed cruising. White fibers are composed of fast-twitch glycolytic fibers and red fibers are composed of slow-twitch oxidative fibers (William & David, 1979). In some fishes, there are more than these two fiber types (Sänger & Stoiber, 2001). The axial muscle consists mainly of fast white fibres, covered by a thin layer of slow-red muscle fibers, with a layer of pink intermediate muscle fibres in between them (Kiessling et al., 1995; Martinez, Bang, Hatlen, & Blix, 1993).

Muscle fiber types are important for determining meat quality parameters such as the temporal change of freshness value, visual color of meat and color stability and drip loss (Ryu & Kim, 2005; Yada et al., 2000). Muscle fibers are the main structural unit and contributor of the physical, chemical, textural, and nutritional properties of fish muscle. The muscle fiber size, distribution, and number are often referred to as muscle cellularity, and are thought to be an important determinant of fish flesh quality and to influence the taste and processing characteristics of the flesh (Fauconneau et al., 1993; Hatae, Yoshimatsu, & Matsumoto, 1990; Hurling & Rodell, 2007; I. A. Johnston, 1999).

2.2.3 Muscle contraction and *rigor mortis*

Myofibrils contain two primary types of protein filaments, actin and myosin. Each myofibril is surrounded by the sarcoplasmic reticulum (SR). The SR contains very high concentrations of Ca^{2+} and when the sarcoplasmic reticulum receives a signal from the nervous system, it releases Ca^{2+} to the sarcoplasm resulting in the binding of the myosin head to the actin filament. Actin then slides in between the myosin filaments with the use of energy released from the dephosphorylation of ATP, and consequently the muscle contracts. When the nerve signal stops, the concentration of Ca^{2+} in the cytosol falls again, the myosin head is released from the actin filament and the contraction ends. In post-mortem muscle, the energy stores are depleted, ATP cannot be regenerated and the sarcoplasmic reticulum is no longer able to maintain a low level of Ca^{2+} in the cytosol. Since the ATP is necessary to remain in a relaxed state, this leads to irreversible cross bridges between actin and myosin. This results in shrinkage and stiffening of the muscle fibrils and swelling of the muscle is resisted by the links between myosin and actin in the rigor state (Mackie, 1993; Offer & Knight, 1988).

The time after death before onset of *rigor mortis* (rigor) is important in relation to the handling and processing (filleting, freezing) of the fish.

2.3 Water in fish muscle

As mentioned before, water is the main constituent of fish flesh, which usually accounts for about 80% of the weight of a fresh white fish muscle (Murray & Burt, 2001). The water is a solvent for salts, enzymes and other non-fat solids. The amount of water and its physical state in foods are important properties, influencing the quality, shelf life and processing yields (Schmidt, 2007). In other words, what determines the shelf life of a food is the availability of water for chemical, microbial or enzymatic activity, and this is measured by the water activity (a_w) of a food. Water activity is defined as 'the ratio of the vapor pressure of water in a food to the saturated vapor pressure of water at the same temperature'. It ranges between 0 and 1 and is a parameter that measures how available the water is in the fish flesh (Fellows, 2009; Hui, 2008). Water activity is connected to moisture content in a non-linear relationship known as a moisture sorption isotherm curve (Figure 5) (Roos, 1993).

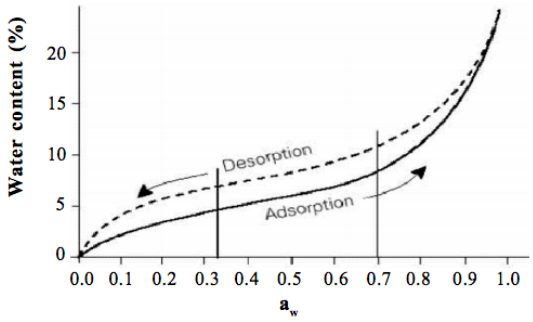

Figure 5. The relationship between food water content and water activity (Damodaran, Parkin, & Fennema, 2007).

By lowering water activity, food can be made safe to store. Every microorganism has a minimum, optimum and maximum water activity for growth. Molds and yeasts can grow at a low water activity. However, 0.85 is believed to be the safe cutoff level for pathogen growth (Fellows, 2009; Roos, 1993).

Water in food may be divided into three different groups according to the mobility and how tight the water molecules are bound to the structure. The term used are nonbound, loosely bound, and tightly bound waters (Hui, 2008). Nonbound water is easily removed or lost and is located between cells. Loosely bound water can take part in chemical reactions and is bound to the monolayer through hydrogen bonds. Loosely bound water is located inside the muscle cells and is maintained there with capillary force and part of this water can be removed. Tightly bound water is water that is not accessible for chemical reactions and is very difficult to remove from proteins and does not freeze at -40°C (at least not all). Tightly bound water is located inside the proteins and forms a strongly bound monolayer (Fellows, 2009). The tightly bound water in fresh fish muscle is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure. After extend chilled or frozen storage, however, the proteins are less able to retain all the water, and some of it, containing dissolved substances, is lost as drip. Frozen fish that are stored at too high a temperature,

will produce a large amount of drip and as a result the quality will suffer. In the living fish, the water content usually increases and the protein content decreases as spawning time approaches. It is possible to estimate the condition of the fish by measuring the water content of the muscle (Murray & Burt, 2001).

2.3.1 Water holding capacity (WHC) in fish muscle

The water holding capacity (WHC) can be defined as the ability of the muscle to hold its own and added water during the application of forces, pressing, gravity, centrifugation or heating. This property depends on the structure of the muscle proteins that bind and interact with the tightly bound water molecules and is therefore influenced by protein changes (Ramanzin, Bailoni, & Giovanni, 1994; Zayas, 1997). The water holding capacity (WHC) of fish has an important effect on its commercial value.

WHC is one of the functional properties that are used to characterize a fish as raw material for further processing (Fennema, 1990). It is an important factor when considering the juiciness and taste of the fish and for drip loss related to thawing. Reduced WHC leads to economic losses caused by reduced sales weight and complications during processing.

There are some factors that have an influence on the water holding capacity of muscle tissue and can be categorized as external or internal factors. External factors are, for example season and location of catching, feeding patterns and handling post slaughter. Internal factors are, for example size, age, muscle type and muscle tissue condition post mortem. Changes in chemical composition during processing are also an important factor that has an influence on WHC, especially in processes like salting. In fact, salting has a major impact on the water holding capacity. Increase in salt concentration up to a certain level leads to increase in water holding capacity (Hamm, 1960; Thorarinsdottir et al., 2004).

2.4 Fishing technique

There are two main fishing techniques used for catching cod, bottom trawling and longlining. Bottom trawling (Figure 6) is a technique that uses a cone shaped net consisting of a body, usually made from two, four or more panels, closed by one or two cod ends and with warps that leads to the fishing boat. A bottom trawl is kept open horizontally by two trawl boards that use the force of the water to spread out. On the top of the bottom trawl is usually a panel to prevent fish from escaping over the top of the net. Bottom trawls can be in a very wide range of depths, ranging from few meters to around 2.000 m (“Bottom Trawling,” n.d., “Fishing gear types. Bottom otter trawls. Technology Fact Sheets. In: FAO Fisheries and aquaculture department [online]. Rome,” 2001).

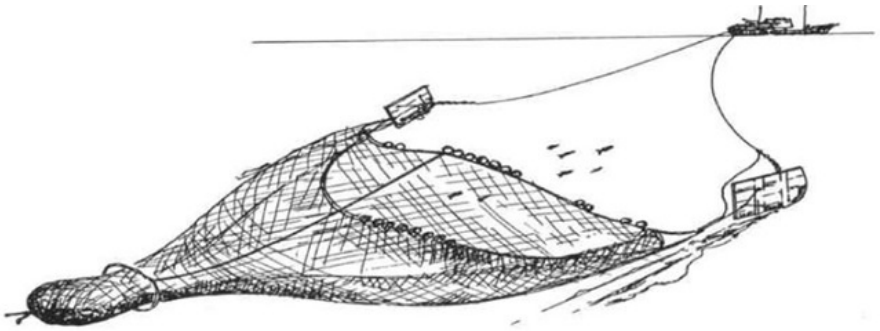

Figure 6. Drawing of a bottom otter trawl ("Seabird bycatch," n.d.)

Longlining is a technique that consists of a main line snoods with series of baited hooks at regular interval, hanging from the main line (Figure 7). The longline is set, in general, on or near the bottom. Its length can range from few hundred meters to more than 50 km. The fish are attracted by bait, hooked and held by the mouth until they are brought aboard the operating vessel ("Fishing gear types. Set longlines. Technology fact sheets. In: FAO Fisheries and aquaculture department [online]. Rome," 2001).

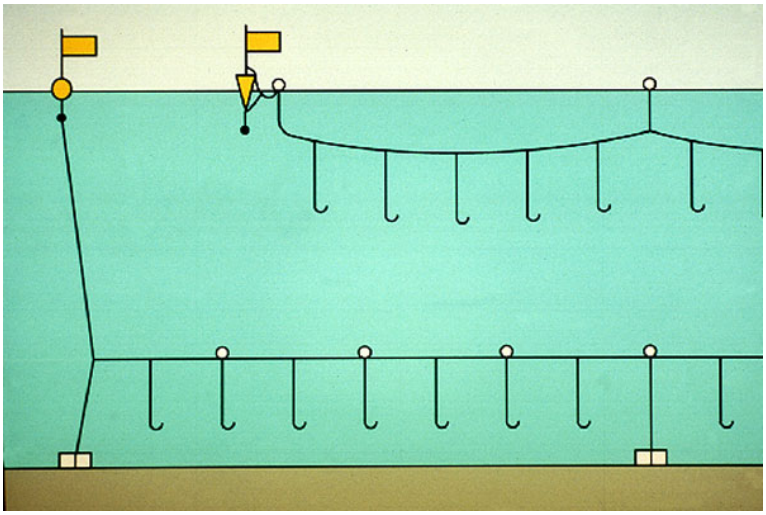

Figure 7. Drawing of longlines ("Fishing gear types. Set longlines. Technology fact sheets. In: FAO Fisheries and aquaculture department [online]. Rome," 2001).

Few studies have compared the quality of cod caught by trawl and longline. Botta and Bonnell (2006) reported that discoloration and final overall grades of cod caught by trawl were significantly lower than of cod caught by longline. However, the fillet odor and texture grades were not significantly different. Furthermore they also reported that cod caught by trawl had higher protein content, as well as lower moisture content than cod caught by longline. Rotabakk, Skipnes, Akse, and Birkeland (2011) also reported that longline caught cod had better overall sensory quality and increased lightness as well as water holding capacity compared to trawled cod. Trawling resulted in poor bleeding and bruises, while longline resulted in gaffing damages.

2.5 Bleeding

It has been shown that proper bleeding of fish directly after catching can improve flesh quality. In order to the make the fish fillet maintain a good appearance, the fish has to be bled. Proper bleeding prevents blood reaching the fish flesh. It is necessary to get the fish to bleed long enough in seawater (15-20 minutes) so that the blood is sufficiently drained, otherwise there is a risk of residual blood in fish muscle. Fish should be bled as soon as possible after catching. Bloodstains are regarded as defects, as the fillet should be white. Insufficiently bled fish becomes dark or reddish in the muscle and, therefore, will be of lower quality. Traditional bleeding is to cut the throat veins and/or arteries leading from the heart to the gills in such a way that the fish is exsanguinated (Adalbjornsson & Vidarsson, 2010; Thordarson, Hognason, & Gestsson, 2012).

2.6 Salting

Salting of food and particularly salting of fish is one of the oldest treatments in food preservation still in use nowadays. The aim of salting was to get a shelf stable product. By lowering the water activity it was possible to store food for several months. Nowadays the goal is to promote important sensory changes that remain during cooking (Andrés, Rodríguez-Barona, Barat, & Fito, 2005).

Salting is a process in which mass transfer, basically water and salt, between cod and its surroundings occurs by diffusion, i.e. the fish muscle absorbs salt and loses water (Andrés & Rodríguez-Barona, 2002; Martínez-Alvarez & Gómez-Guillén, 2006; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2001).

Salting of foods leads to increased diffusion of water and swelling of the muscle fibers (myofibrils), thus changing the water distribution in the tissue and muscle properties, such as the WHC, which increases with an increasing salt concentration (Bocker, Kohler, Aursand, & Ofstad, 2008; Fennema, 1990; Offer & Trinick, 1983). Salting is, therefore, a good method to decrease water drip during storage and so is often used prior to freezing in order to counteract the negative effects of freezing. The properties of the muscle are, however, highly dependent on the salting procedure and the amount of salt used in the salting process (Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2004).

Many factors, including quality and condition of the raw material, the type, concentration and quality of salt, as well as the method used for salting, are believed to influence the quality and characteristics of the final product (Andrés et al., 2005; Pedro et al., 2002; Thorarinsdottir et al., 2001; Thorarinsdottir et al., 2004). Studies have shown that the salt uptake depends on many factors including muscle type, chemical composition, fish size, physical state of the muscle, fillet thickness, physiological state, salting method, brine concentration, duration of salting process, and fish-to-salt ratio, ambient temperature, freezing and thawing (Barat & Rodríguez-Barona, 2006; Wang, Tang, & Correia, 2000).

Production of salted fish is a simple process where the raw fish is filleted or ‘butterfly’ split and then heavily salted. Regular salted cod (heavy salted cod) contains 55-58% water and 18-21% salt, compared with approximately 80% water and 0,3% salt in the raw material. Prior to consumption of the fish, it is soaked in water, usually for 1-5 days, which results in an uptake of water and desalting (Bogason, 1987; Van Klaveren & Legendre, 1965).

Studies have shown that the processing yield of fish that is salted before rigor is worse than when the fish is salted after rigor. Apparently more water and soluble proteins are lost when fish goes through rigor state in the brining process. Therefore, it is not desirable to salt fish before rigor (Matís ohf, n.d.).

2.6.1 Lightly salted

A new product, lightly salted cod fillets entered the market about 15-20 years ago, with only ≈ 2% salt content. This new product has been growing in popularity, especially in Spain (Gudjonsdottir et al., 2010). The reason for this growing popularity may be because the rehydration of the heavy salted fish is time-consuming, something that does not suit the modern lifestyle in Spain. The lightly salted fish does not need rehydration, and is therefore much more suitable for immediate consumption or short preparation times. Lightly salted products also allow for more focus on health, as too much salt has been considered unhealthy. The lightly salted fillets consist of 2 days of processing, compared to the traditional process, which consists of 24 days of processing. Therefore, the modern product is sold for less than half the price of the traditional product and the buyer can get a cheaper product.

The characteristics and process of the lightly salted products vary greatly from the traditional bacalao (heavily salted cod). The lightly salted products are only injected and/or brined and then stored as frozen. Therefore, the characteristic texture and flavor, which are formed as fats and proteins are degraded during the traditional curing of heavy salted cod, are missing. The fish gets whiter and production characteristics are milder (Lindkvist et al., 2008).

The brine is made up of water, salt, phosphates and sometimes other functional ingredients and the brining time is often 24-48 hours (Thorkelsson et al., 2008; Xiong, 2005).

2.7 Freezing and frozen storage

Freezing is recognized as one of the best methods for the long-term preservation of foods, but it must be emphasized that it does not improve product quality (Fennema, 1990). In most cases all changes slow down with a decrease in temperature, but the activity of some enzymes is very high in a certain temperature range of freezing due to increased concentration of dissolved substances (Bilinski, Jonas, & Peters, 1981). As an example, the enzyme which causes degradation of trimethylamine N-oxide (TMAO) is very active down to freezing temperature of salt (NaCl) which is -21,6°C. The concentration of salt in solution increases with decreasing temperature and counteracts to some extent a decline in activity due to a decrease in temperature. The best storage temperature for fish should be targeted at -25°C as a maximum temperature to minimize enzyme activity (Arason & Asgeirsson, 1984). In addition to a right storage temperature, it is also important to minimize any temperature fluctuations because it can affect the formation of free fatty acids and fat oxidation (Bilinski et al., 1981).

Removal of heat is required of freezing, and fish from which heat is removed, drops in temperature like shown in Figure 8. The temperature falls rather rapidly to just below 0°C, during the first stage of cooling, (down to freezing point of water). During the second stage, more heat requires to be removed. In order to turn the major part of the water to ice, the temperature changes by a couple degrees and this stage is known as the period of “thermal arrest”. Throughout the third stage most of the remaining

water freezes because the temperature again begins to drop rapidly, when about 55% of the water has been turned to ice. During this third state, a relatively small amount of heat has to be removed (Johnston et al., 1994).

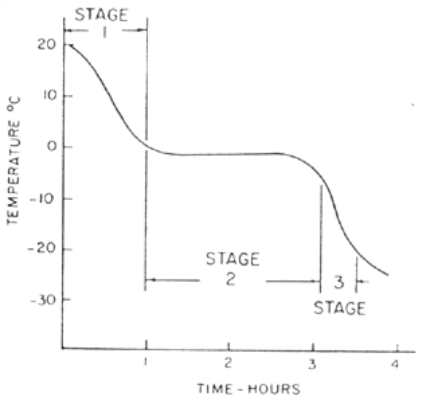

Figure 8. Temperature-time graph for fish during freezing (W. A. Johnston et al., 1994).

When water in fish freezes out as pure ice crystals, the remaining unfrozen water contains an increasing concentration of salts and other compounds which are normally present in fish flesh. The effect of this increasing concentration in the remaining unfrozen water is that unlike pure water, the entire change to ice is not achieved at a stable temperature of 0°C, but proceeds over a range of temperature. By the time the fish temperature is reduced to -5°C about 70% of the water is frozen. At a low temperatures, such as -30°C, a part of the water in the fish muscle still remains in the unfrozen state (Johnston et al., 1994).

It has been reported that ice crystals are formed between the muscle cells and within them during freezing (Howgate, 1979). Quickly frozen fish results in a formation of small intra-cellular ice crystals while larger intra-cellular ice crystals are formed, during slow freezing. Formation of large ice crystals during freezing has been shown to result in texture degradation and organoleptic quality losses. The rate of freezing and the formation of small ice crystals in freezing are critical to minimize tissue damage and drip loss in thawing. When ice crystals form in cells, the cytoplasm is distorted by the expansion that accompanies freezing. Biological structures are largely excluded from the ice and are moved along with the advancing liquid-solid interface. The most successful approaches to avoid such damage is simply to freeze samples quickly. If the temperature drops rapidly enough, there is not enough time for water molecules to diffuse onto a growing crystal surface before they have lost so much kinetic energy that they no longer move appreciably (Li & Sun, 2002; McIntosh, Nicastro, & Mastronarde, 2005; Zhu, Ramaswamy, & Le Bail, 2005).

Microbial growth is stopped during freezing. Physical, chemical and biochemical processes leading to irreversible changes will still occur, but at a very slow rate. Changes taking place in the lipids of the frozen fish will also occur at a very slow rate. Lipid oxidation occurs in lean as well as fatty species and can cause quality deterioration such as yellow/brownish discoloration of the flesh surface. Oxidation of the phospholipids in lean species results in cold-store flavor and oxidation of triglycerides results in a rancid taste and odor (Mercedes Careche et al., 1998; Careche et al., 1999; Chichester &

Stewart, 1981; Kim & Hearnsberger, 1995; Lauritzsen et al., 1999; Sikorski et al., 1976). Whole lipids, free fatty acids (FFA) and oxidized lipids can interact with proteins, in some cases resulting in quality deterioration of especially lean species (Haard, 1992; Mackie, 1993; Shenouda, 1980).

Due to hydrolysis, FFA accumulates in the tissue during frozen storage (Aubourg, 1999; Aubourg, Piñeiro, & González, 2004; Rodríguez et al., 2007). Fluctuating storage temperatures may result in the lysis of lysosomes and thereby increased activity of some endogenous lipases resulting in increased rates of FFA accumulation (Geromel & Montgomery, 1980). Accumulation of FFA have been shown to interrelate with lipid oxidation and have been proposed to have a pro-oxidant effect on lipids but does not in itself affect quality attributes of the product (Aubourg & Medina, 1997; Han & Liston, 1987; Miyashita & Takagi, 1986; Rodríguez et al., 2007; Yoshida, Kondo, & Kajimoto, 1992).

During freezing and frozen storage, some transfer of moisture from the fish product is unpreventable, which leads to dehydration of the fish. It has been shown that fluctuating temperature are a major cause of dehydration. In practice, the more severe cases of drying occur during frozen storage rather than during freezing. In extreme dehydration, the frozen fish obtain a dry wrinkled look, the flesh become spongy and the flesh tends to become pale or white in color. This characteristic appearance is called, inappropriately, 'freezer burn'. From an economic point of view, the weight loss is serious and dehydration will speed up the other important changes, like oxidation as well as protein denaturation. Frozen fish may dry slowly in cold storage even under good operating conditions. This is undesirable, not only because the product will lose weight, but also because drying accelerates oxidation of the fat and denaturation of the protein in the fish. Even completely waterproofed wrappers used to protect the fish product do not give full protection if the cold store operation condition are favorable for desiccation within the pack (Johnston et al., 1994).

2.7.1 Glazing

Glazing is the term used to describe the application of layer of ice to the surface of a frozen product to protect the product from the effects of oxidation and dehydration during cold storage. The glazing is carried out after freezing by dipping in cold water, spraying or brushing chilled water onto the surface of the fish. The ice layer sublimes rather than the fish below and protects the surface of the fish from any contact with air and thereby slows down the rate of oxidation. Glaze, however, evaporates over time, and the fish itself begins to dry. The fish may require to be recooled in a freezer before being transferred to the cold store, because of the heat added by the glazing process (Seafish, the authority in seafood, 2008; Whittle & Howgate, 2000).

2.8 Polyphosphate

In some countries chemicals are used to treat fresh fish in order to assist with things like color retention, preservation, or even addition, of fluids (Johnston et al., 1994). Legally permitted additives, like polyphosphates, are widely used to improve eating quality of many foods, particularly fish and meat products. Most countries do have limits on the amount that can be added during processing and exporters must bear this in mind. It has been reported that phosphates are not considered to be toxic. However, when used improperly, too much moisture absorption can be characterized as consumer

fraud. This subject is under regular review both in the European Economic Community and in the United Kingdom and it is possible that restrictions will be submitted in the future.

Different properties are received from different types of phosphates. The main function of polyphosphates lies in improving the retention of water by the protein in fish but their effect is mainly on the surface of the fish through an immersion treatment. Longer immersion time leads to unjustified increase in weight and the risk of off flavor or worsening texture. Polyphosphate treatment of fish before freezing often reduces the amount of 'thaw drip'. Whilst this isn't necessarily a problem in good quality frozen fish, poor quality fish, when thawed, will drip more, therefore application of polyphosphates in poor quality fish will finally mask the original quality. In chilled fillets the addition of polyphosphates in high quality fillet will reduce the amount of 'drip loss' during processing and distribution but can also improve the dull appearance of poor quality fish, thus similarly masking poor quality (Aitken, 2001).

The use of phosphate is one of the explanation for how Iceland took over the Spanish market of salted fish. According to Bjørkevoll et al. (2012) Iceland has enforced the prohibition against the use of phosphate in lightly and fully salted fish differently from other European countries. Knowledge of the use of phosphates in the production of salted fish is mainly based on trials carried out by producers and only in few cases documented in controlled, scientific trials.

