UNIVERSITY OF NORDLAND

MASTER THESIS

An investigation of the biochemical, microbiological and quality changes during ice storage of Atlantic salmon (Salmo salar)

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AK306F MSc in AQUACULTURE

Faculty of Bioscinces and Aquaculture
May 2014

Acknowledgment

Foremost, I would like to express my very great and deep appreciation to my supervisor Professor Marit Bjørnevik, for her infinite patience and enthusiasm, extensive knowledge, the valuable and constructive suggestions and advices during the development of this project. Words cannot express my gratitude for your encouragement and willingness to help me all over the time of my research and writing of this thesis, especially during last weeks. I cannot imagine having a better supervisor and mentor than you and it was a great opportunity to work with you.

Special thanks to my co-supervisor Professor Ørjan Hagen for your extensive knowledge and undeniable help to run the analyses of enzymatic activities. I am grateful for all your guidance. I learnt a lot from you how to work with maximum efficiency.

My hearty thanks to Professor Christel Solberg for her great enthusiasm, abundant and gigantic support and advices for PCA analysis. It was happiness for me to work with you. Your deep and extensive knowledge inspired me during the entire project.

My heartfelt gratitude also extends to Chris Andre Johnson for his help during chemical analyses and histological studies, Renate Karlsen for their help in microbiological studies.

My sincere gratitude goes to the staff in the FBA for providing a good working environment and giving me warm welcome.

I am indebted to Kristin Dahlen and Mainstream, Norway, for providing support. I would like to offer my sincere thanks to the Norwegian Government for offering me a scholarship under the Quata Program.

Most special thanks to my parents, grandfather, dearest brother and my friends for your support, love and understanding.

Abstract

Over the last years, Atlantic salmon has become one of the most intensively farmed fish species, however, complains about softening and gaping have increased.

The main goal of this Master thesis was to characterize some of the biochemical, microbiological and flesh quality changes taking place in ice stored Atlantic salmon during two different seasons. In addition the effect of early and late loading of fish from the harvest well boat was to be explored.

Farmed Atlantic salmon were sampled from Mainstream (Norway) and stored on ice for 22 days in February (winter, n=60) and October (autumn, n=60) 2014. Various biochemical characteristics affecting the quality of farmed Atlantic salmon harvested in winter and autumn were studied. The quality index, color, texture, fillet gaping, liquid loss, pH, proximate composition, microscope observation and cathepsin B+L/H activities were performed at 1, 4, 7, 10, 16 and 21 days of ice storage in winter sampling and 1, 5, 8, 12, 15 and 22 days on ice in autumn sampling. In addition to these analyses total volatile nitrogen (TVN) was analyzed at day 1, 10 and 22, and total bacteria count (TBC), and specific spoilage bacteria analyzed at day 10 and 22 of ice storage experiment (specific spoilage bacteria in autumn only).

The results showed that among all parameters QI scores, liquid loss, fillet gaping, total bacteria count, specific spoilage bacteria and the myofibre-myofibre detachment increased significantly with storage time ($p<0.05$) in both sample periods. Texture of salmon decreased significantly from day 1 to 4 and thereafter remained stable throughout the storage in both sample periods. Only small changes were observed in protein content and flesh color a* and b* values, while no significant changes were found in fat and water content, flesh lightness, TVN and cathepsin activity in respect to ice storage in both autumn and winter sampling. Season did not have any significant influence on the quality parameters with exception of the QI being higher in winter compared with autumn. Fish sampled at late loading had a slightly softer texture, higher liquid loss and lower cathepsin activity compared with early loading, suggesting a small stress effect from loading procedures.

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1. Introduction

1.1 Atlantic salmon farming

Atlantic salmon (*Salmo Salar*) is one of the most intensively farmed species in modern industrialized aquaculture (Asche and Bjørndal 2011). Atlantic salmon is among top species in most major markets and consumed all over the world, especially in the European Union and USA, due to its delicious taste, nutritious, high quality content of protein and marine omega-3 fatty acids. Today, farming of Atlantic salmon is a global industry, and the share of salmon in world production has increased substantially during the last 25 years. At the beginning of the 1980s, the supply was mainly from wild salmon (Asche and Bjørndal 2011). Today, all commercially available Atlantic salmon is farmed (MarineHarvest 2012). Atlantic salmon farming started on an experimental level in Norway in the 1960s, and became commercial in the 1980s (Stickney, 1991). The global production of Atlantic salmon was nearly 1.5 million tons in 2010 (Stickney 1991, FAO 2012). Production of farmed Atlantic salmon considerably increased in Norway in the last decades, with brief stops in 1986-1987, 1990-1992 and 2001-2002, mostly as a result of diseases. Norway produced 1.18 million tons in total and export 965 100 tons to the EU, Russian and Asian markets in 2012 (FAO 2012). Markets for farmed Atlantic salmon have expanded geographically. The EU and the US are the largest traditional markets for Atlantic salmon.