2.9 Color

In the food industry, the visual appearance is a very important property. For white fishes, a delicate white appearance is preferred, and even if it is only slightly darkened or colored it may be rejected. It has been stated that a distinguish change in flesh color can be caused by an altered reflection due to the change in surface properties with altered fibre area. Studies have so far not been able to confirm this hypothesis (Love, 2001b).

2.10 Study objectives

The main objectives of this project were to investigate the stability of lightly salted fillets during frozen storage. The aim was to explore what processing method would be best to increase yield of the fillets and thereby increase quality and value. The main variables evaluated were glazing content, drip loss, water holding capacity, cooking yield, color and chemical analysis

3 Materials and methods

3.1 Experimental design

A total of 180 fillets of cod were used in this study. Most of the fillets were lightly salted. All the samples were received from two processors. Fish processor A collected the samples in July and used a long line fishing technique and fish processor B collected the samples in October and used a bottom trawling fishing technique.

To test the effect of different bleeding methods on product quality after frozen storage, two different bleeding methods were used, traditional bleeding and insufficient bleeding. The effect of different fillet sizes on product quality after frozen storage was also tested. The sizes were divided to small and large fillets after their weights, small fillets ranged from 500 to 1000 grams and the large fillets weighed over 1500 grams. Another parameter tested on product quality after frozen storage was the age of the fillets until they were processed. In samples collected in July the age of the fillets until they were processed were 1 day and 4 days and in samples collected in October the fillets were 3 days and 6 days post catch.

The lightly salted products were brine injected, brined for 48 hours, individual quick frozen (IQF) and then glazed. After glazing, the fish was packed in cardboard boxes (Figure 9) before refrigeration. The total weight of each packaging was about 11 kg. Each group of fillet was stored at different temperatures of -18°C and -25°C. Five fillets of each group were analyzed for physical and chemical properties (Table 1 and Figures 10 and 11) after frozen storage for 1 week, 3 months and 6 months of frozen storage. Thawing of the fillets was carried out at +4°C for approximately 48 h. Each fillet was identified with a numbered plastic tag and weighed before and after thawing. Before chemical analysis, the fillets (without the loin part) were skinned by hand and minced in a mixer (Braun Electronic, type 4262, Kronberg, Germany).

Figure 9. Packaging of the cod in cardboard boxes before refrigeration.

Table 1. Experimental design and sampling.

Sample collection	Weight of fillets (grams)	Slaughter	Age of the raw material	Storage temp. (°C)	Sampling	CODE
Summer	500-1000	Traditional bleeding	1 day	--	At the beginning	
	500-1000	Traditional bleeding	1 day	-18	After 3 and 6 months	A
	500-1000	Traditional bleeding	1 day	-25	After 3 and 6 months	
	500-1000	Traditional bleeding	4 days	--	At the beginning	
	500-1000	Traditional bleeding	4 days	-18	After 3 and 6 months	B
	500-1000	Traditional bleeding	4 days	-25	After 3 and 6 months	
	500-1000	Insufficient bleeding	1 day	--	At the beginning	
	500-1000	Insufficient bleeding	1 day	-18	After 3 and 6 months	C
	500-1000	Insufficient bleeding	1 day	-25	After 3 and 6 months	
	500-1000	Insufficient bleeding	4 days	--	At the beginning	
	500-1000	Insufficient bleeding	4 days	-18	After 3 and 6 months	D
	500-1000	Insufficient bleeding	4 days	-25	After 3 and 6 months	
	500-1000	Traditional bleeding	4 days	--	At the beginning	
	500-1000	Traditional bleeding	4 days	-18	After 3 and 6 months	E*
	500-1000	Traditional bleeding	4 days	-25	After 3 and 6 months	
Autumn	500-1000	Traditional bleeding	3 days	--	At the beginning	
	500-1000	Traditional bleeding	3 days	-18	After 3 months	F
	500-1000	Traditional bleeding	3 days	-25	After 3 months	
	500-1000	Traditional bleeding	6 days	--	At the beginning	
	500-1000	Traditional bleeding	6 days	-18	After 3 months	G
	500-1000	Traditional bleeding	6 days	-25	After 3 months	
	> 1500	Traditional bleeding	3 days	--	At the beginning	
	> 1500	Traditional bleeding	3 days	-18	After 3 months	H
	> 1500	Traditional bleeding	3 days	-25	After 3 months	
	500-1000	Traditional bleeding	3 days	--	At the beginning	
	500-1000	Traditional bleeding	3 days	-18	After 3 months	I*
	500-1000	Traditional bleeding	3 days	-25	After 3 months	
	> 1500	Traditional bleeding	3 days	--	At the beginning	
	> 1500	Traditional bleeding	3 days	-18	After 3 months	J*
	> 1500	Traditional bleeding	3 days	-25	After 3 months	

*Untreated control fillets

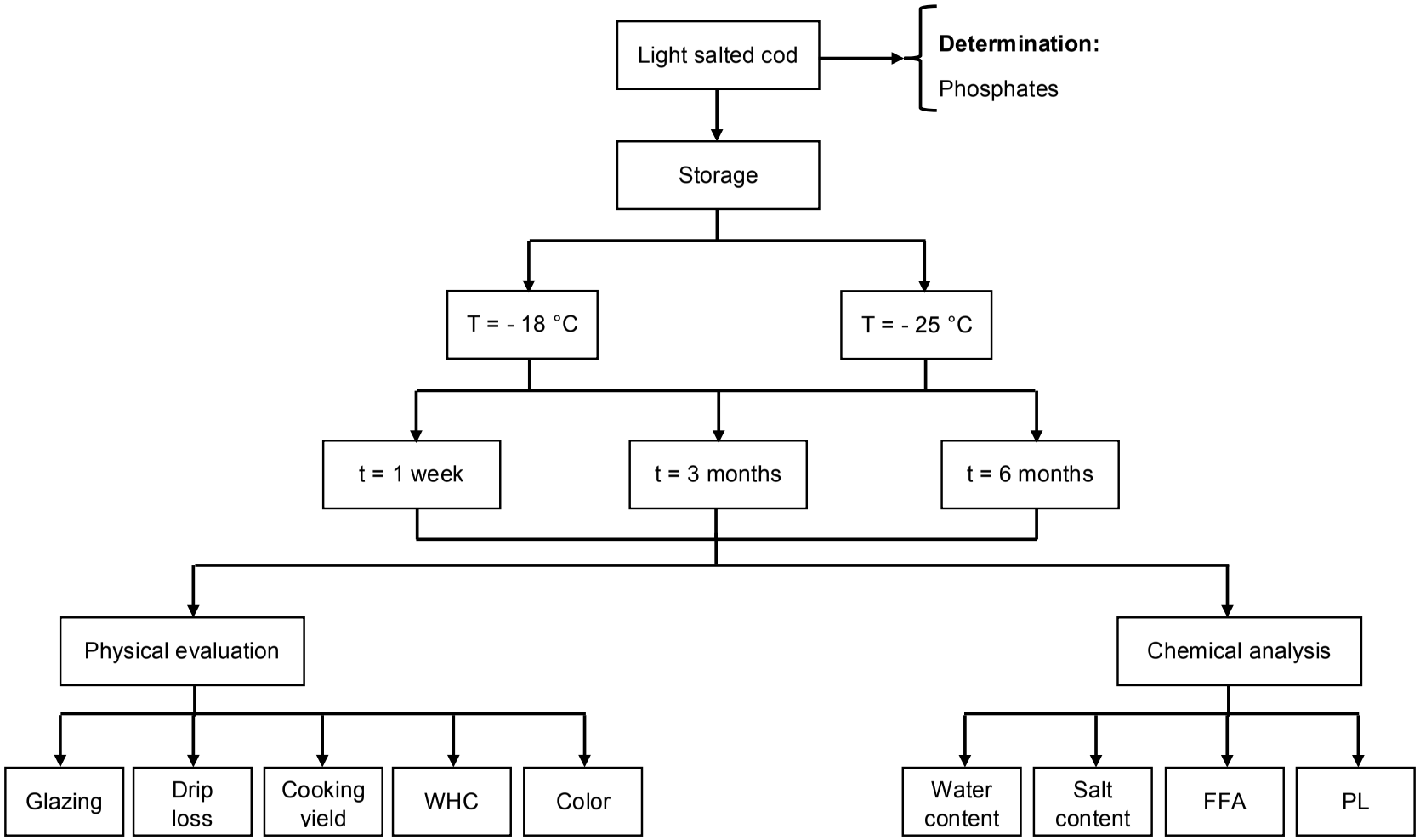

Figure 10. The flowchart of the experiments storage of lightly salted cod.

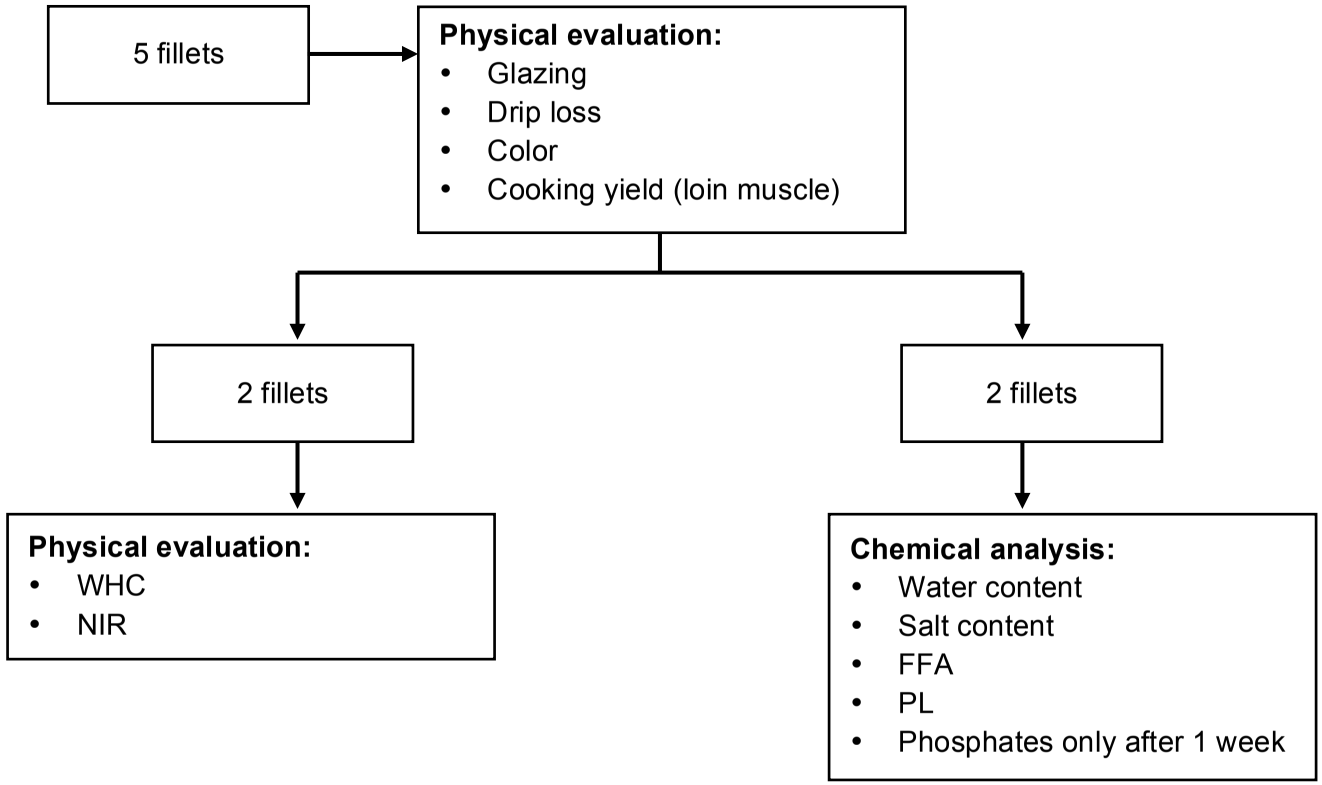

Figure 11. The flowchart of total fillets used from each group for physical evaluation and chemical analysis.

3.2 Temperature measurements

Temperature mappings were performed with the help of Micro-T DS1922L temperature loggers from NexSens Technology (Dayton, OH, USA, see Figure 12). This logger has an accuracy of ±0.5 °C and a resolution of 0.0625 °C and an operating range of -40 to 85 °C. The diameter is 17 mm and the thickness is 5 mm.

Figure 12. Micro-T DS1922L temperature loggers used in the study.

The temperature was recorded with 10 min intervals, by data loggers placed at three to four different positions inside the fish tubs, during brining of fillets collected in October. Two to three loggers were placed at the corners of every fish tub in a latex glove and tied with a nylon fishing line at the top of the tub in different heights. One logger was also placed in a latex glove and tied with a nylon fishing line to a float and placed in the middle of the tub. The temperature was also recorded with 10 min intervals, by data loggers placed inside three fillets in each fish tub. The red circle in Figure 13 shows the position of the temperature records inside the fillets.

Figure 13. Position of temperature records inside fillets.

3.3 Physical analysis

3.3.1 Glazing content

The Codex standard was used for instruction on how to evaluate the amount of glazing content on the samples (*Codex Alimentarius*, 2001). Frozen fillets were weighed and placed under a gentle spray of cold water (Figure 14). The fillets were agitated carefully so that the product would not be broken. Fillets were sprayed until all ice glaze that could be seen or felt was removed. Adhering water was removed by a paper towel and weighed.

$$\textbf{Glazing content}\ (\%) = \frac{\text{g frozen fillets} - \text{g fillets after gentle spray of cold water}}{\text{g frozen fillets}} \times 100$$

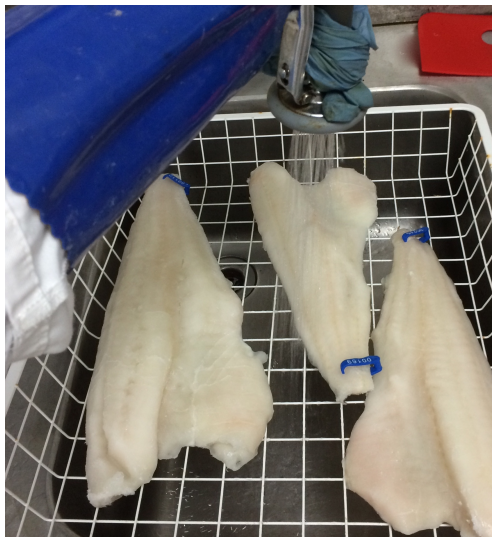

Figure 14. Frozen fillets under a gentle spray of cold water to remove the glazing.

3.3.2 Drip loss

Drip is the liquid separated from the frozen fish or meat after being thawed because the protein network, cell membranes and so forth are affected or to some degree disrupted by the formation of ice crystals, thereby altering the WHC. The drip measured in this study was “Free drip” which is separable naturally. The larger the amount of those separated liquid becomes, the lower the commercial value of the frozen goods because drips contain a large amount of various nutritive chemical components. (Akiba, 1961).

Frozen fillets were weighed before and after thawing. Frozen fillets were placed on plastic racks as seen in Figure 15, with plastic film on the top to prevent drying of the fillets. The samples were thawed at +4°C for approximately 48 hours.

Figure 15. Frozen fillets on plastic racks during thawing.

Drip loss was measured as the weight loss during the thawing time after the glazing had been removed. The mass of the drip was divided by the initial mass of the product.

$$\textbf{Drip loss}\ (\%) = \frac{\text{g frozen fillets without glazing} - \text{g thawed fillets}}{\text{g frozen fillets}} \times 100$$

3.3.3 Cooking yield

Cooking yield (CY) is defined as the amount of liquid lost during cooking. The loin part of the fillet was cut from each fillet for evaluation of cooking yield. Each loin muscle was weighed and heated in a steaming oven at approximately 95°C for 8 minutes (Convotherm, Elektrogeräte CmbH, Eglfing, Germany). After the cooking period, the samples were drained for 15 minutes at room temperature (25°C) before weighing for cooking yield determination. The cooking yield (CY) was calculated as the weight of the cooked sample divided by the weight of the sample before cooking.

$$\textbf{Cooking yield}\ (\%) = \frac{\text{g cooked sample}}{\text{g raw sample}} \times 100$$

3.3.4 Total yield

Evaluation of total yield of the fillets was determined by multiplying the cooking yield and drip loss.

$$\textbf{Total yield}\ (\%) = \frac{\text{g cooked sample}}{\text{g raw sample}} \times \frac{\text{g thawed fillets}}{\text{g frozen fillets}} \times 100$$

3.3.5 Color

The color of the surface of raw cod fillets was measured by a color machine vision system (CMVS), consisting of a light box and a CCD color camera connected to a computer with a firewire connection. Fish fillets were placed in the light box and the digital camera captured a picture of the samples for each analysis time point. Images were captured on the CMVS software program LensEye (Engineering & Cyber Solutions, Gainsville, FL, USA), and color results obtained based on the CIE L*a*b system. L describing lightness (L = 0 for black, L = 100 for white), a describing intensity in red (a*>0), b describing intensity in yellow (b*>0) (Figure 16) (Hutchings, 1999).

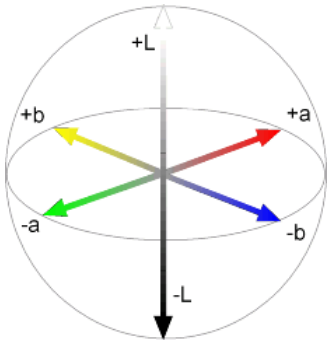

Figure 16. Description of color measurement using the CIE L*a*b system (Hutchings, 1999).

The CMVS was calibrated using ColorChecker® (X-Rite, Grand Rapids, MI, USA), an array of 24 scientifically designed colored plates. Average L* (lightness), a* (redness), b* (yellowness) values of the light and dark muscle surfaces were calculated using the LensEye software. In addition, whiteness of the samples was calculated as described by Park (1994) as follows:

$$\text{Whiteness (\%)} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

3.3.6 Water holding capacity (WHC)

Water holding capacity (WHC) was determined by a centrifugation method (Eide, Børreses, & Strøm, 1982). The sample glasses were made from plexi-glass and their dimensions were: height 62 mm, inner diameter 19 mm and outer diameter 25 mm. The sample glasses were put into holsters for certain size of rotor (Heraeus #3335) for appropriate centrifuge (Biofuge Stratas, Thermo electron corporation, Germany). Glass balls were put in the bottom of the holsters. The sample glass was weighed empty and then approximately 2 g of the minced sample was weighed accurately and immediately centrifuged at 210 x g for 5 min, with temperature maintained at 4°C. After centrifugation, the sample glass was weighed again with the sample in it. The weight loss after centrifugation was divided by the water content of the fillet and expressed as %WHC.

$$\textbf{WHC}\ (\%) = \frac{(\%\text{water} \times \text{g sample}) - (\text{g weight loss})}{\%\text{water} \times \text{g sample}} \times 100$$

Where the weight loss is defined as:

$$\textbf{Weight loss}\ (\%) = \frac{\text{g weight loss in centrifuge}}{\text{g original sample weight}} \times 100$$

3.4 Chemical analysis

3.4.1 Lipid extraction

The lipid extraction of the samples was based on a method by Bligh and Dyer (1959) with adaption. Twenty five g of grounded sample was weighed out into a 250 mL centrifuge bottle. 25 mL of chloroform and 50 mL of methanol was added to the centrifuge bottle with the sample and homogenized for 2 min. Another 25 mL of chloroform was added to the mixture and mixed for 1 min. 25 mL of 0,88% KCl was then added to the mixture and mixed for 1 min and centrifuged at 2500 rpm for 20 min at 4°C. After centrifugation, the lower chloroform phase containing the fat was absorbed with glass pipettes and filtrated on a glass microfiber under suction. The suction flask content was then poured into a 50 mL volumetric flask. Every trace of the upper phase was removed and the 50 mL volumetric flask filled with chloroform to the mark. The content (chloroform phase) was then

poured into a 50 mL test tube and stored in freezer for later analysis because the chloroform phase was used further for determination of total lipid content, phospholipid content and free fatty acid content.

Duplicate was made of each sample by adding 3 mL of chloroform from the lipid extraction in 2 screw cap glass tube and any solvent present was removed at 55°C using a nitrogen jet. The weight of the glass tube was recorded before adding the chloroform and after all of the solvent had been removed. The difference in weight of the glass tube before adding the chloroform and after all of the solvent had been removed was the determination of the fat contend.

Total fat content (%) = Weight of the empty glass tube –
weight of the glass tube after all of the solvent had been removed

3.4.2 Free fatty acid content

The method used for determination of free fatty acids (FFAs) content was the method from Lowry and Tinsley (1976) with modification made by Bernárdez, Pastoriza, Sampedro, Herrera and Cabo (2005). The duplicate sample in the 2 screw cap glass tube with the removed solvent from the lipid extraction (Bligh & Dyer, 1959) was used and 3 mL of cyclohexane and 1 mL of cupric acetate-pyridine reagent was added. The mixture were vortexed for 40 seconds and centrifuged at 2000 rpm for 10 min at 4°C. The upper layer was read at 710 nm. Quantification was based on a calibration curve constructed from oleic acid standards.

$$\textbf{Free fatty acid content}\ (\%) = \frac{\text{Oleic acid x } 282{,}46 \text{ x } 1*10\text{-}6}{\text{g lipid in the sample}} \times 100$$

Where oleic acid is the oleic acid amount in µmol; 282,46 is the molecular weight of oleic acid.

3.4.3 Phospholipids content

The method used for estimation of phospholipids content was the colorimetric method, based on the formation of a complex between phospholipids and ammonium ferrothiocyantate (Stewart, 1980). Duplicate was made of each sample. 2 mL of chloroform was added to 15 mL plastic tube with a screw cap. 10 µL of chloroform from the lipid extraction was added to the 2 mL of chloroform in the plastic tube and 1 mL of thiocyanate reagent. The mixture was vortexed for 1 min and centrifuged at 2000 rpm for 5 min at 4°C. The lower layer was read at 480 nm and compared with known amounts of a standard phospholipid solution.

3.4.4 Water content

The water content was determined by accurately weighing out 5 g of minced sample in a ceramic bowl with sand. The sample was then mixed with the sand and dried in an oven at 101-105 °C for 4 hours. The water content was based on weight differences before and after drying (ISO 6496. 1999, 2007).

3.4.5 Salt content

The salt content was determined by the method of Volhard according to AOAC 937.18 (2000). Approximately 5 g of minced sample was weighed into 250 mL plastic bottles and then 200 mL of distilled water was added. The bottles were then shaken for 45 minutes in an electric shaker. After sedimentation, 20 mL of the solution were pipetted into a 100 mL beaker along with 20 mL of HNO_3 solution. The solution was then titrated with 0.1 N $AgNO_3$ in a 716 DMS Titrino device.

3.4.6 Phosphates content

The phosphorus was determined by colorimetry as phosphate vanadomylbdate at 420 nm (AOAC, 1990). Accurately 5 g of minced sample was weighed into a ceramic bowl. The sample was then mixed with 1 g of CaO and dried in an oven at 100 °C for approximately 1 hour. The samples was then annealed on a heat plate in an oven at 500 °C until the entire sample had turned to ash. The ash was poured into 250 ml beaker with 10 ml of water, 12 ml HCl and 5 ml of HNO_3 and boiled for 10 minutes. When the solution has cooled down, it is poured into 250 ml flask and filled up to the limit with water. The solution is then filtered and the first 10 – 20 ml are not used. 10 ml of the filtered solution is pipetted into 100 ml flask with 25 ml of vanadate/molybdat solution and fillet up to the limit with water. The solution is than allowed to stand for 10 minutes before color measurements.

3.5 Statistical analysis

Microsoft Excel 2011 was used to calculate the means and standard deviations (SD) as well as percentages for all multiple measurements and to generate graphs. Statistical analysis were performed in StatPlus, build for Microsoft Excel for mac OS. Analysis of variance (1-way ANOVA) was used to test whether groups were significantly different with regards to temperature and storage time. Significance of differences was defined at the 5% level ($p<0.05$). When referring to the results of the 1-way ANOVA in relation to tables and figures, the values from the 1-way ANOVA are not shown.

Pearson's correlation analysis was performed to observe correlation between measured variables. The correlation between two variables reflects the degree to which the variables are related.

4 Results

4.1 Temperature profiles of raw fillets collected during processing

The temperature profiles of raw fillets were only collected in October. The loggers were placed in each corner of the fish tub and also in the middle. Unfortunately, the position of each logger was not written down.

4.1.1 Temperature profiles inside fish tubs during brining

The temperature of the brine in each fish tub that was stacked up was rather stable. Figure 17 shows that there was a small difference in temperature inside each fish tub.

The brine temperature in the small fillets, 3 days fish tub varied from 1.1°C - 3.9°C and 6 days, small fillets fish tub varied from 0°C – 1.9°C. The brine temperature in the large fillets, 3 days fish tub varied from -0.9°C – 5.4°C.

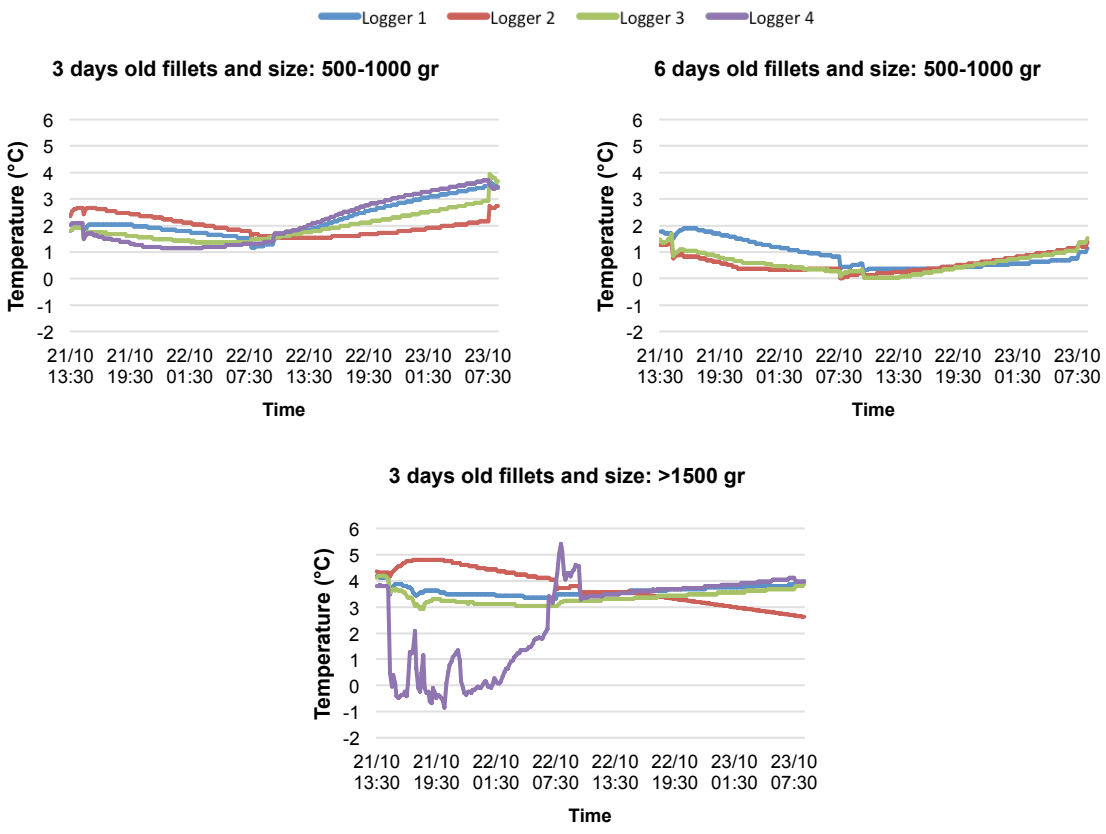

Figure 17. Temperature profiles during brining in 3 large fish tubs, where each sample group was all inside one tub. The fish tubs were stacked up and the small, 3 days fillet tub was at the top. The tub with the small, 6 days fillets tub was in the middle and finally the large, 3 days fillets tub was at the bottom.

4.1.2 Temperature profiles inside raw fillets during brining

The temperature inside the fillets in each tub that were stacked up was quite stable (Figure 18).

The temperature inside the small, 3 days fillets varied from 1.3°C – 3.9 °C and inside the small, 6 days fillets the temperature varied from 0.3°C – 3.1°C. The temperature inside the large, 3 days fillets varied from 3.2°C – 5.8°C.

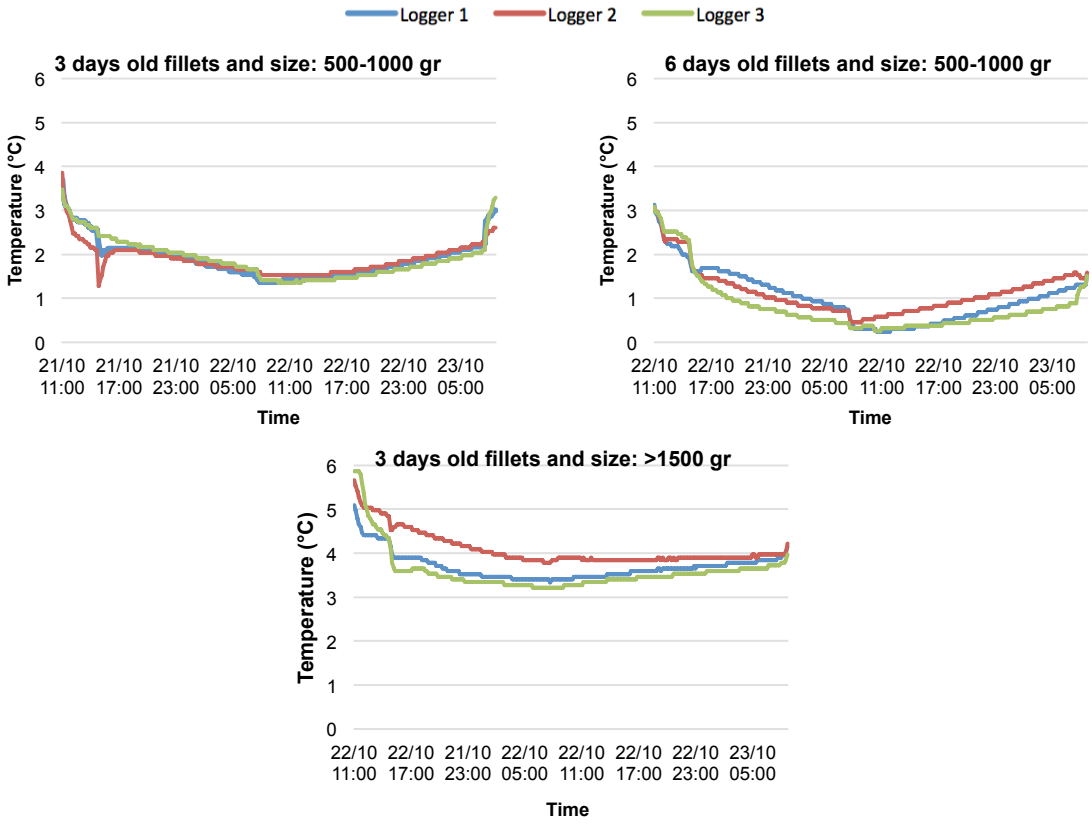

Figure 18. Temperature profiles inside raw fillets during brining in 3 large fish tubs, where each sample group was all inside one tub. The fish tubs were stacked up and the small, 3 days fillet tub was at the top. The tub with the small, 6 days fillets tub was in the middle and finally the large, 3 days fillets tub was at the bottom.

4.1.3 Average temperature profiles inside fish tubs and raw fillets during brining

Figure 19 shows the difference in average temperature between the fish tubs that were stacked up, both inside the fillets and the tubs. There was a small fluctuation in the temperature while brining. The average temperature in the fish tub with the small, 3 days fillets was 2.1°C and 1.9°C inside the fillets. In the fish tub with the small, 6 days fillets the average temperature was 0.7°C and 1°C inside the fillets. Finally in the fish tub with the large, 3 days fillets the average temperature was 3.3°C and 3.8°C inside the fillets.

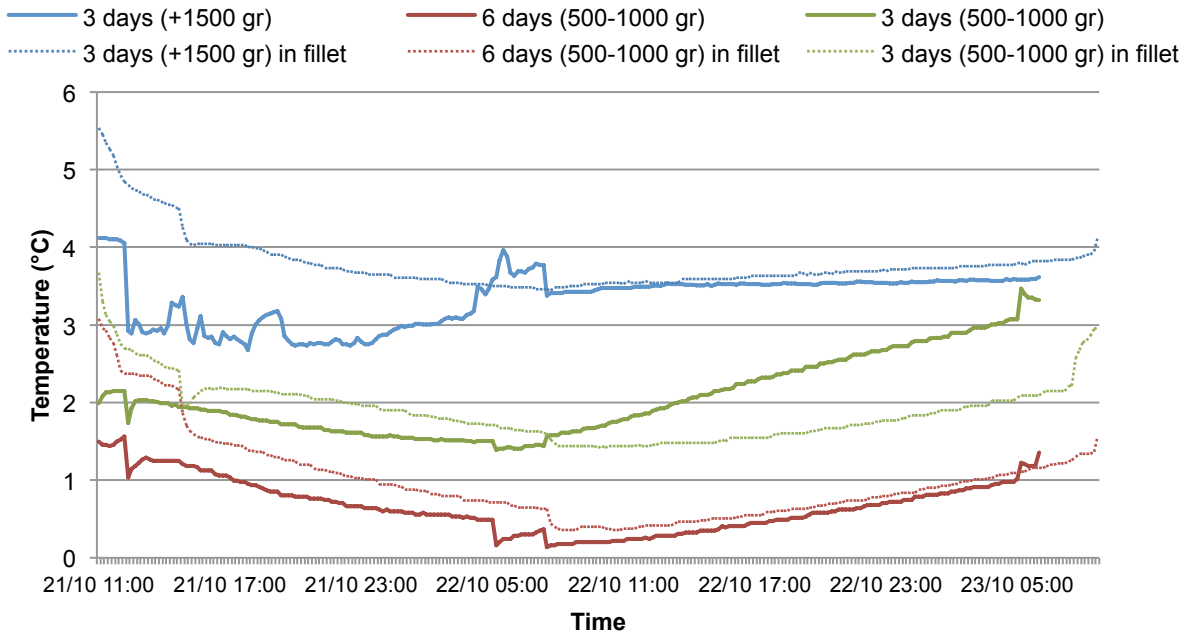

Figure 19. Average temperature profiles uring brining, both inside raw fillets (dotted line) and inside the fish tubs (solid line). The brining was conducted in 3 large fish tubs, where each sample group was all inside one tub. The fish tubs were stacked up and the small, 3 days fillet tub was at the top. The tub with the small, 6 days fillets tub was in the middle and finally the large, 3 days fillets tub was at the bottom.

4.2 Physical changes

4.2.1 Glazing content

Glazing content of the lightly salted cod fillets collected in July is shown in Figure 20. The glazing content in the sample collected in July ranged from 1% to 11% and the median glazing content was 4%.

Glazing content of most of the fillets increased or decreased during frozen storage. All of the groups, except the control group and the 1 day, traditional bled fillet group collected in July showed a difference in glazing content within the group during the storage time ($p < 0.05$).

The difference between control fillets and lightly salted fillets collected in July was significant ($p > 0.05$) in two groups. Those groups were 4 days fillets, both traditionally bled and insufficient bled. The control group had more glazing content than traditionally bled, 4 days fillets but lower glazing content than insufficient bled, 4 days fillets.

Comparison between traditional bled fillets and insufficiently bled fillets collected in July, showed that the traditional bled fillets had lower glazing content than the insufficient bled fillets ($p < 0.05$). Four days fillets were also significant different ($p < 0.05$) from 1 day fillets, where 4 days fillets had overall a higher glazing content.

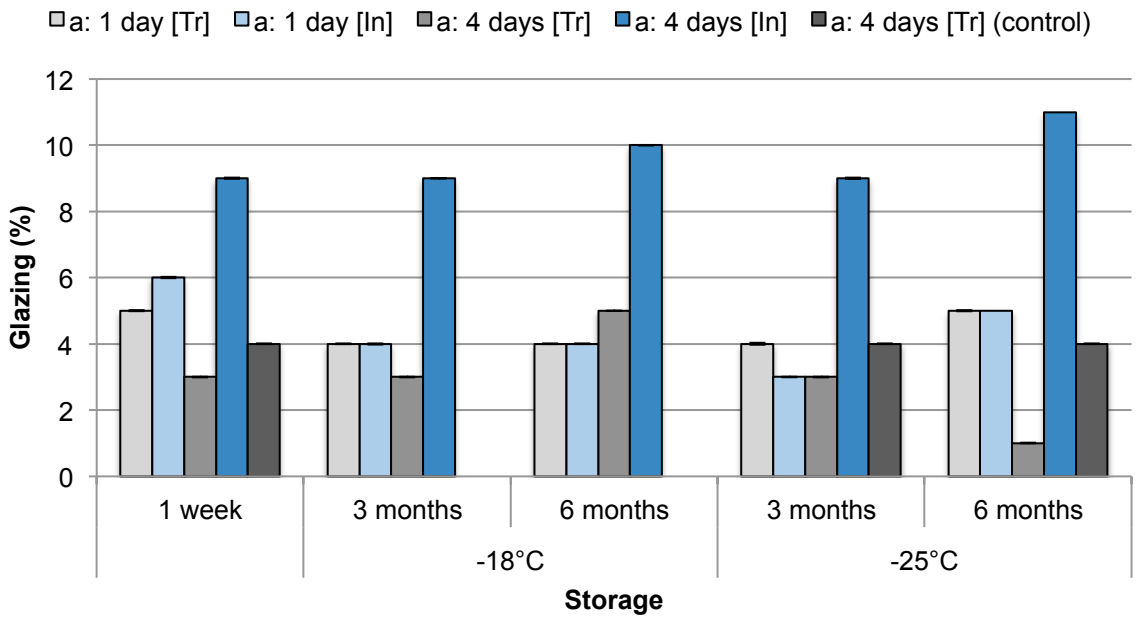

Figure 20. The average glazing content (%) of lightly salted cod fillets (n=5 per group) after various times at frozen storage (-18 °C and -25 °C). Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

Glazing content of the lightly salted cod fillets collected in October is shown in Figure 21. The glazing content in the sample collected in October ranged from 5% to 10% and the median glazing content was 7%.

Glazing content of most of the fillets increased or decreased during frozen storage. Small, 3 and 6 days fillet groups collected in October showed a difference ($p < 0.05$) in glazing content within the group during the storage time.

The difference between control fillets and lightly salted fillets collected in October was significant ($p > 0.05$) in two groups. Those groups were both of the small fillet groups. The control group had a lower glazing content.

Comparison between large fillets and small fillets collected in October showed that small fillets had a lower glazing ($p < 0.05$).

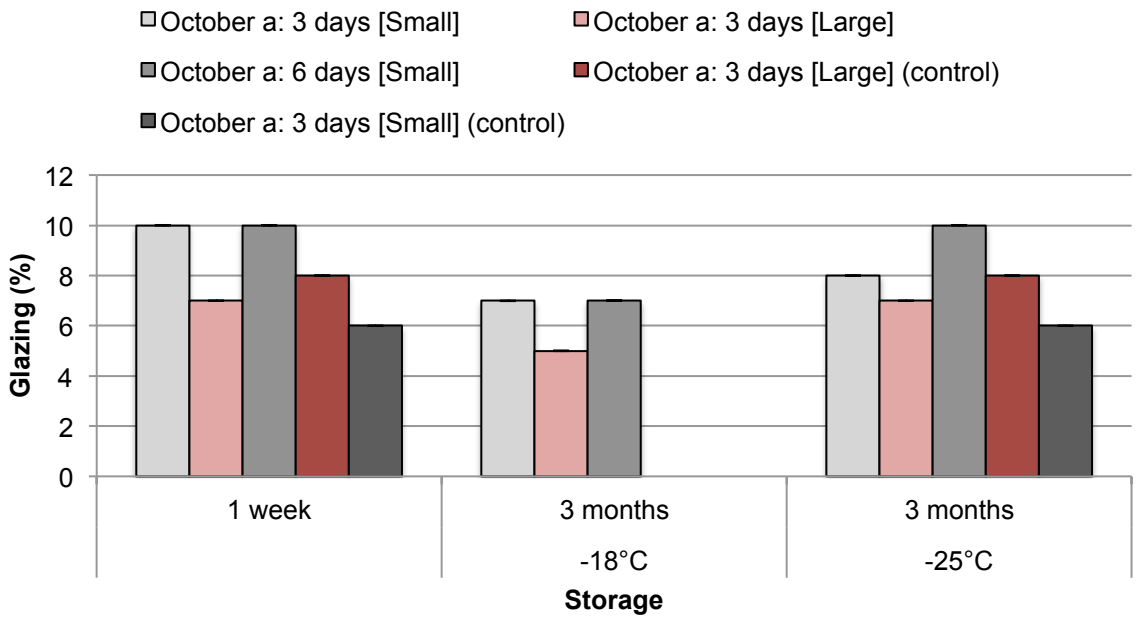

Figure 21. The average glazing content (%) of lightly salted cod fillets (n=5 per group) after various times at frozen storage (-18 °C and -25 °C). Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between fillets collected in July and fillets collected in October showed a significant difference where October fillets had a much higher glazing content.

4.2.2 Drip loss

The drip loss was measured as the weight loss in lightly salted cod during the storage time. Generally the drip loss was high for all the samples (4-11%), especially for samples collected in July (average 8%) as seen in Figure 22.

Drip loss had the tendency to increase during frozen storage. The group with the insufficient bled, 4 days lightly salted cod and the control group collected in July showed a difference in drip loss within the group during the storage time ($p < 0.05$).

The difference between control fillets and lightly salted fillets was not significant ($p > 0.05$). An exception was the insufficient bled, 1 day lightly salted cod, collected in July. In those samples the drip loss was greater compared with the control fillets ($p < 0.05$).

Comparison between traditional bled fillets and insufficient bled fillets collected in July, showed that the traditional bled fillets had less drip loss than the insufficient bled fillets ($p < 0.05$).

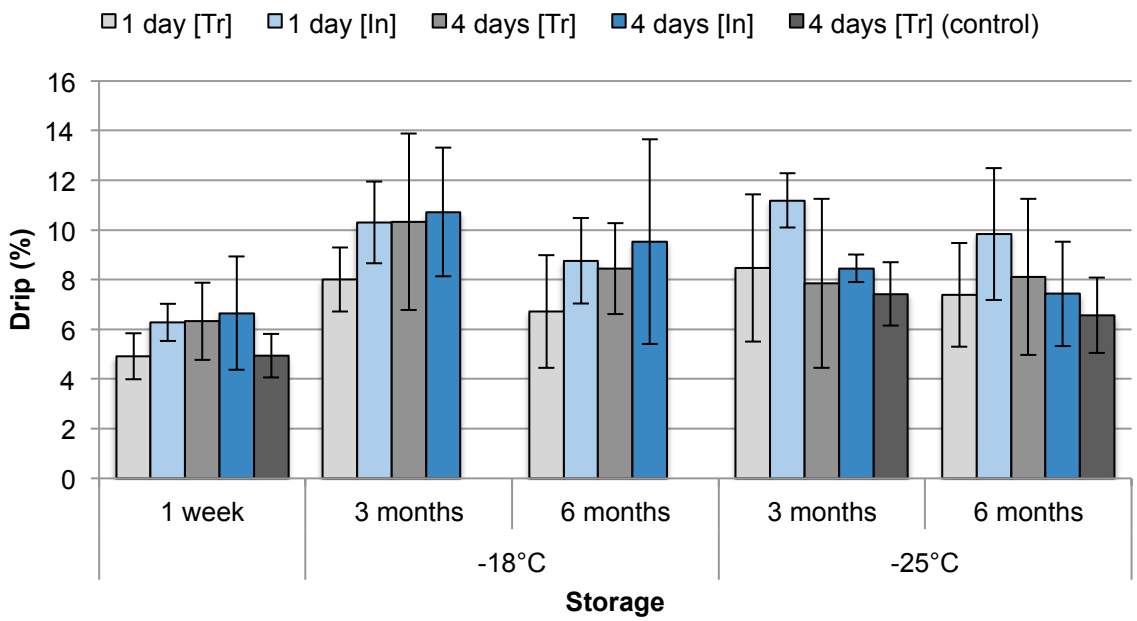

Figure 22. The average drip loss (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

Average drip loss in October was 6% (Figure 23). Drip loss of most of the fillets had the tendency to increase during frozen storage but there were only one group that showed a significant difference ($p < 0.05$) in drip loss within each group collected in October and that was the large, traditional bled, 3 days lightly salted cod.

The difference between control fillets and lightly salted fillets collected in October was not significant ($p > 0.05$).

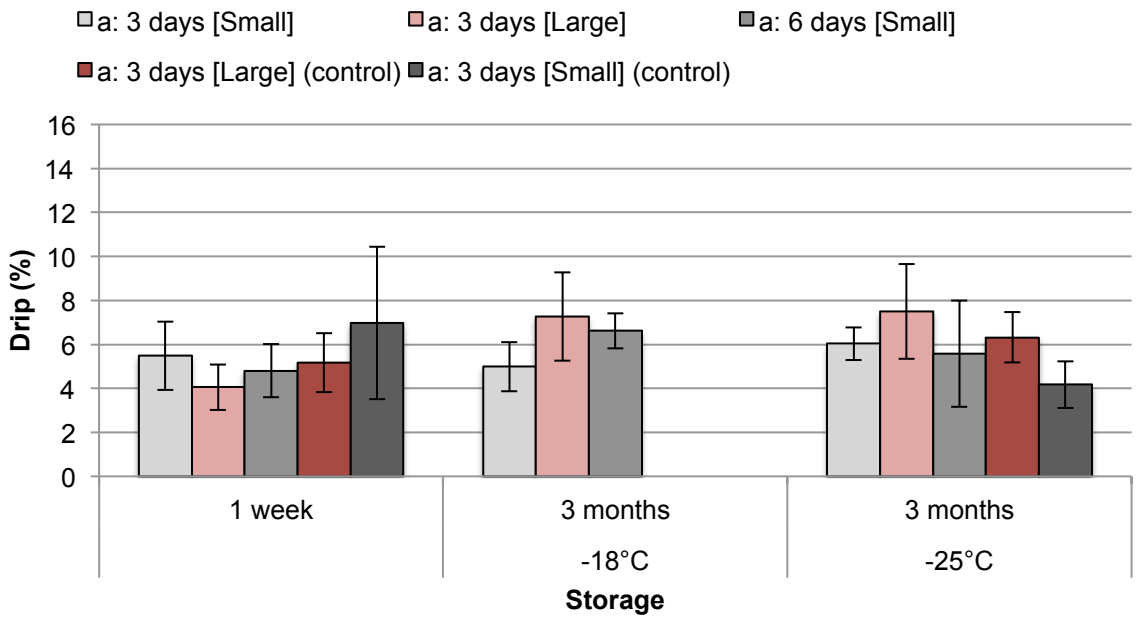

Figure 23. The average drip loss (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between fillets collected in July and fillets collected in October showed a significant difference ($p < 0.05$) where July fillets had a much higher drip loss.

4.2.3 Cooking yield

The cooking yield (CY) for fillets collected in July, ranged from 70.4 – 86.2%, with the average of 79% (Figure 24). The values of cooking yield decreased significantly ($p < 0.05$) for the insufficient bled, 1 day fillets collected in July after frozen storage. No significant difference in CY was found in other groups after frozen storage ($p > 0.05$).

When the groups of lightly salted fillets collected in July were compared to the control fillets, they all showed a significant difference ($p < 0.05$), except one group. No significant difference was found between the insufficient bled, 4 days lightly salted cod samples and the control fillets ($p > 0.05$). Overall for the fillets collected in July, lightly salted cod fillets showed none or a decrease in yield after cooking compared with control fillets.

Comparison between traditional bled fillets and insufficient bled fish, both collected in July showed no significant difference ($p > 0.05$). On the other hand, comparison between 1 day fillets and 4 days fillets, both also collected in July showed a significant difference ($p < 0.05$), where 4 days fillet showed a better CY compared to 1 day fillets.

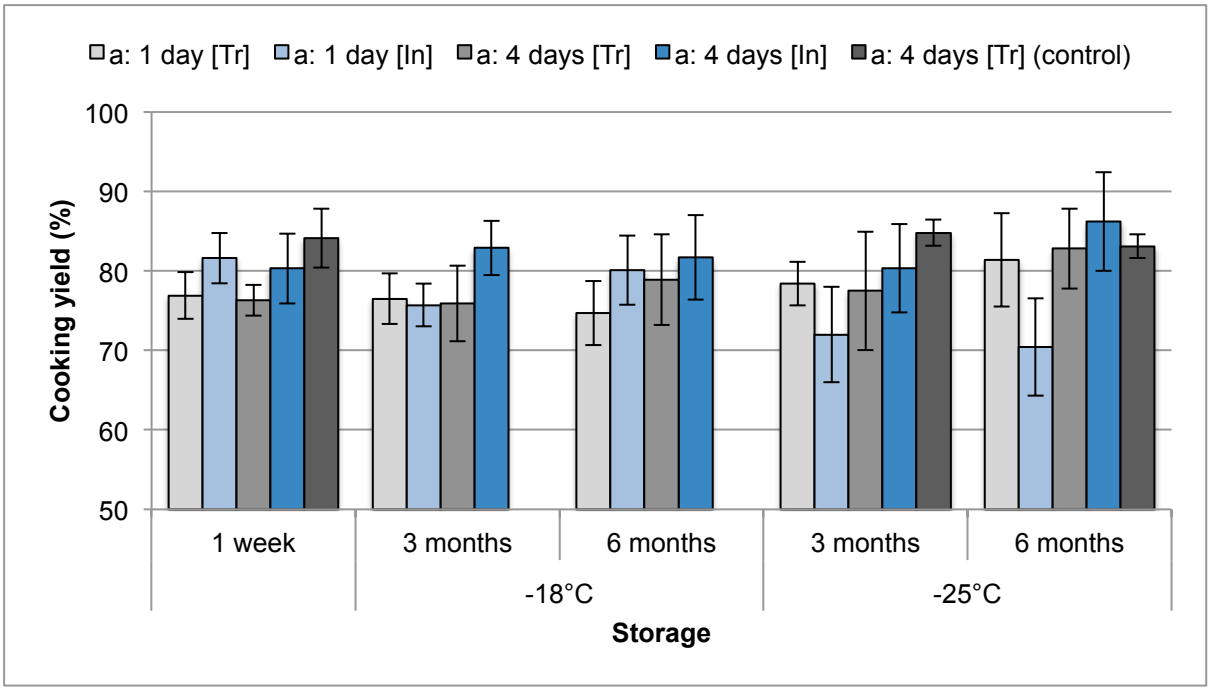

Figure 24. The average cooking yield (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The CY for fillets collected in October, ranged from 80.5 - 92.5% and had the average of 85% (Figure 25). The values of cooking yield increased significantly ($p < 0.05$) for the large, traditional bled, 3 days fillets collected in October after frozen storage, but only at -25°C. No significant difference in CY was found in other groups after frozen storage ($p > 0.05$).

No significant difference was found between the groups of lightly salted fillets collected in October and the control fillets ($p > 0.05$). Overall for the fillets collected in October, lightly salting cod fillets showed none effects on yield after cooking compared with control fillets.

Comparison between large fillets (weight of raw fillets > 1500 grams) and small fillets (weight of raw fillets 500-1000 grams), collected in October showed a significant difference ($p > 0.05$), where large fillets have better CY than small fillets.

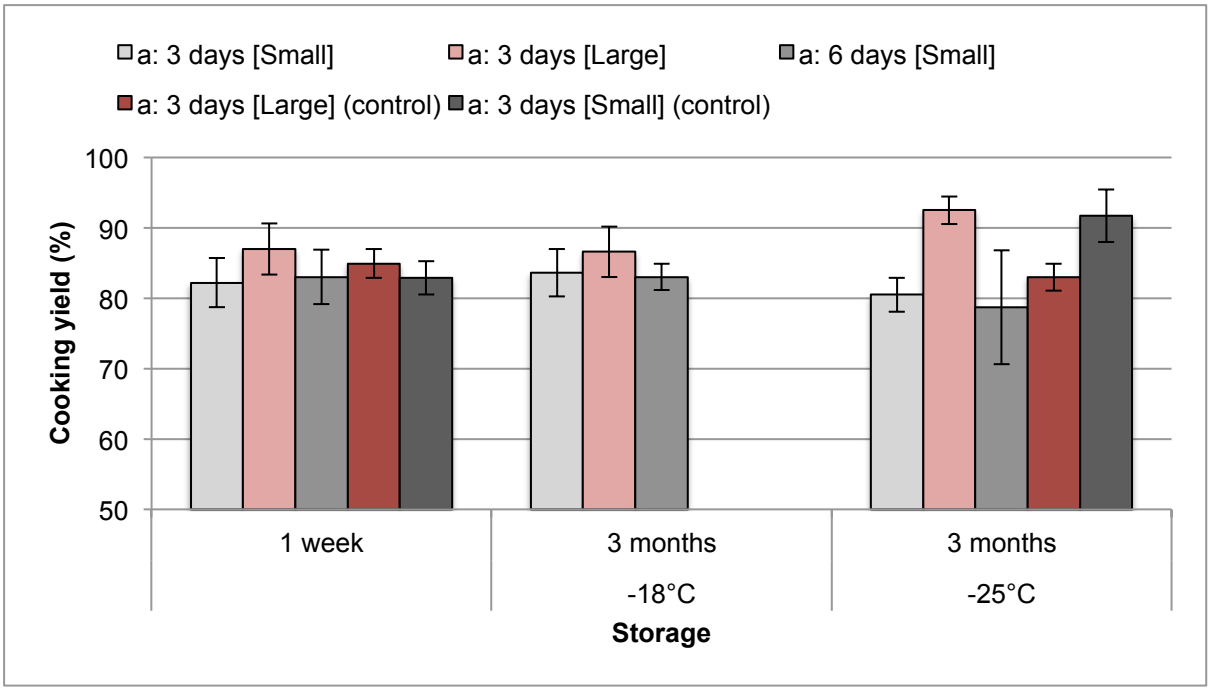

Figure 25. The average cooking yield (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B **and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.**

Comparison between cooking yield in fillets collected in July and cooking yield in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher Cooking yield.

4.2.4 Total yield

TY for fillets collected in July (Figure 26), ranged from 63.5 – 80%, with the average of 73%. Fillets, collected in July had the tendency to decrease in TY with storage time. They also had the tendency to increase their TY after 6 months of storage in -25°C but there was only one group that showed a significant difference ($p < 0.05$) within the group. The group that showed the difference within the group was the insufficient 1 day fillet group.

3 out of 4 groups showed a difference ($p < 0.05$) in TY compared to the control group where the control group had about 5% - 10% better yield. The only group that did not show a significant difference ($p > 0.05$) was the 4 days insufficient bled fillets group. Comparison between 1 day fillets and 4 days fillets showed a significant difference ($p < 0.05$) where 4 days fillets had a better results in total yield. There was no significant difference ($p > 0.05$) between insufficient bled and traditional bled fish.

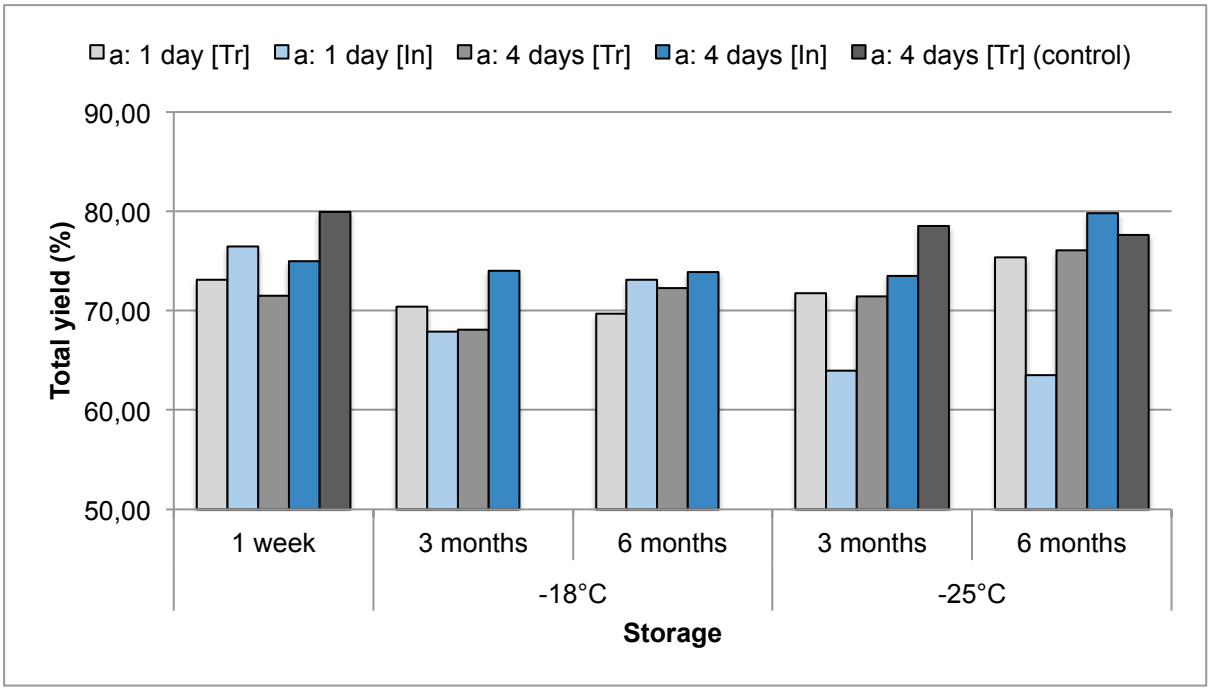

Figure 26. The average total yield (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color).

TY for fillets collected in October (Figure 27), ranged from 74 – 88% and had the average of 79%. All the lightly salted fillet groups collected in October had the tendency to show a slight decrease in TY with storage time, but there was no significant difference ($p > 0.05$) within the groups. The total yield seemed to be higher in -25°C compared to -18°C. The control groups had the tendency to decrease their TY.

There was no significant difference ($p > 0.05$) between lightly salted fillets collected in October compared to the control group. There was a significant difference ($p < 0.05$) between large and small fillets, where large fillets had about 6% higher TY.

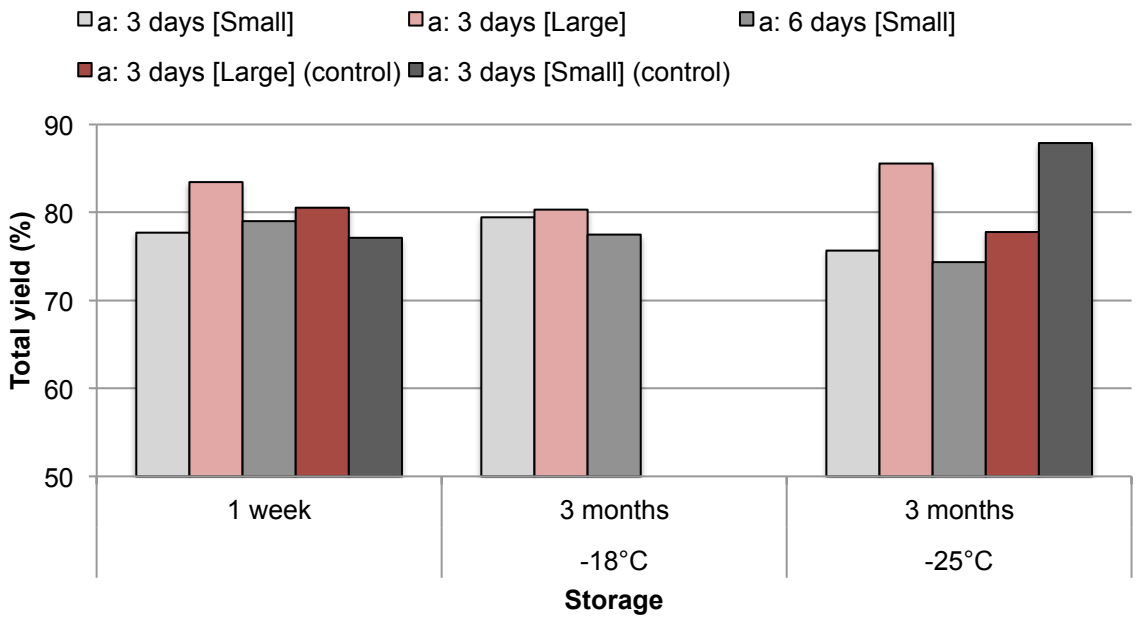

Figure 27. The average total yield (%) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color).

Comparison between total yield in fillets collected in July and total yield in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher total yield.

4.2.5 Color

The L* value or lightness of the fillets was measured on a scale from 0 to 100 (from black to white). The average value of lightness for samples collected in July was 92.7 (Figure 28).

The difference in lightness within each group during the storage time was only significant ($p < 0.05$) for the control group and insufficient bled, 4 days fillet group collected in July. Generally the lightness of the lightly salted cod fillets increased with storage time, especially in -25°C.

Both the insufficient bled, 1 day and traditional bled, 1 day fillets collected in July resulted in a lower value of lightness compared to the control fillets ($p < 0.05$). The difference between control fillets and other fillets collected in July was on the other hand not significant ($p > 0.05$).

Comparison between traditional bled fillets and insufficient bled fish, both collected in July showed a significant difference ($p < 0.05$), where traditional bled fish had a slight higher value of lightness compared to insufficient bled fish. On the other hand, comparison between 1 day fillets and 4 days fillets, both also collected in July showed no significant difference ($p > 0.05$).

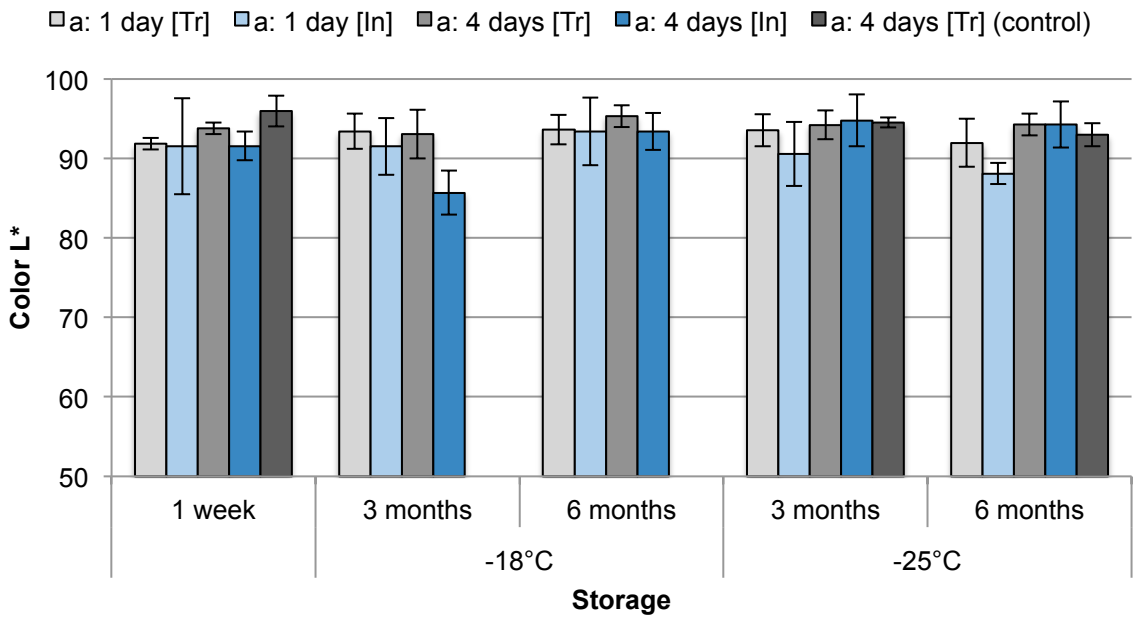

Figure 28. The average lightness (L* value) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The average value of lightness for samples collected in October was 87.4 (Figure 29). The difference in lightness within each group during the storage time was not significant ($p > 0.05$) for fillets collected in October.

All of the fillet groups collected in October resulted in a higher value of lightness compared to the control fillets ($p < 0.5$). Comparison between large fillets (weight of raw fillets > 1500 grams) and small fillets (weight of raw fillets 500-1000 grams), showed also a significant difference ($p < 0.05$), where small fillets resulted in higher value of lightness compared to large fillets.

Comparison between lightness in fillets collected in July and lightness in fillets collected in October showed a significant difference ($p < 0.05$) where July fillets had a much higher value of lightness.

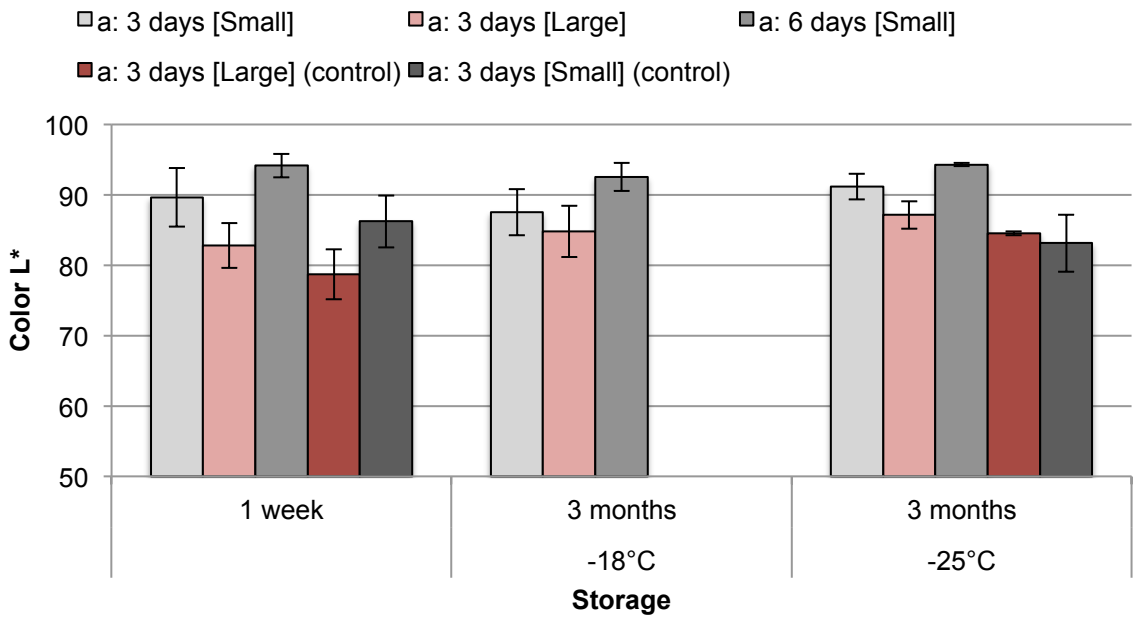

Figure 29. The average lightness (L* value) of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

The a* value describes the intensity in green color (negative) and in red color (positive) of lightly salted cod fillets. Evaluation of a* value for fillets collected in July is shown in Figure 30. The average a* value for samples collected in July was -1.5.

Generally, the a* value of all the samples after storage collected in July, decreased but there was only two groups that showed a significant different ($p < 0.05$) within each group during the storage time. The groups that showed a significant difference within each group was insufficient bled, 4 days fillets and the control group. Insufficient bled fillets had higher a* values than traditional bled fish ($p < 0.05$). Insufficient bled, 1 day fillet was the only sample group collected in July that showed significant difference ($p < 0.05$) compared to the control fillets. That group was higher in a* value compared to the control fillets.

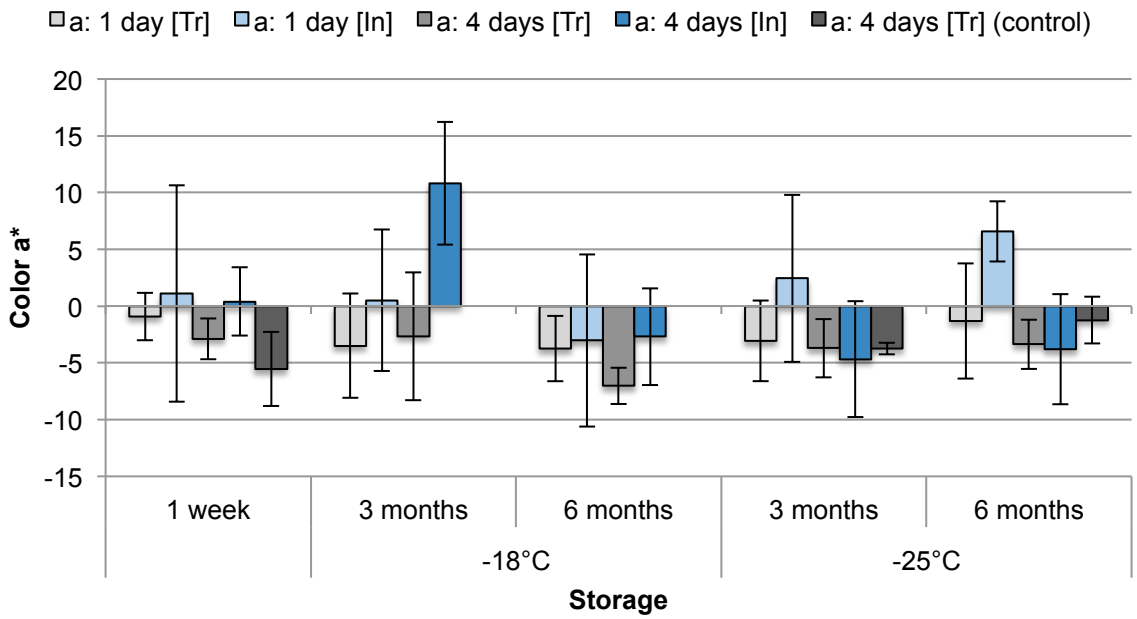

Figure 30. The average a* value of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

Evaluation of a* value for fillets collected in October is shown in Figure 31. The average value of lightness for samples collected in October was 6.4

Generally, the a* value of all the samples after storage collected in October, decreased but there was no significant different ($p > 0.05$) within each group during the storage time. Comparison between large and small fillets did not show significant difference ($p > 0.05$).

All sample groups collected in October showed a significant different ($p < 0.05$) compared to the control fillets. Control fillets showed at all times the lowest a* value.

Comparison between a* value in fillets collected in July and a* value in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher value of a* value.

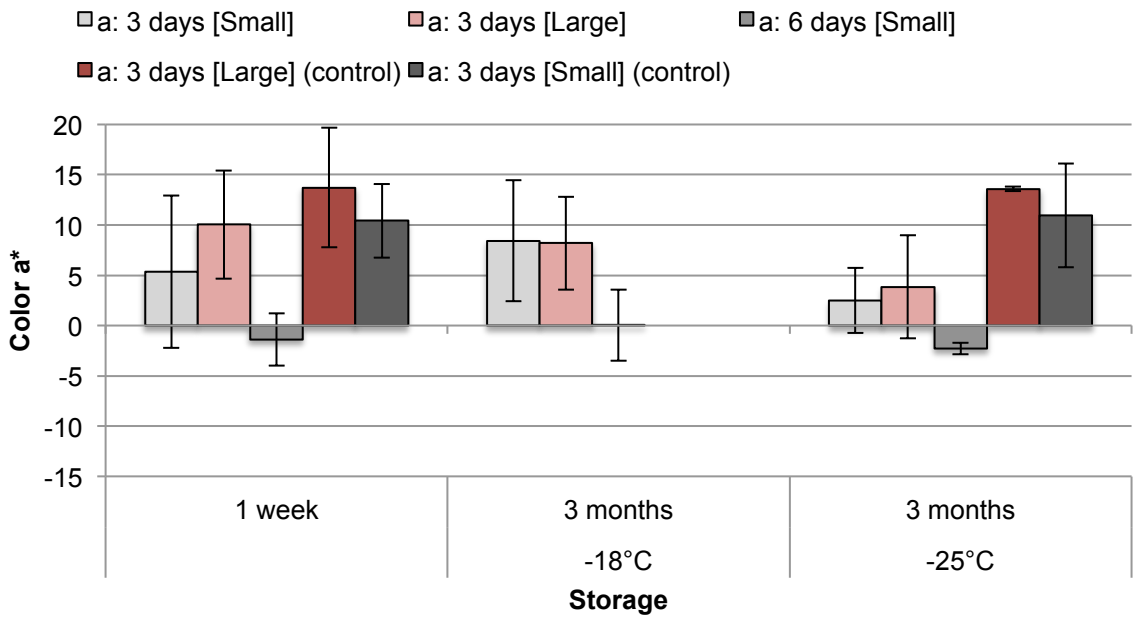

Figure 31. The average a* value of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

The b* value describes intensity in blue (negative) and in yellow (positive) of the lightly salted cod. For samples collected in July the average b* value was 35.1 (Figure 32).

Generally, the b* value increased with storage time, but there was only one sample group that showed a significant difference ($p < 0.05$) within each group during the storage time. The group that showed significant difference within each group during the storage time was traditional bled, 4 days fillets collected in July. Control fillets showed at all times lower b* value compared to the groups collected in July.

Comparison between insufficient bled and traditional bled fillets collected in July showed that insufficient bled fillets had a lower b* value compared to the traditional bled fillets ($p < 0.05$). Comparison between 4 days and 1 day fillets showed that 4 days fillet had a lower b* value compared to 1 day fillets ($p < 0.05$).

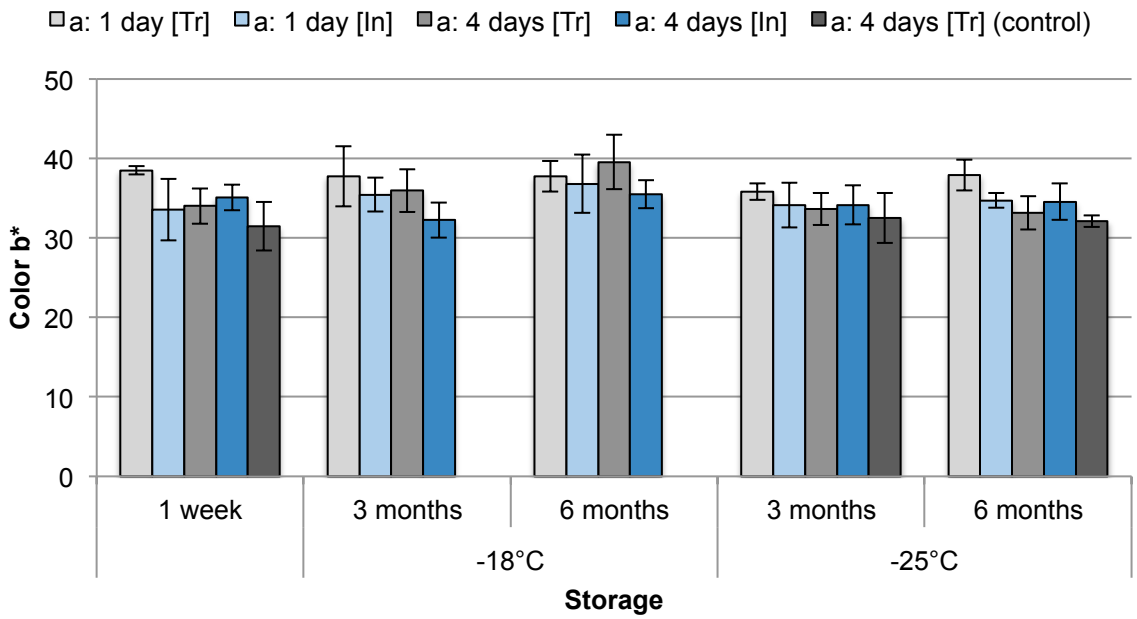

Figure 32. The average b* value of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

For samples collected in October the average b* value was 29.5 (Figure 33). In most cases in samples collected in October, the b* value had the tendency to increase after frozen storage but there was no significant difference ($p > 0.05$) within the groups during storage time.

The difference in b* value between the large fillets and small fillets during the storage time was not significant ($p > 0.05$).

Comparison between b* value in fillets collected in July and b* value in fillets collected in October showed a significant difference ($p < 0.05$) where July fillets had a much higher value of b* value.

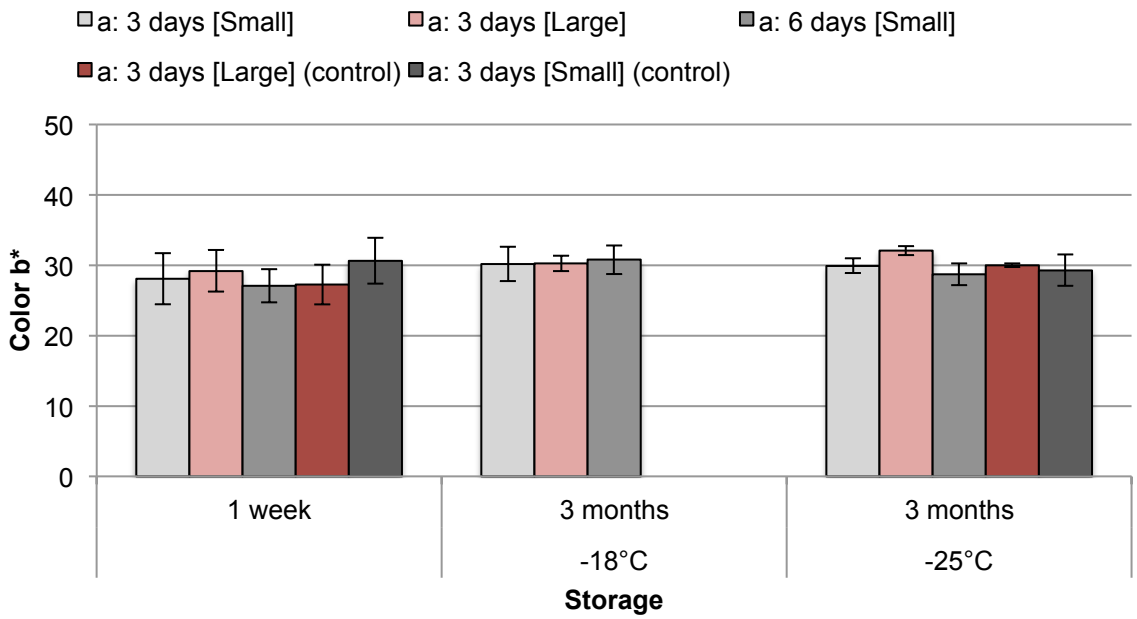

Figure 33. The average b* value of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

The average whiteness for the lightly salted cod fillets collected in July was 63.5 and is shown in Figure 34. Generally the whiteness of the fillets collected in July was rather stable during the storage time. Comparison between the groups collected in July showed that control fillets had the highest value of whiteness at all times ($p < 0.05$).

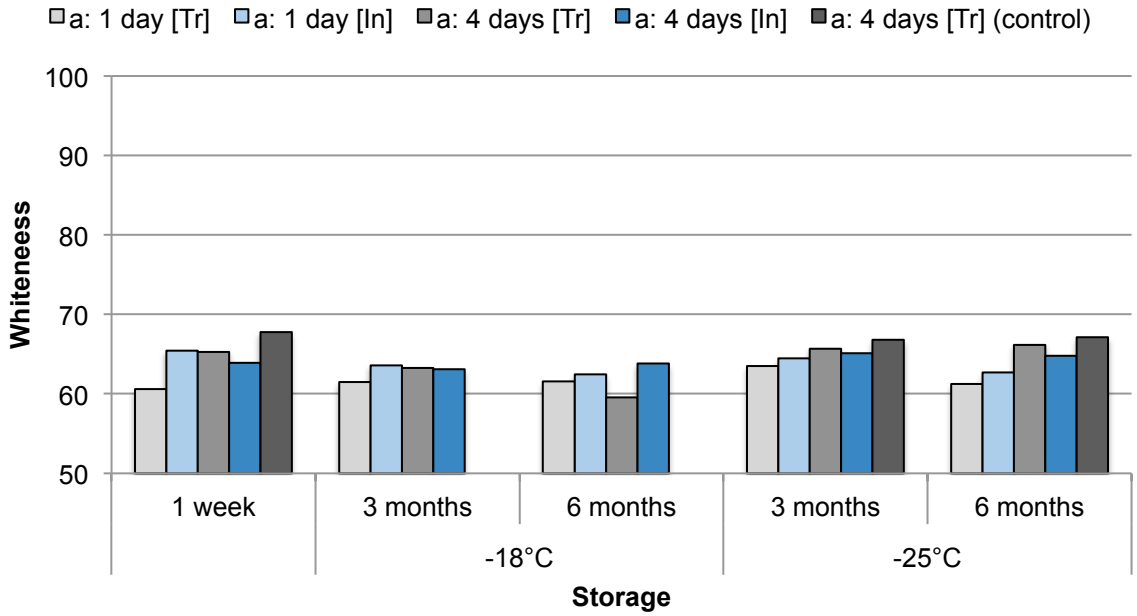

Figure 34. The average whiteness of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color).

The average whiteness for the lightly salted cod fillets collected in October was 66.3 and is shown in Figure 35. Generally the whiteness of the fillets collected in October was rather stable during the storage time, with the exception of small, 3 days fillets where the whiteness decreased a lot after 3 months of storage, both at -18°C and -25°C. Small fillets seemed to have higher value of whiteness compared to large fillets.

Comparison between whiteness in fillets collected in July and whiteness in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a higher value of whiteness.

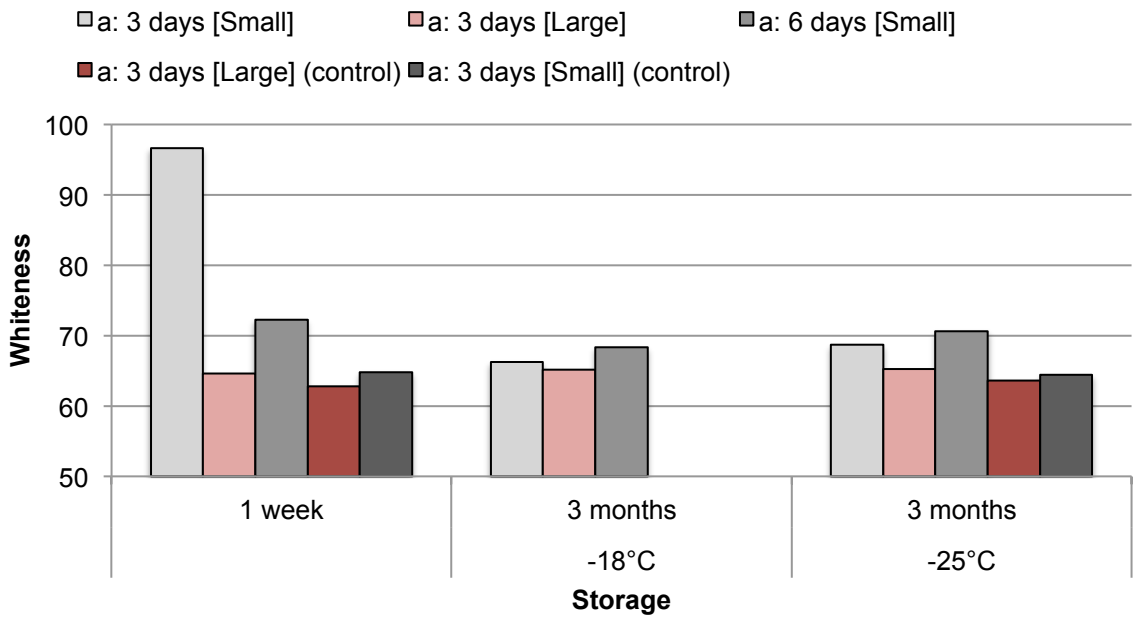

Figure 35. The average whiteness of lightly salted cod sample (n=5 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color).

4.2.6 WHC

The water holding capacity (WHC) of lightly salted cod fillets and control fillets collected in July is shown in Figure 36. The average water holding capacity obtained for all the fillets collected in July was 77.8%. The difference within the groups in WHC of lightly salted fillets and control fillets after frozen storage was not significant ($p > 0.05$). The lowest value of water holding capacity for fillets collected in July after frozen storage was obtained in the control group. The highest value after frozen storage was obtained from the traditional bled, 4 days fillets. The difference between the groups and the control fillets was not significant ($p > 0.05$).

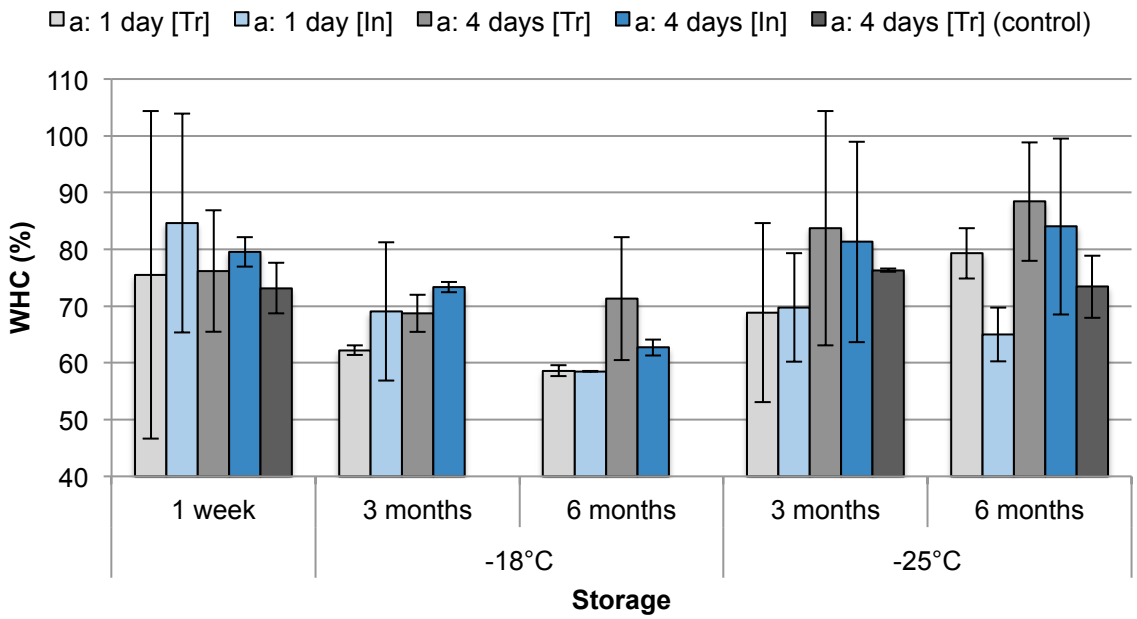

Figure 36. The average water holding capacity (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The water holding capacity (WHC) of lightly salted cod fillets and control fillets collected in October is shown in Figure 37. The average water holding capacity obtained for all the fillets collected in October was 85.7%. The difference in WHC of lightly salted fillets and control fillets after frozen storage was not significant ($p > 0.05$). The lowest value of water holding capacity for fillets collected in October after frozen storage was obtained in the control groups. The highest value after frozen storage was obtained from the large, 3 days fillets and small, 6 days fillets. The difference between the groups and the control fillets was not significant ($p > 0.05$). The WHC tends to be higher for small fillets compared to large fillets but did not show significant difference ($p > 0.05$).

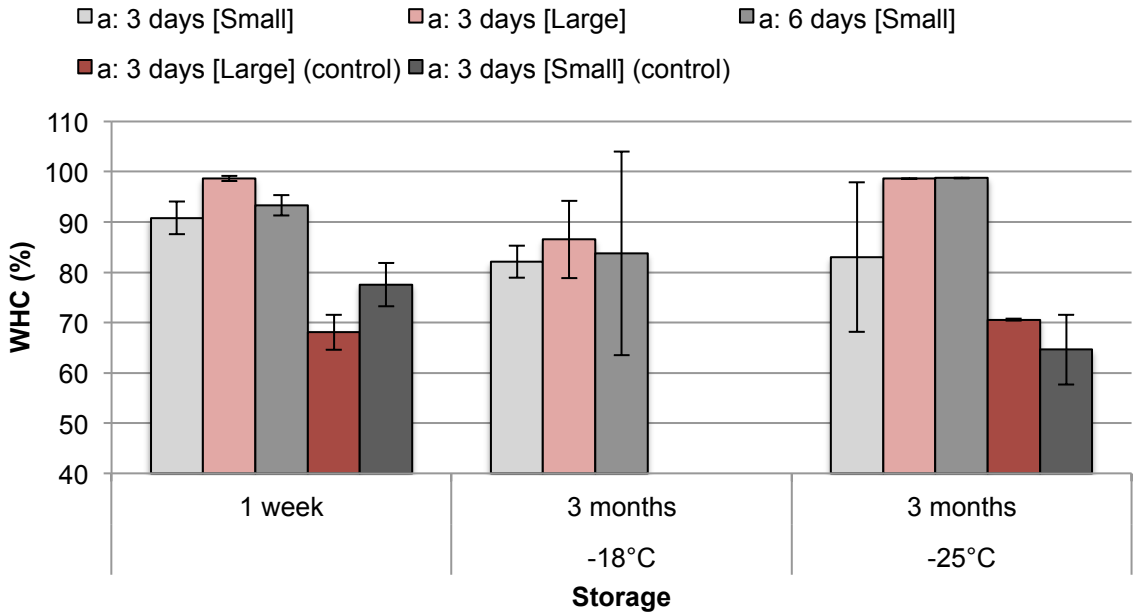

Figure 37. The average water holding capacity (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between WHC in fillets collected in July and WHC in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher WHC.

4.3 Chemical changes

4.3.1 Lipid content

Total lipid contents of lightly salted cod collected in July are shown in Figure 38. The total lipid content in fillets collected in July arranged from 0.2% – 1.5% and the average lipid content was 0.5%.

The effect of the frozen storage time on total lipid content measured showed a significant difference ($p < 0.05$) in 4 days fillets collected in July, both insufficient and traditional bled and the control group. Those fillet groups showed a general increase in lipid content after 3 months of frozen storage, especially in -25°C, but a decrease after 6 months. There was no significant difference ($p > 0.05$) in total lipid contents between the groups and the control fillets collected in July.

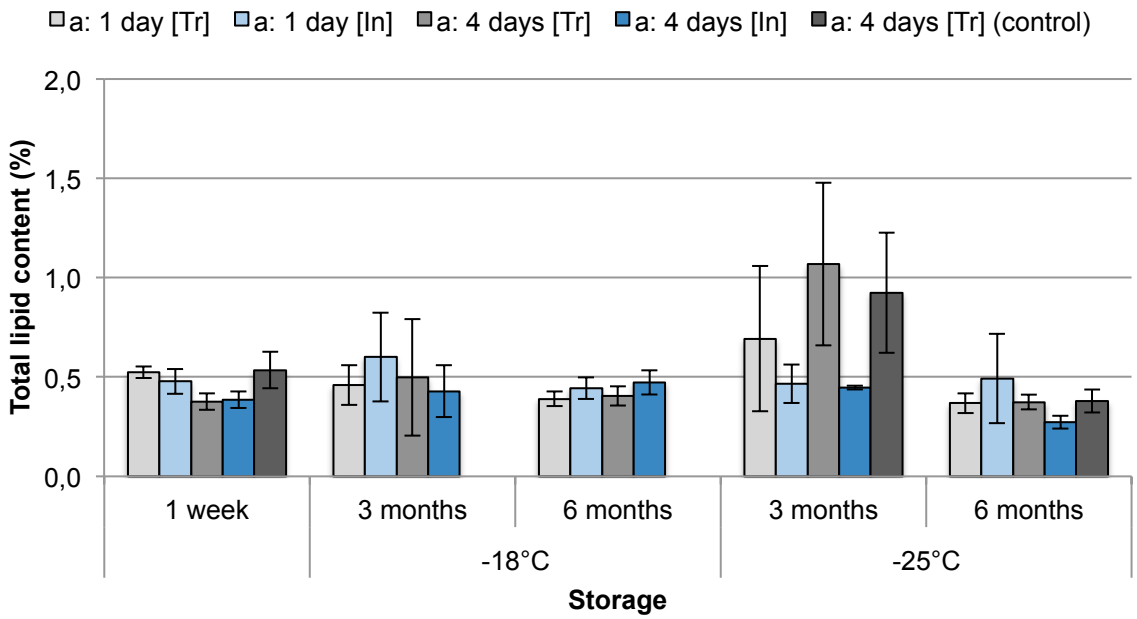

Figure 38. Average change of lipid content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The total lipid content in fillets collected in October (Figure 39) arranged from 0.4% – 2.1% and the average lipid content was 0.7%.

The effect of the frozen storage time on total lipid content measured showed a significant difference ($p < 0.05$) in small, 6 days fillets collected in October and both the control groups. Those fillet groups showed a general decrease in total lipid content after 3 months of frozen storage. The lipid content decreased more in -25°C compared to -18°C. There was no significant difference ($p > 0.05$) in total lipid contents between the groups and the control fillets collected in October.

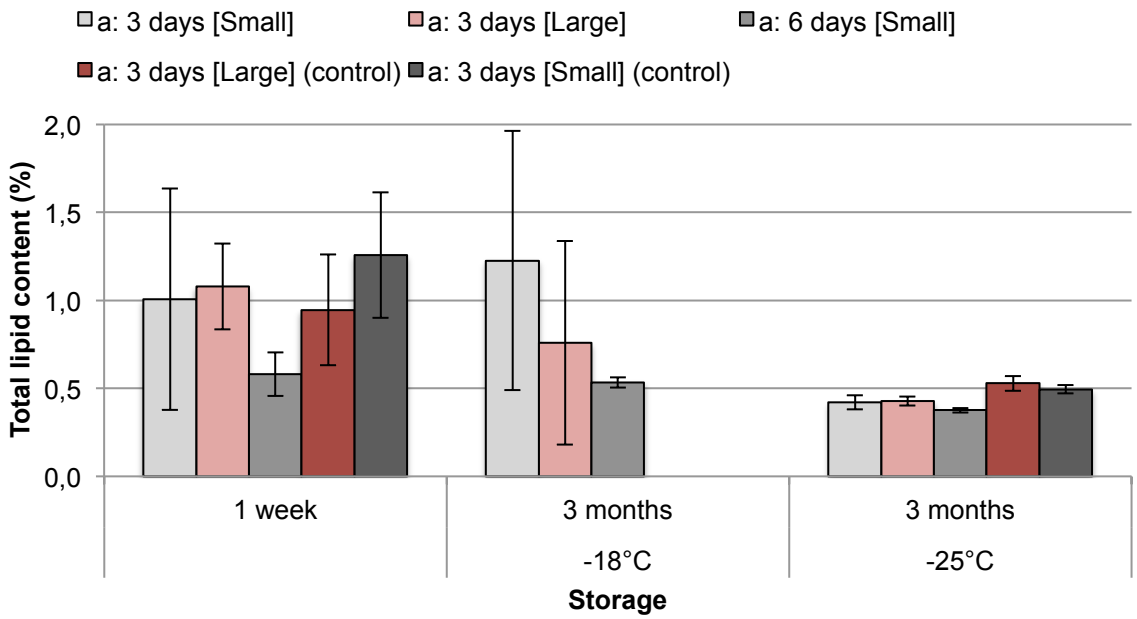

Figure 39. Average change of lipid content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between total lipid content in fillets collected in July and total lipid content in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher total lipid content.

4.3.2 Free fatty acid content

The effect of frozen storage time on FFA content measured showed a significant difference ($p < 0.05$) in traditional bled, 1 day and 4 days fillets collected in July (Figure 40). The fillets had the tendency to increase in FFA formation as a result of frozen storage. Furthermore, the FFA content increased more in -18°C compared to -25°C. Insufficient bled fillets had higher value of FFA compared to traditionally bled fillets ($p < 0.05$), both collected in July. No significant difference ($p > 0.05$) was found between the groups collected in July.

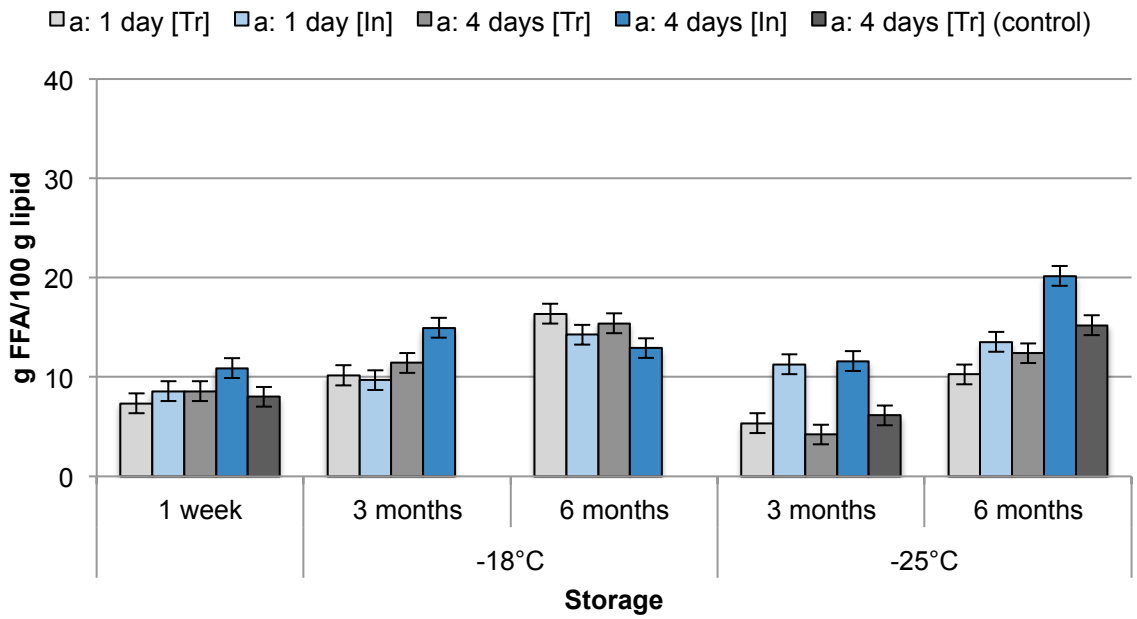

Figure 40. Average change in g FFA/100 g lipids of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The FFA content increased significantly ($p < 0.05$) after three months of frozen storage within all groups collected in October (Figure 41). The FFA content increased from 6.7% in the first week to 17.6% of total lipid as oleic acid after 6 months of frozen storage in -25°C. No significant difference ($p > 0.05$) was found between the groups collected in October.

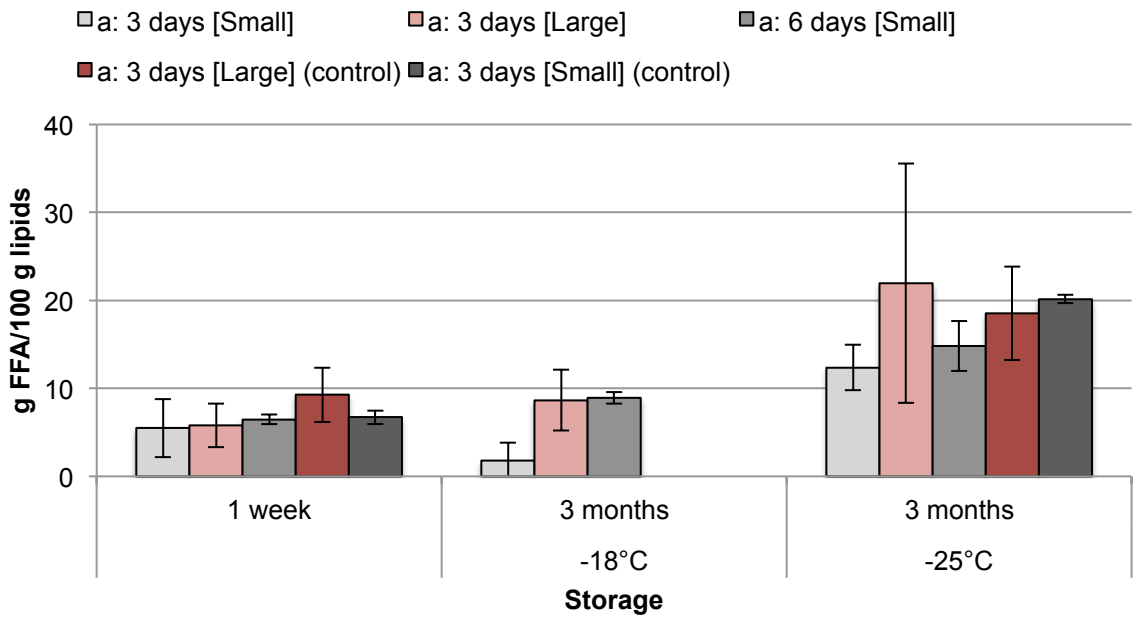

Figure 41. Average change in g FFA/100 g lipids of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between FFA in fillets collected in July and FFA in fillets collected in October did not show a significant difference ($p > 0.05$).

4.3.3 Phospholipids content

Average phospholipids content in all fillets collected in July (Figure 42) after one week was 13%. The highest average value obtained for PL content after frozen storage was after 6 months in -25°C or about 17%. All groups collected in July showed a significant difference ($p < 0.05$) in PL content within each group, except the control group. PL content in fillets collected in July had the tendency to decrease after 3 months of frozen storage and decrease after 6 months of frozen storage, especially in -25°C. There was a significant difference ($p < 0.05$) between the lightly salted fillet groups and the control group collected in July. Traditional bled, 4 days fillet was an exception and did not show a significant difference ($p > 0.05$) compared to the control group. The control group had the lowest value of phospholipids content (7.2%) and the traditional bled, 1 day fillet group had the highest value of PL content (15.5%).

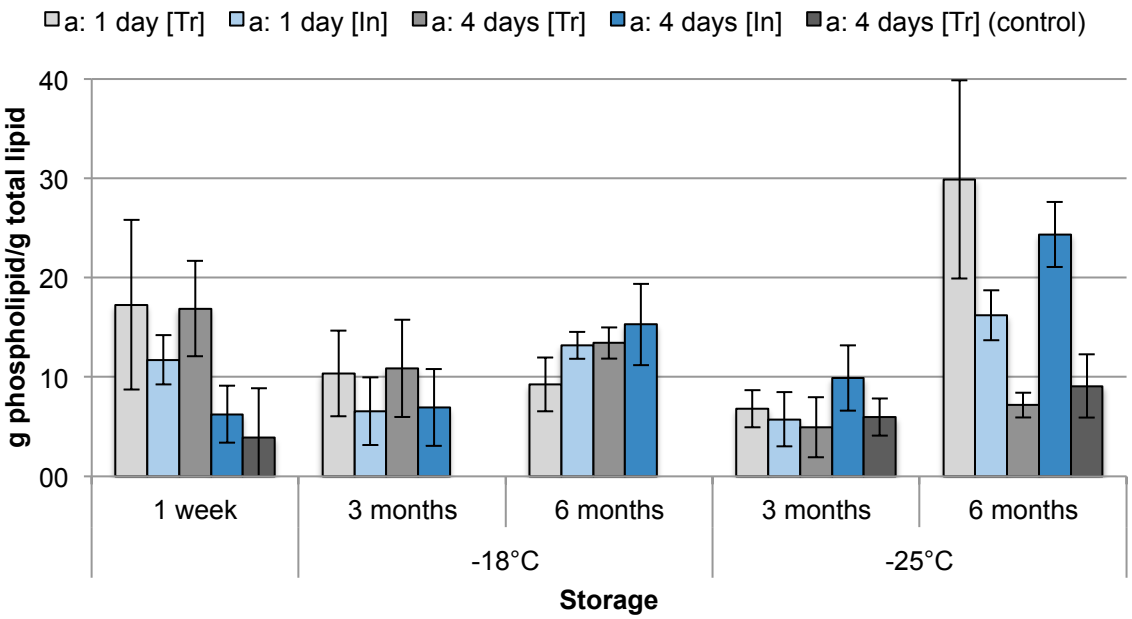

Figure 42. Average change in g phospholipid/g total lipid g of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

Average phospholipids content in all fillets collected in October (Figure 43) after one week was 7.4%. The average value for PL content in fillets collected in October after 3 months of frozen storage was 13.9% in -18°C and 21.3% in -25°C. All groups collected in October showed a significant difference ($p < 0.05$) in PL content within each group. PL content in fillets collected in October increased after 3 months of frozen storage. Furthermore the PL content increased more in -25°C compared to -18°C. The control groups had the tendency to have a higher value of PL content compared to other groups but there was not a significant difference ($p > 0.05$) between the groups collected in October.

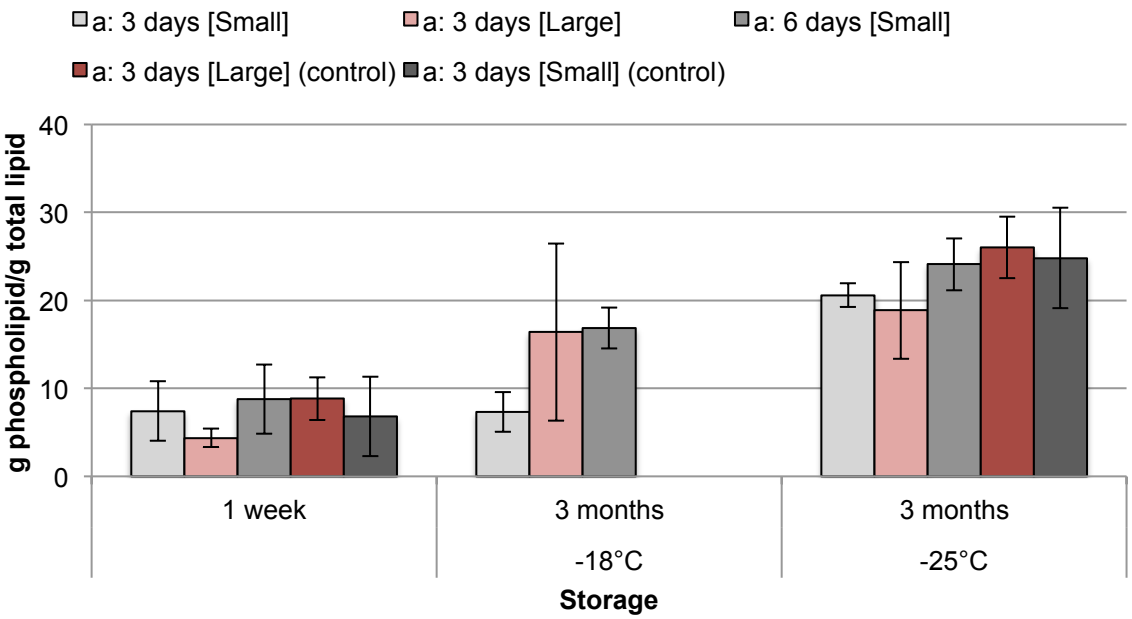

Figure 43. Average change in g phospholipid/g total lipid g) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between phospholipid content in fillets collected in July and phospholipid content in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher phospholipid content.

4.3.4 Water content

The variations in the water content in fillets collected in July with the frozen storage time are illustrated in Figure 44. Water contend in lightly salted fillets collected in July ranged from 84.2% to 87.4% compared to 81.2% to 82.4% in control fillets. Average water content for all of the lightly salted cod fillets collected in July was 85.6% and for control fillets, the water content was 81.8%. There was no significant difference ($p > 0.05$) in water content within the groups collected in July during frozen storage. Comparison in water content between the groups and the control group collected in July showed a significant difference ($p < 0.05$), where the control group resulted in a lower value of water content.

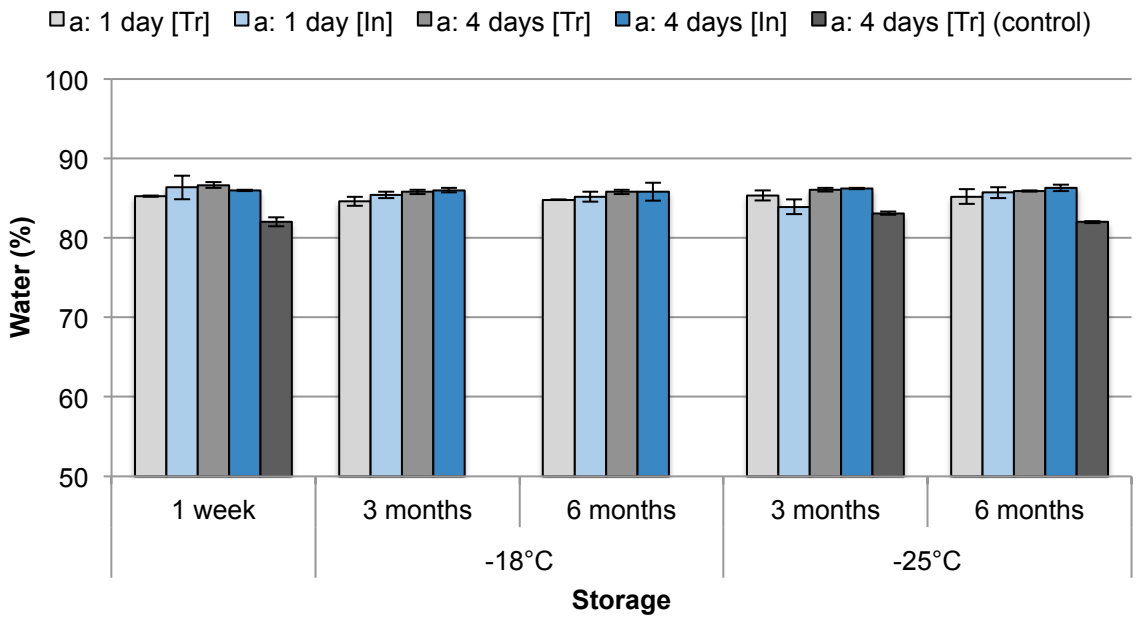

Figure 44. The average water content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The variations in the water content in fillets collected in October with the frozen storage time are illustrated in Figure 45. Water contend in lightly salted fillets collected in October ranged from 84.0% to 86.3% compared to 80.7% to 81.6% in control fillets. Average water content for all of the lightly salted cod fillets collected in October was the same as in July or 85.6% and for control fillets, the water content was about 81.3%. There was no significant difference ($p > 0.05$) in water content within the groups collected in October during frozen storage. Comparison in water content between the groups and the control groups collected in October showed a significant difference ($p < 0.05$), where the control group resulted in a lower value of water content.

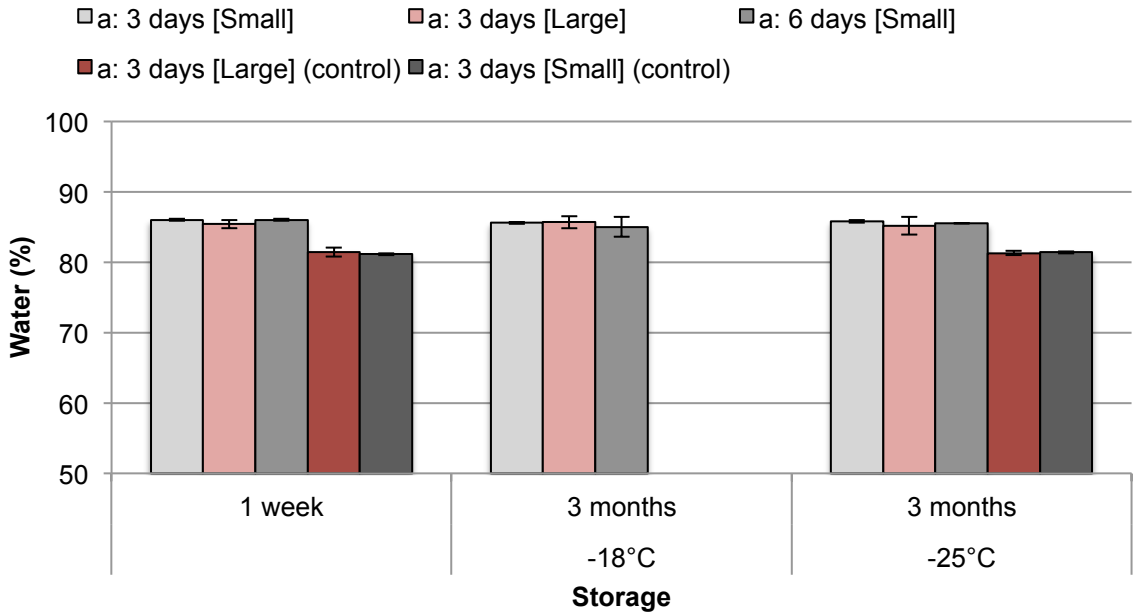

Figure 45. The average water content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

Comparison between water content in fillets collected in July and water content in fillets collected in October did not show a significant difference ($p > 0.05$).

4.3.5 Salt content

The variations in salt contents of the controls and injected fillets collected in July are listed in Figure 46. The salt content in the lightly salted fillets ranged from 1.4% to 2.3% compared to 0.2% to 0.3% in control fillets collected in July. Furthermore, average salt content for all of the lightly salted cod fillets collected in July was 1.9% and for control fillets, the salt content was 0.3%. Comparison in salt content between the groups and the control group collected in July showed a significant difference ($p < 0.05$), where the control group resulted in a lower value of salt content as expected. There was a significant difference ($p < 0.05$) in salt content during frozen storage within traditional, 4 days fillets collected in July. The difference was found between the first week and frozen storage in -25°C, both after 3 months and 6 months, where the salt content decreased after frozen storage.

Comparison between traditional bled fillets and insufficient bled fillets, both collected in July showed a significant difference ($p < 0.05$) where the traditional bled fillets obtained in a higher value of salt content. There was also a significant difference between 1 day and 4 days fillets collected in July, where 4 days fillets had a higher value of salt content.

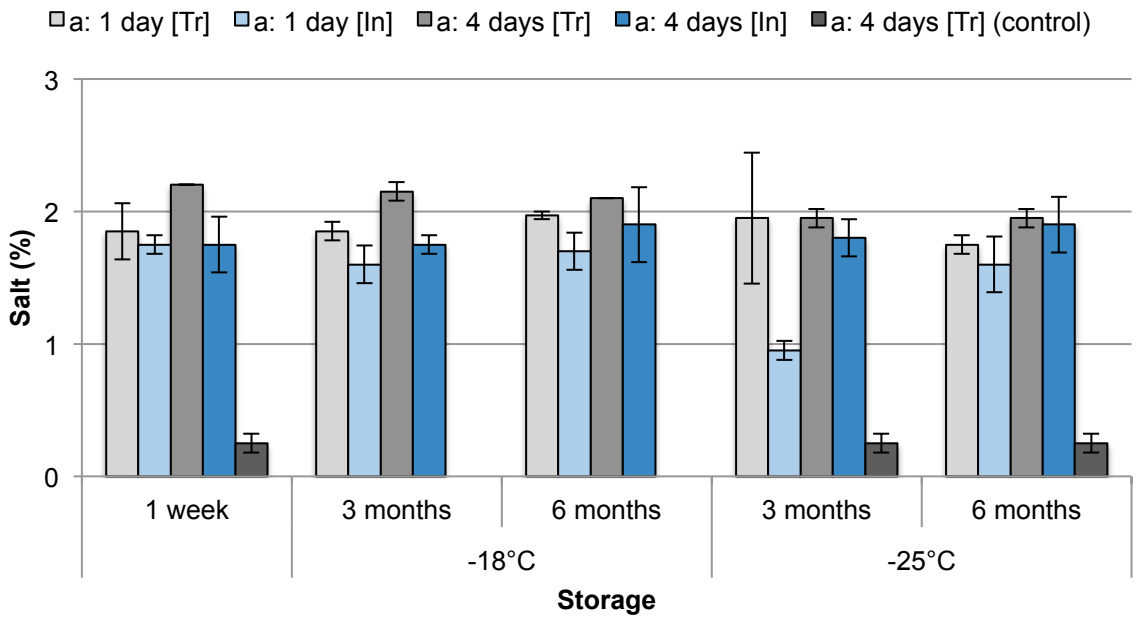

Figure 46. The average salt content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The variations in salt contents of the controls and injected fillets collected in October are listed in Figure 47. The salt content in the lightly salted fillets ranged from 1.4% to 1.8% compared to 0.2% to 0.4% in control fillets collected in October. Furthermore, average salt content for all of the lightly salted cod fillets collected in October was 1.6% and for control fillets, the salt content was about 0.3%. Comparison in salt content between the groups and the control group collected in October showed a significant difference ($p < 0.05$), where the control group resulted in a lower value of salt content as expected. There was no significant difference ($p < 0.05$) in salt content during frozen storage within the groups collected in July.

Comparison between large and small fillets collected in October did not show a significant difference ($p > 0.05$).

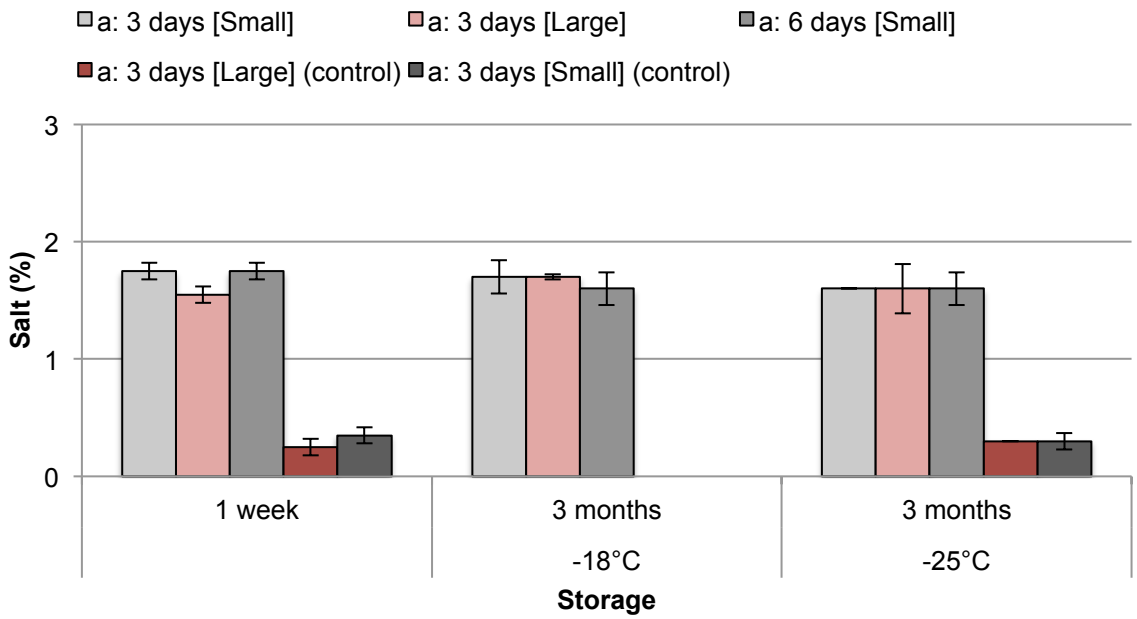

Figure 47. The average salt content (%) of lightly salted cod sample (n=2 per group) after various times at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

4.3.6 Phosphates content

The variations in phosphates content in fillets collected in July with the frozen storage time are illustrated in Figure 48. The phosphates content in the lightly salted fillets ranged from 2.0% to 5.8% compared to 4.8% to 6.4% in control fillets collected in July. Furthermore, average phosphates content for all of the lightly salted cod fillets collected in July was 3.5% and for control fillets, the phosphates content was 5.3%. Comparison in phosphates content between the groups and the control group collected in July did not show a significant difference ($p > 0.05$), although the control group had a tendency to have a higher value of phosphates content. There was not a significant difference in phosphates content during frozen storage within the fillet groups collected in July.

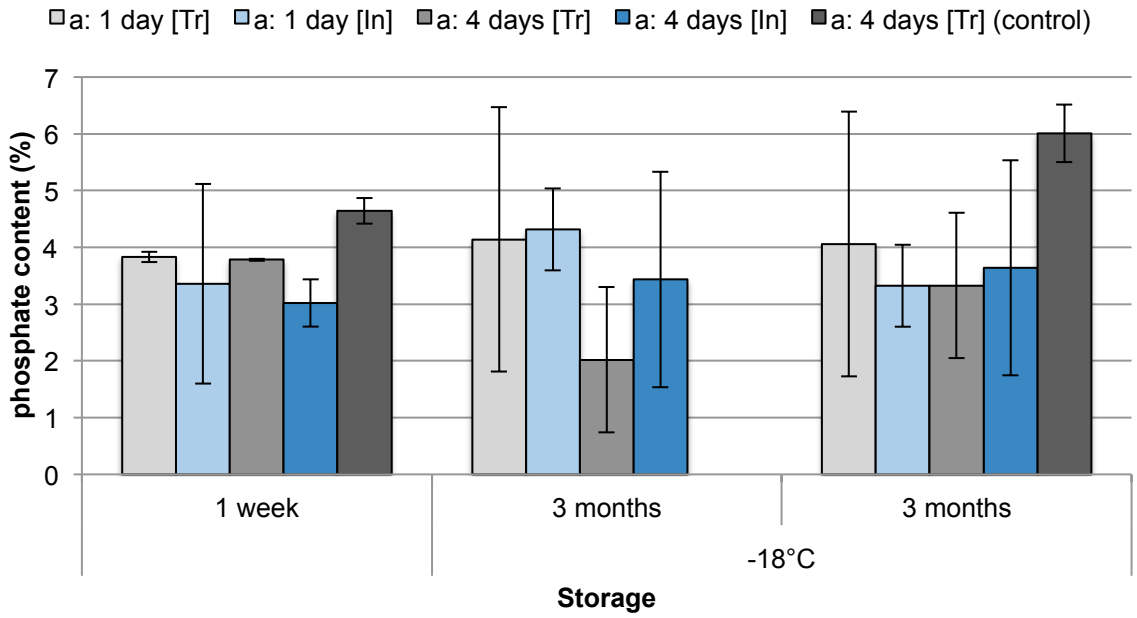

Figure 48. The average phosphate content (%) of lightly salted cod sample (n=2 per group) after 1 week at frozen storage. Samples were received from processor A and collected in July 2014. a = age of raw material, [Tr] = Traditional bleeding (blue color), [In] = Insufficient bled (grey color). The error bars represent the standard deviation of uncertainty.

The variations in phosphates content in fillets collected in October are illustrated in Figure 49. The phosphates content in the lightly salted fillets ranged from 2.4% to 3.0% compared to 4.9% to 5.0% in control fillets collected in October. Furthermore, average phosphates content for all of the lightly salted cod fillets collected in October was 2.7% and for control fillets, the phosphates content was 5%. Comparison in phosphates content between the groups and the control groups collected in October showed a significant difference ($p < 0.05$) between the large fillet group compared to the large control group.

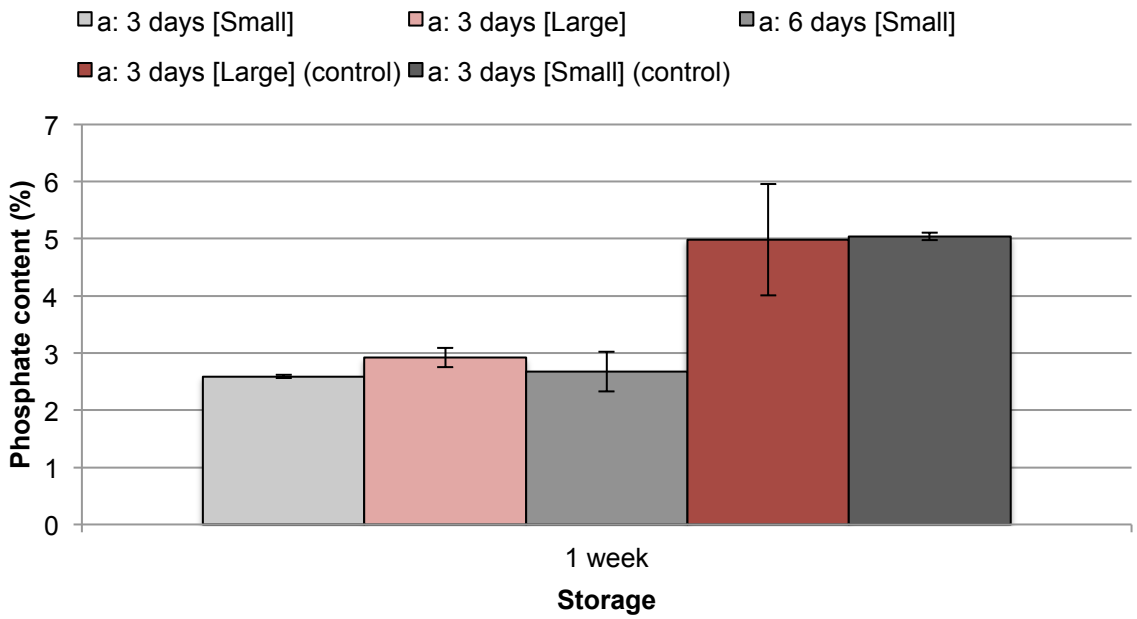

Figure 49. The average phosphate content (%) of lightly salted cod sample (n=2 per group) after 1 week at frozen storage. Samples were received from processor B and collected in October 2014. a = age of raw material, [Small] = weight of raw material: 500-1000 grams (grey color), [Large] = weight of raw material: 1000-1500 grams (red color). The error bars represent the standard deviation of uncertainty.

4.4 Correlation between different parameters

Pearson's correlation analysis was performed for the samples of cod fillets. The correlation between two variables reflects the degree to which the variables are related. The correlation coefficient and corresponding p-value are listed in Table 2 for the cod fillets collected in July and Table 3 for cod fillets collected in October. The pair of variables with positive correlation coefficients tends to increase together. For the pairs with negative correlation one variable tends to decrease while the other increases.

Table 2. Pearson's correlation between measured variables of frozen fillets collected in July 2014 and received from processor A.

	Color L	Color a	Color b	Whiteness	Drip	Cooking yield	Total yield	Water	Salt	Phosphate	WHC	Glazing	Total lipid	FFA	PL
Color L	1.00														
Color a	**− 0.98*****	1.00													
Color b	0.15	**− 0.31*****	1.00												
Whiteness	0.20*	− 0.04	**− 0.89*****	1.00											
Drip	0.18	− 0.15	− 0.10	0.14	1.00										
Cooking yield	**0.20***	− 0.15	**− 0.27****	**0.29****	**0.34*****	1.00									
Total yield	**0.23***	− 0.17	**− 0.27****	**0.29****	**0.61*****	**0.95*****	1.00								
Water	− 0.23	0.22	0.06	− 0.13	− 0.18	− 0.23	− 0.25	1.00							
Salt	− 0.17	0.13	0.24	− 0.29	− 0.14	**− 0.31***	**− 0.31***	**0.87*****	1.00						
Phosphate	0.18	− 0.19	0.05	− 0.08	0.11	0.32	0.30	**− 0.56****	**− 0.58****	1.00					
WHC	0.09	0.04	− 0.29	**0.32***	0.21	0.27	0.29	0.18	0.04	**− 0.47***	1.00				
Glazing	− 0.15	0.16	− 0.06	− 0.05	0.03	0.15	0.13	**0.33***	0.18	− 0.17	0.16	1.00			
Total lipid	0.05	− 0.02	− 0.12	0.14	0.07	0.05	0.07	− 0.28	− 0.19	0.19	0.02	**− 0.22***	1.00		
FFA	− 0.03	0.01	0.14	− 0.16	− 0.13	0.03	− 0.02	0.23	**0.29***	− 0.26	− 0.10	0.20	− 0.60	1.00	
PL	0.01	− 0.04	0.20	− 0.19	0.20	− 0.03	0.04	0.29	**0.28***	− 0.22	− 0.02	0.041	− 0.29	**0.25***	1.00

**<p0.05; **p<0.01; ***p<0.001*

Table 3. Pearson's correlation between measured variables of frozen fillets collected in October 2014 and received from processor B.

	Color L	Color a	Color b	Whiteness	Drip	Cooking yield	Total yield	Water	Salt	Phosphate	WHC	Glazing	Total lipid	FFA	PL
Color L	1.00														
Color a	**− 0.91*****	1.00													
Color b	0.30	**− 0.37***	1.00												
Whiteness	**0.85*****	**− 0.76*****	− 0.19	1.00											
Drip	− 0.04	0.08	**− 0.33***	0.10	1.00										
Cooking yield	**− 0.47****	0.31	0.07	**− 0.46****	− 0.04	1.00									
Total yield	**0.18****	**− 0.08***	0.19	0.05	0.00	**0.95*****	1.00								
Water	**0.52***	**− 0.50***	0.24	**0.52***	− 0.22	− 0.15	0.18	1.00							
Salt	**0.53***	**− 0.52***	0.23	**0.55***	− 0.25	− 0.14	0.23	**0.98*****	1.00						
Phosphate	− 0.61	**0.74***	− 0.22	**− 0.71***	0.08	− 0.11	− 0.37	**− 0.98*****	**− 0.97*****	1.00					
WHC	0.33	− 0.37	0.13	**0.44****	− 0.19	− 0.12	0.14	**0.67*****	**0.72*****	**− 0.89****	1.00				
Glazing	**0.59*****	**− 0.42***	− 0.04	**0.56*****	**0.26***	**− 0.49*****	0.08	0.26	0.23	**− 0.55****	0.24	1.00			
Total lipid	− 0.23	**0.33***	− 0.07	− 0.20	0.18	− 0.04	− 0.01	− 0.25	− 0.24	0.35	− 0.06	− 0.05	1.00		
FFA	− 0.06	− 0.05	0.23	− 0.13	− 0.07	**0.30***	0.08	− 0.22	− 0.24	0.58	− 0.09	− 0.15	**− 0.56*****	1.00	
PL	0.14	− 0.20	0.26	0.05	− 0.31	0.06	0.15	− 0.17	− 0.16	− 0.02	− 0.15	− 0.12	**− 0.56*****	**0.67*****	1.00

**<p0.05; **p<0.01; ***p<0.001*

5 Discussion

5.1 Temperature during brining

The temperature of the brine in each fish tub that was stacked up was rather stable. The tub with the large, 3 days old fillet had one logger that was much lower than the other loggers. The reason for that might be because logger 4 may have been sitting on an ice cube that was added to the brine. The temperatures inside the fillets are therefore a better indicator on what the temperature really was. Additionally, the brine temperature in that fish tub varied from -0.9°C – 5.4°C and the temperature inside the large, 3 days old fillets varied from 3.2°C – 5.8°C. The brine temperature in the small fillets, 3 days fish tub that was at the top of the stacked fish tubs varied from 1.1°C - 3.9°C and the temperature inside the fillets varied from 1.3°C – 3.9°C. The brine temperature in the 6 days old, small fillets fish tub that was in the middle of the stacked fish tubs varied from 0°C – 1.9°C and the temperature inside the fillets varied from 0.3°C – 3.1°C. The average temperature inside the fish tubs and inside the fillets are listed in Table 4.

Table 4. Average temperature inside the fish tubs and inside the fillets as well as average salt conent in the fillets and position of the fish tubs. The temperature measurement was only collected from processor B.

Position of the fish tubs	Fillets:		Average temperature		Average salt content
	Age (days)	Size	Inside the fish tub	Inside the fillets	
Top	3	Small	2.1°C	1.9°C	1.7
Middle	6	Small	0.7°C	1.0°C	1.7
Bottom	3	Large	2.3°C	3.8°C	1.6

Temperature in the middle fish tub had the lowest average temperature and that is most likely because the other two fish tubs insulated that tub. That result is in a good agreement with studies that were studying the temperature in stacked boxes (Moureh, 2002; Margeirsson, 2012; Moureh & Derens, 2000). Furthermore, since the central fish tub is better insulated to the ambient air, changes in ambient temperature effects the tub to a smaller degree than the other tubs. That is in line with the fact that the middle fish tub had the lowest temperature variation.

According to Einarsdóttir, Gudjónsdóttir, and Arason (2008) salt concentration of the brine has a greater impact on the salt uptake of the cod muscle, than the temperature of the brine. Furthermore, salt uptake of the cod muscle is faster at -2°C than at 5°C. In this study it looks like the temperature of the brine did not have an effect on the salt uptake of the fillets. However, it was not possible to do a statistical analysis between the yield of the fillets or the salt content and the temperature of the brine. Furthermore, it is also hard to make an assumption about the effect of the temperature in the brine and the salt content in the fish flesh because many other factors, like the size and the condition of the raw material, are also believed to influence salt uptake (Andrés et al., 2005; Pedro et al., 2002).

5.2 Glazing fluctuation during storage

Reducing the exposure to oxygen can reduce the rate of oxidation. This can be achieved by introducing a barrier at the surface of the fish and the addition of an ice glaze can work as the barrier. Infect ice glazing of the fish surface is a well known method to protect sea products from oxidation and dehydration during long term storage (Jacobsen & Fossan, 2001; Johnston et al., 1994).

Another argument for implementing glazing is that if the product is subject to inadequate cold storage, the glaze will evaporate instead of the tissue water itself. It has been clearly established that fluctuation in temperature while storage are a major cause of dehydration. This effect is usually much more than the one caused during freezing (Boonsumrej, Chaiwanichsiri, Tantratian, Suzuki, & Takai, 2007; Campañone, Roche, Salvadori, & Mascheroni, 2002; Campañone, Salvadori, & Mascheroni, 2001; Jacobsen & Fossan, 2001; Johnston et al., 1994). In this study, glazing prevented dehydration in fillets collected in July, because there was a moderate positive correlation between water content and glazing content ($r = 0.33$ and $p < 0.05$) in those fillets. However, there was only a weak positive correlation between glazing content and drip in fillets collected in October ($r = 0.26$, $p < 0.05$), but no correlation was found between drip loss and glazing content in fillets collected in July.

During the experimental period of storage, the samples did not show a similar glazing percentage fluctuation and both decreased and increased significantly ($p < 0.05$) theirs percentage. The results showed that 5 out of 10 groups showed significant ($p < 0.05$) glazing fluctuation within each group.

Large fillets obtained to have a lower glazing content compared to small fillets ($p < 0.05$) which is in correlation with Jacobsen and Fossan (2001) findings. According to their results, the ice glaze uptake on a product is dominated by factors like surface area/volume ratio and residence time. As a rule, the smaller size of the product, the larger the relative amount of glaze.

Glazing of seafood products is a conventional method to prevent changes in color, odor, and texture of the fish flesh (Jacobsen & Fossan, 2001). In this experiment there was a strong positive correlation between glazing content and whiteness of fillets collected in October ($r = 0.56$, $p < 0.001$). Furthermore, there was a strong positive correlation between the glazing content and the lightness of the fillets collected in October ($r = 0.59$, $p < 0.001$) and strong negative correlation between the glazing content and the redness of the fillets collected in October ($r = -0.42$, $p < 0.05$).

5.3 Drip loss

There were only 3 groups out of 10 that showed a significant difference in drip loss within the groups during storage. The drip loss of the cod fillets in this experiment had the tendency to increase during frozen storage but was however not higher for samples stored at higher temperature. Normally the drip loss tend to increase during storage and be higher for samples stored at higher temperature due to enzymes in the fish which are controlled by temperature (Huss, Boerresen, Dalgaard, Gram, & Jensen, 1995).

There was only one group that showed a significant higher drip loss compared to control group (unsalted fillets) but the control group had the tendency to have a lower drip loss compared to the lightly salted fillet groups. Thorarinsdottir et al. (2004) also showed that salt injected cod fillets had

higher drip loss, compared to control fillets. Furthermore there was no correlation between salt content and drip loss in this study. There was also no correlation between WHC and drip loss in this study. That is in contrast with previous studies. Addition of salt into fish prior freezing has shown to increase water holding capacity and decrease drip loss (Ragnarsson, 1987). Phosphates have also been used to reduce drip loss as was shown in Thorarinsdottir *et al.* (2004) study. Thaw drip has been linked to partial denaturation of proteins during freezing, which leads to decreased water holding capacity. In this study there was no correlation between drip loss and water holding capacity and also not with phosphate content.

Comparison between fillets collected in July and fillets collected in October showed a significant difference where July fillets had a much higher drip loss. The reason is most likely due to the fact that in July the cod is feeding intensively which causes a lower pH in the flesh. When pH is low in the flesh, the cod is firm and the flesh is watery and therefore not in a good condition for freezing. By October/November cod are in top condition, and yield is at a peak (Love, 2001a; Mello & Rose, 2005). Another reason for the difference between the fillets is most likely because almost half of the fillets in July where insufficiently bled compared to non-in October. Furthermore, comparison between traditional bled fillets and insufficient bled fillets collected in July, showed that the traditional bled fillets had less drip loss than the insufficient bled fillets ($p < 0.05$). Insufficient bled fillets are said to have lower flesh quality due to more risk of residual blood in the fish muscle, which may partially explain the difference (Adalbjornsson & Vidarsson, 2010; Thordarson et al., 2012). It has been claimed that thaw drip can be reduced by using high quality raw materials and good control of storage conditions (Cormier & Léger, 1987). During *rigor mortis* the glycolysis leads to formation of lactic acid and the pH is lowered. As the pH drops, the net surface charge on the muscle proteins is reduced, causing them to partially denature and lose some of their water holding capacity. A low pH value can cause rupture of the connective tissue that underlies gaping (Love, 1997). The decrease in pH occur a lot faster for insufficient bled fillets which can explain the difference between traditional and insufficient bled fillets (Huss et al., 1995).

5.4 Cooking yield (CY)

Cooking yield of food products is an important concern to the consumer. It is important for the consumer that the product does not shrink during cooking and becomes less juicy. Addition of salt before frozen storage did not improve the cooking yield in fillets compared with the control fillets. Infect there was moderate negative relationship between salt content and cooking yield in fillets collected in July ($r = -0.31$, $p < 0.05$). The results are surprising where it have been claimed that increased salt concentration increase cooking yield (Jittinadana et al. 2002).

Large fillets showed a much better CY compared to small fillets. That might be, due to the reason that muscle of larger fish is intrinsically tougher than smaller fish (Hall, 1997).

The fillets had the tendency to increase in cooking yield after frozen storage, but only 2 out of 10 fillet groups showed a significant difference in cooking yield after frozen storage within the group. That is in contrast with other studies (Thorarinsdottir et al., 2001b). It is possible that variations between

individuals can affect the results because the number of individuals measured in each time was only 5. However, the trend in almost all groups was in same direction.

Comparison between fillets collected in July and fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had much better CY. The reason for that might be due to the fact that almost half of the fillets collected in July where 1 day old before processing compared to 3 - 6 days old fillets in October. Furthermore, comparison between 1 day fillets and 4 days fillets, both also collected in July showed a significant difference ($p < 0.05$), where 4 days fillets showed a better CY compared to 1 day fillets. There might be that the 1 day fillets have not fully gone through rigor mortis. It is believed that rigor can take from 20-65 hours after death, based on the storage temperature of 0°C. Furthermore, 1 day old fillets obtained a lower value of salt content compared to 4 days fillet, that indicates that 1 day old fillets had not gone through rigor (Arason & Eyjolfsdottir, 1995). However, fillets collected in July had lost more drip during thawing and therefore should have less water to loose during cooking. There was a moderate positive relationship between drip loss and cooking yield in fillets collected in July ($r = 0.34$, $p < 0.001$). In other words, fillets collected in July that had lost more of their loosely bound water during thawing, had less water to lose during cooking.

There was no significant difference ($p > 0.05$) in cooking yield between traditionally bled fillets and insufficiently bled fillets collected in July.

5.5 Total yield (TY)

Total yield (TY) considers two steps, i.e. the drip loss and the cooking yield. Those two steps were multiplied together to get the TY.

The lightly salted fillets had the tendency to decrease in TY after 3 months of storage, but increase their TY again after 6 months of storage at -25°C. However there was only one group out of 7 that showed a significant difference ($p < 0.05$) within the group. Most of the control groups had the opposite results, because they had the tendency to increase their TY after storage but there was no group that showed a significant difference ($p < 0.05$) within the group.

3 out of 7 groups showed a difference ($p < 0.05$) in TY compared to the control group and those groups where all collected in July. The control group had the tendency to have a better yield and when there was a significant difference between control group and the lightly salted group, the control group had about 5% - 10% better yield. That is unexpected results because it has been claimed that increased salt concentration can increase yield and WHC of the muscle (Jittinandana, Kenney, Slider, & Kiser, 2002; Paterson, Parrish, & Stromer, 1988). From those results it seems like fillets collected in July are not suitable to obtain a better TY after lightly salting. Perhaps a higher salt concentration (> 2%) is required for injection into cod fillets to obtain desirable results.

There was a significant difference ($p < 0.05$) between large and small fillets, where large fillets had about 6% higher TY. This was expected, because as mentioned before, the large fillets had a much better cooking yield compared to small fillets, which of course reflects the results in total yield.

Comparison between 1 day fillets and 4 days fillets showed a significant difference ($p < 0.05$) where 4 days fillets had a better result in total yield. The reason for this is because the 4 days fillet had a better CY compared to 1 day fillets and that reflects in better TY.

Comparison between total yield in fillets collected in July and total yield in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a much higher total yield. That is a reflection of the results from the CY and drip loss where fillets collected in July, both resulted in a higher drip loss and lower cooking yield and the reasons for that are discussed in previous sections.

5.6 Color

Change in color of salted cod is a problem in the fishing industry concerning the storage of the fish. The color of the raw fish flesh is also one of the most important indicators by which consumers use to evaluate the freshness and quality of the fish (Haard, 1992; Pearson & Dutson, 1995). The L* value or lightness of the fillets was measured on a scale from 0 to 100 (from black to white). This research showed that after 3 and 6 months of frozen storage, the lightness of lightly salted cod had the tendency to increase with storage time, especially when stored at -25°C. The redness of the fillets (a* value) had the tendency to decrease with storage time while the yellowness (b* value) had the tendency to increase with storage time. The increase in yellow and red color is thought to be due to the oxidation of pigment in the fish muscle by oxygen and enzyme oxidation (Hamre, Lie, & Sandnes, 2003; Khayat & Schwall, 1983). However, there was only one group that showed a significant difference ($p < 0.05$) within the group in L*, a* and b* value during the storage time.

Lightly salted fillets collected in July had the tendency to have a lower value of lightness compared to the control fillets but only 2 out of 4 groups showed a significant difference ($p < 0.05$) compared to the control fillets. The same groups also had the tendency to have a higher a* and b* value compared to the control fillets but not all of the groups showed a significant difference ($p < 0.05$) compared to the control groups. All sample groups collected in October showed a significant difference ($p < 0.05$) in L* and a* value compared to the control groups. The a* value was higher and the L* value was lower in control fillets compared to the lightly salted fillets.

No significant difference was found in b* value between control fillets and lightly salted fillets collected in October. However, two groups in July showed a significant difference ($p < 0.05$) compared to the control fillets. Those two groups both contained 1 day old fillets, traditionally bled and insufficiently bled and the fillets in the control group where from 4 days old fish before processing. That might be the reason for the differences because the 1 day old fillets, have not gone through rigor properly and fillets that are salted before rigor has proven to be not as good as fish that is salted after rigor (Lauritzsen et al., 2004; Matís ohf, n.d.). Furthermore, comparison between 4 days and 1 day fillets showed that 4 days fillet had a lower b* value compared to 1 day fillets ($p < 0.05$).

The opposite result was obtained for fillets collected in October, where the lightly salted fillets had higher values of lightness compared to the control fillets and all of the groups showed a significant difference ($p < 0.05$) compared to the control groups. Furthermore, there was a strong positive correlation between lightness and salt content in fillets collected in October ($r = 0.52$ $p < 0.05$).

Comparison between fillets collected in July and fillets collected in October showed a significant difference ($p < 0.05$) in L*, a* and b* value of the fillets. July fillets had a much higher value of lightness, lower a* value and higher b* value. It means that July fillets was more reddish on the surface and was also more yellow. The reason for this is most likely because almost half of the fillets in July where insufficiently bled compared to October were all of the fillets were traditionally bled. Another reason might be because some of the fillets in July were processed after 1 day from catch and those fillets have probably not gone through rigor mortis.

Comparison between traditional bled fillets and insufficient bled fillets, showed a significant difference ($p < 0.05$) in L*, a* and b* value. Traditional bled fillets had a slight higher value of lightness, lower a* value and higher b* value. It is not surprising that insufficient bled fillets resulted to have a more red color because insufficiently bled fish becomes dark or reddish in the muscle and, therefore will be of lower quality. (Mercedes Careche et al., 1998; Careche et al., 1999; Chichester & Stewart, 1981; Digre et al., 2011; Kim & Hearnsberger, 1995; Lauritzsen et al., 1999; Sikorski et al., 1976).

Comparison between large and small fillets, showed a significant difference ($p < 0.05$) in L* value where small fillets resulted in higher value of lightness.

Generally the whiteness of the fillets was rather stable during the storage time. Comparison between whiteness in fillets collected in July and whiteness in fillets collected in October showed a significant difference ($p < 0.05$) where October fillets had a higher value of whiteness. There was a moderate positive correlation between whiteness and WHC in fillets collected in July and strong positive relationship in fillets collected in October ($r = 0.32$, $p < 0.05$ and $r = 0.44$ and $p < 0.01$ respectively). That might be an explanation why fillets collected in October had a higher value of whiteness compared to fillets collected in July, because fillets collected in October had a much higher WHC compared to fillets collected in July. The main reason is probably due to the fact that almost half of the fillets in July are 1 day old fillets and almost half of the fillets are also insufficiently bled.

Phosphate is said to give a whiter fish (Thorarinsdottir, Bjørkevoll, & Arason, 2010) that is in contrast with findings in this study where a very strong negative relationship was found between phosphate content and whiteness ($r = -0.71$ $p < 0.05$). Furthermore, there was a very strong positive correlation between phosphate content and yellowness in fillets collected in October ($r = 0.74$ $p < 0.05$).

5.7 Water holding capacity (WHC)

The water holding capacity (WHC) is one of the quality factors which the consumers appreciate. The WHC of lightly salted cod in all groups stored at -18°C and -25°C did not change significantly ($p > 0.05$) during the storage period. Frozen storage, in particular, has been found to decrease the water holding capacity. Throughout the whole freezing process the muscle cells shrink, causing liquid to leak out of the cells to inter-cellular space (Bello et al., 1981; Estévez, 2011; Hurling & Mcarthur, 1996; Mercier, Gatellier, & Renerre, 2004; Mariana Utrera, Rodríguez-Carpena, Morcuende, & Estévez, 2012; Utrera, Armenteros, Ventanas, Solano, & Estévez, 2012; Xia, Kong, Liu, & Liu, 2009). The relatively small sample size and therefore low statistical power may have affected the strength and

associations of the findings and the probability of finding a statistically significant difference between groups.

Comparison between fillets collected in July and fillets collected in October showed a significant difference ($p < 0.05$) in WHC. Fillets collected in October had much better water holding capacity compared to fillets collected in July. It has been found that the WHC of fish is influenced by season and is often related to the nutritional status affecting the ultimate muscle pH. Intensive feeding leads to low muscle pH, which results in a low WHC, which is most likely the case with the fillets collected in July (Ang & Haard, 1985; Ingolfsdottir, Stefansson, & Kristbergsson, 1998). There was no statistical difference ($p > 0.05$) between WHC in 1 day old fillets and WHC in 4 days old fillets. That was not expected because fish that is salted before rigor, is expected to have a lower WHC. Additionally, there was no statistical difference ($p > 0.05$) between WHC in traditionally bled fillets and insufficiently bled fillets. That underlines that the reason for the difference between October and July fillets in WHC is most likely due to the fact that July fillets are in worse condition nutritionally as mentioned before.

In recent years the additions of polyphosphates to seafood products have been used to improve the WHC in the muscle. Surprisingly, in this experiment there was a strong negative correlation between WHC and phosphate content of fillets collected in July and a very strong negative correlation in fillets collected in October ($r = -0.47$ and $p < 0.05$, $r = -0.89$ and $p < 0.01$, respectively). That means that when phosphate content decreases the WHC content increases. Those results are in contrast with other findings (Lindsay, 1996; Offer & Knight, 1988; Thorarinsdottir et al., 2001b).

Salting is known to have a major impact on the water holding capacity. Increase in salt concentration up to a certain level (6%) leads to increase in water holding capacity (Hamm, 1960; Offer & Knight, 1988; Thorarinsdottir et al., 2001). The salt content in the lightly salted fillets was 1.6% - 1.9%. In this experiment there was a very strong positive correlation between WHC and salt content in fillets collected in October ($r = 0.72$ and $p < 0.001$). However, comparison of WHC between the control fillets and the lightly salted fillets was not significant ($p > 0.05$).

It is interesting that there was a strong positive correlation between WHC and water content, only in fillets collected in October ($r = 0.67$ and $p < 0.001$).

5.8 Water, salt and phosphates content

The water content in the lightly salted cod ranged from 84.0% to 87.4% compared with 80.7% to 81.6% in control fillets. Comparison in water content between the lightly salted fillet groups and the control groups showed a significant difference ($p < 0.05$), where the control group resulted in a lower value of water content. The salt content in the muscle of lightly salted cod can explain this. According to Offer and Trinick (1983), Wilding et al. (1986) and Slabyj et. al (1987) the salting is used to increase water binding and obtain higher yields. As expected there was a very strong positive relationship between salt and water content both in fillets collected in July and in October ($r = 0.87$ and $p < 0.001$, $r = 0.98$ and $p < 0.001$, respectively). Furthermore the lightly salted fillets were also injected with water and salt in the salting process, that might be the explanation.

The water in fresh fish muscle is tightly bound to the proteins in the structure but after prolonged chilled or frozen storage, however, the proteins are less able to retain all the water. Salting of foods leads to increased diffusion of water and swelling of the muscle fibers (myofibrils), thus changing the water distribution in the tissue and muscle properties. Salting is, therefore, a good method to decrease water drip during storage and so is often used prior to freezing in order to counteract the negative effects of freezing (Bocker et al., 2008; Fennema, 1990; Offer & Trinick, 1983; Thorarinsdottir et al., 2004). In this study, there was no significant difference ($p > 0.05$) in water content within the groups during frozen storage.

The water content in lightly salted fillets collected in July and in October was similar. The water content in fillets in July has been documented to be higher than in fillets collected in October (Love, 2001a). The reason for why there was no difference ($p > 0.05$) between water content in lightly salted fillets collected in July and in October may be due to the fishing technique used. The fishing technique used in October was trawl compared to longline in July and it has been reported that cod caught by trawl have a lower moisture content than cod caught with longline (Botta & Bonnell, 2006).

The salt content in the lightly salted fillets ranged from 1.4% to 2.3% compared to 0.2% to 0.4% in control fillets. As expected, there was a significant difference ($p < 0.05$) between the lightly salted fillet groups and the control groups.

Comparison between traditional bled fillets and insufficient bled fillets, both collected in July showed a significant difference ($p < 0.05$) where the traditional bled fillets obtained in higher value of salt content. There was also a significant difference between 1 day and 4 days fillets collected in July, where 4 days fillets had a higher value of salt content. That underlines the fact that many factors, including quality and condition of the raw material are believed to influence the quality and characteristics of the final product (Andrés et al., 2005; Pedro et al., 2002; Thorarinsdottir et al., 2001; Thorarinsdottir et al., 2004).

One of phosphates main value is to improve the retention of water by the protein in fish (Aitken, 2001). According to Thorarinsdottir et al. (2001) the phosphate increases the yield after the salting process by increasing the water intake. However, in this study there was no correlation between yield and phosphate content, but there was a correlation between water holding capacity and phosphate content in this study, as discussed in previous section. The phosphates content in the lightly salted fillets ranged from 2.0% to 5.8% compared to 4.8% to 6.4% in control fillets. Comparison in phosphates content between the lightly salted fillet groups and the control groups only show a significant difference ($p < 0.05$) in one group, but the control groups had a tendency to have a higher value of phosphates content. It is therefore obvious that the processors are using brine containing phosphates in the salting process because it has been confirmed that natural phosphorus are lost during the salting process (Bjørkevoll et al., 2012). Furthermore, there was a strong negative relationship between salt and phosphate content in fillets collected in July and a very strong negative correlation in fillets collected in October ($r = -0.58$ and $p < 0.01$, $r = -0.97$ and $p < 0.001$, respectively). There was also a strong negative relationship between water and phosphate content in fillets collected in July and a very strong negative correlation in fillets collected in October ($r = -0.56$ and $p < 0.01$, $r = -0.98$ and $p < 0.001$, respectively). The results were not in correlation with other studies where increase

in phosphate content decreased the water content (Bjørkevoll et al., 2012; Gudmundsdottir et al., 2003).

5.9 Lipid content and free fatty acids (FFA)

Changes in lipids during frozen storage of fish can, directly or indirectly, lead to quality deterioration. Lipid contents ranged between 0.2% to 1.5% in July and 0.4% to 2.1% in October. Differences obtained could be explained as a result of lipid content variations among individual fishes and not as a result of salt treatment or frozen storage time. The fat content varies with season and is also influenced by the size of the fish (Botta & Bonnell, 2006; Dambergs, 2011). The fat content for cod is higher in October than in July (Ingolfsdottir et al., 1998). As expected, total lipid (TL) content was significantly higher ($p < 0.05$) in fillets collected in October (average TL = 0.7%) compared to fillets collected in July (average TL = 0.5%). The fish collected in July is most likely in its heavy feeding state, after the spawning period, where the fish regenerate its fat supplies (Botta & Bonnell, 2006; Dambergs, 2011; Love, 1962, 2001a).

The effect of the frozen storage time on total lipid content measured, showed a significant difference ($p < 0.05$) in 3 out of 7 lightly salted fillet groups and in all of the control groups. The fillets usually showed a general increase in lipid content after 3 months of frozen storage, especially in -25°C, but a decrease after 6 months. Furthermore, the lipid content decreased more in -25°C compared to -18°C. The reason for the increased lipid content after frozen storage may be due to the fact that it was easier to remove the lipid from the fish after frozen storage.

The effect of frozen storage time on free fatty acid (FFA) content measured of total lipid as oleic acid, showed a significant difference ($p < 0.05$) in traditionally bled, 1 day and 4 days old fillets collected in July. The fillets had the tendency to increase in FFA formation as a result of frozen storage, indicating extensive hydrolysis of lipids. Accumulation of FFA is said to contribute to off flavor of the product and cause textural alterations by complexing with proteins (Mai & Kinsella, 1980). Due to lipid hydrolysis, FFA accumulate in the tissue during frozen storage, especially at high temperatures around -10 to -20°C (Aubourg & Medina, 1999; Aubourg et al., 2004; Rodríguez et al., 2007). In present study, the FFA content in fillets collected in July increased more at -18°C compared to -25°C. However, the FFA content in fillets collected in October increased at higher level in samples stored at -25°C compared to -18°C. The reason for the higher FFA content in fillets collected in October stored at -25°C compared to -18°C, can be because of fluctuating storage temperature that can result in the lysis of lysosomes and thereby increased activity of some endogenous lipases resulting in increased rated of FFA accumulation (Geromel & Montgomery, 1980).

Insufficient bled fillets had higher value of FFA compared to traditionally bled fillets ($p < 0.05$), both collected in July. There is more likelihood of residual blood in insufficient bled fillets compared to traditionally bled fillets. Therefore those results are expected, where lipid oxidation is accelerated by hemoglobin, which is one of the predominant hem proteins in red blood cells (Hultin, 1994; Richards, 2000).

In the present study, no significant difference ($p > 0.05$) was found between lightly salted fillets compared to control fillets. However there was a weak positive correlation between FFA and salt content in fillets collected in July ($r = 0.29$ and $p < 0.05$).

Phospholipids (PL) undergo faster hydrolysis and oxidation than neutral lipids and though lean species only contain up to 2% lipids, most of these are phospholipids, making them prone to oxidation despite the low lipid content (Han & Liston, 1987). During frozen storage, lipid oxidation occurs and can cause quality deterioration such as yellow/brownish discoloration of flesh surface. In present study, all fillet groups showed a significant difference ($p < 0.05$) in PL content within each group, except the control group collected in July. PL content in fillets had the tendency to decrease after 3 months of frozen storage and increase after 6 months of frozen storage in fillets collected in July but increase after 3 months of frozen storage in fillets collected in October. The changes were more dramatic in -25°C compared to -18°C.

Salt content within fish has been reported to enhance lipid oxidation of the highly unsaturated lipids directly related to the production of off flavors and odors, protein denaturation, and texture changes (Ackman, 1989; Davis, Goodwin, Smith, & Hole, 1993; Hsieh & Kinsella, 1989; Mackie, 1993; Takiguchi, 1989). Almost all lightly salted fillet groups collected in July showed a significant difference ($p < 0.05$) in PL content compared to the control group, where the lightly salted fillets had a higher PL content. Furthermore, there was a weak positive correlation between salt content and phospholipid content in fillets collected in July ($r = 0.28$ and $p < 0.05$) in this study. However, comparison between lightly salted fillets collected in October compared to the control groups, showed no significant difference ($p > 0.05$) and no correlation was found between salt content and PL in fillets collected in October.

6 Conclusion

The results indicate that different bleeding method have an effect on a product quality after frozen storage. Insufficient bled fillets have more drip loss, lower lightness, higher redness (a* value) and lower yellowness (b* value) and therefore lower whiteness compared with traditional bled fillet. Insufficient bled fillets also obtained a lower value of salt content and a higher value of free fatty acid compared with traditional bled fillet.

The age of the fillet before processing was another parameter that affected the product quality. Four days old fillets, before processing, had better cooking yield, total yield, less yellowness and absorbed salt better than when the fillets were 1 day old before processing. This can mainly be due to the fact that 1 day fillets have not fully gone through rigor mortis. It is believed that rigor can take from 20-65 hours after death, based on the storage temperature of 0°C

These findings above, indicate that a good quality fillets for processing are fillets that were traditionally bled and more or the same as 3 days old before processing

Glazing was a successful method for reducing the drip loss, but only in good quality fillets. Glazing was also successful method for preventing dehydration in bad quality fillets. Moreover, glazing was a conventional method for preventing changes in lightness, reduce redness and therefore also preventing changes in whiteness of lightly salted cod fillets.

The effect of different fillet sizes on product quality after frozen storage was obtained. Large fillets had a higher glazing content, lighter fillet color, much better cooking yield and therefore total yield compared to small fillets.

There was a big difference between season groups on stability of lightly salted fillets in frozen storage. Lightly salted fillets in October had a whiter appearance, better water holding capacity, higher lipid content, higher phospholipid content and lower drip loss, better cooking and total yield compared to fillets collected in July. However, the water content were similar between the season groups. But it must be kept in mind that all fillets collected in July were small, half of them were insufficiently bled and half of them where only 1 day old before processing. Meanwhile, the fillets collected in October were all traditionally bled, one third were large fillets and the age of the fillets were 3 or 6 days old before processing.

Lightly salted, good quality fillets had higher value of lightness and whiteness compared to control fillets that were not salt treated. Lightly salted, good quality fillets also had a higher water holding capacity and there was a positive correlation between WHC and salt content in those fillets compared to untreated control fillets. However, light salt injection did not have any effect on drip loss in any of the lightly salted fillets compared to the untreated control fillets. Furthermore, lightly salted, good quality fillets did not have any effect on cooking yield and decreased cooking yield in most fillets collected in July. As expected, salting did have an effect on water content, as more salt content in the fillets resulted in more water content.

Phosphate content in the fillets did not have any effect on yield nor drip loss. The higher content of phosphate in the fillets resulted in lower water content and also water holding capacity. Furthermore,

phosphate content had effect on the yellow color of the fillet, by making the fillets more yellow with increased phosphate content, and therefore less whiteness, but only in fillets collected in October.

The drip loss of the lightly salted cod fillets had the tendency to increase during frozen storage but was however not higher for samples stored at higher temperature. The lightness of lightly salted cod fillets had the tendency to increase with frozen storage time, especially when stored at -25°C. The redness of the fillets had the tendency to decrease with storage time while the yellowness had the tendency to increase with storage time. The WHC and the water content of lightly salted cod in all groups stored at -18°C and -25°C did not change during the storage period. PL content in fillets had the tendency to decrease after 3 months of frozen storage and increase after 6 months of frozen storage in fillets collected in July but increase after 3 months of frozen storage in fillets collected in October. The changes were more dramatic in -25°C compared to -18°C. Furthermore, the fillets had the tendency to increase in FFA formation as a result of frozen storage, indicating extensive hydrolysis of lipids. The increase in FFA content in fillets collected in July increased more at -18°C compared to -25°C. However, the FFA content in fillets collected in October increased at higher level in samples stored at -25°C compared to -18°C.

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