Production of farmed salmon is essentially dominated by Norway. Among various product forms, fresh and smoked salmon are the most sought-after in the European market. Fresh salmon is in the great request and provides the highest price to the producer. However, Norwegian fresh salmon meet competition from cheaper Chilean frozen salmon in the European and US market. Two-thirds of the salmon are sold as a fresh (fillet or whole fish) and one third as frozen and smoked (MarineHarvest 2012). In spite of this it is expected that fresh salmon will continue to be the preferred form in the market. Quality of fresh salmon is one of the factors affecting the price of the final product and consumer acceptance. A major part of Norwegian salmon is sold as gutted and iced. In later years there has been an increasing focus on exports of fillets. Post-rigor filleting is more common, the fish being filleted 4 - 5 days after slaughter of ice storage. However, it is suggested that pre-rigor processing affects positively on the quality characteristics, therefore, tendency toward to pre-rigor filleting increases (Veiseth-Kent, Hildrum et al. 2010). The loss of salmon quality occurs between harvest and consumption (Kestin and Warriss 2000). At most processing

plants, packing and transporting of salmon to the markets are occurring on the same day (Erikson, Misimi et al. 2011). Sveinsdottir, Martinsdottir et al. (2002) determined that maximum shelf-life of salmon is 24 days in ice. During storage up to 3 weeks, spoilage becomes evident. Loss of freshness and spoilage during storage and transporting of fish creates problems for producers. Freshness as well as food safety of fish products is a key quality aspect of fish and fish products. To decrease the degradation during transport from the fish cage to the market is of large importance. During the last years, complains about quality degradation associated with softening and gaping in fresh fish harvested during autumn and winter time have increased (Johnsen, Hagen et al. 2011). In addition to the seasonal effect, the ice storage can result in the loss of flesh quality. Present work is focused on changes in various attributes of salmon quality related with season, storage and loading.

1.2 Challenges during salmon production, stress effect

Production of farmed Atlantic salmon in Norway and all over the world includes few steps. Among them, transport of live fish in boats from the sea cages to the plants at high densities, loading, capture, netting or pumping of the fish might have negative effects on flesh quality and physiological reactions (Erikson, Sigholt et al. 1997). It is well-documented that loading and the first few hours of transport created the greatest stress (Erikson, Sigholt et al. 1997). According to numerous researches stressed fish can show a propensity to rapid drop in pH, poor color and liquid-holding capacity, gaping and soft texture (Erikson, Sigholt et al. 1997, Skjervold, Fjæra et al. 2001, Matos, Gonçalves et al. 2010, Merkin, Roth et al. 2010). After slaughter, one of the most significant changes of muscle is the drop in muscle pH *post mortem*. Short-term stress leads to a faster drop of muscle pH (Skjervold, Fjæra et al. 2001). It is known that low pH associated with accumulation of lactic acid leads to the soft texture, gaping, high cathepsin activity and liquid loss (Love 1988, Erikson, Sigholt et al. 1997, Einen and Thomassen 1998, Skjervold, Fjæra et al. 2001, Bahuaud, Mørkøre et al. 2010). Einen and Thomassen (1998) suggested that duration of pre-slaughter stress affects the texture, where short-term stress have demonstrated softening of fish muscle, whereas the long-term stress increases the muscle firmness. Moreover, changes in freshness of fish is also associated with stress, where stressed fish showed reducing fish freshness with stress compared to unstressed fish (Erikson, Sigholt et al. 1997, Ådland Hansen, Rødbotten et al. 2012). Stress and struggle of fish before or at slaughter adversely affect the fish quality. Therefore, it is interesting to know how early and late loadings from the well boat affects the sensorial and biochemical attributes of fish quality.

1.3 Muscle architecture

The swimming muscle of teleost fish is the most abundant tissue; making up 60 % of the total body mass, 65% in Salmonids (Johnston 2001). The body musculature of fish is a series of segmental myotomes of complex shape separated from each other by collagenous sheets, called myosepta (Hoar and Randall , Johnston, Alderson et al. 2000, Johnston, Manthri et al. 2004). Muscle fibres are orientated at angles up to 40^{o} to the longitudinal axis and, hence, myotomes have complex three-dimensional structure (Fig. 1). Myotomal shape is the deep W-shape and the orientation of fibres is not random to contract at about the same rate (Hoar and Randall).

Fish musculature is composed of bundles of muscle fibres (long multinucleated cells). Each muscle fibre is composed of bundles of myofibrils containing the contractile materials, myosin and actin filaments. The muscle fibre number and size are one of the characteristics affecting the flesh texture. It is believed that the muscle fibre size and fibre density is correlated to the firmness of the fish flesh (Johnston, Alderson et al. 2000, Periago, Ayala et al. 2005). According to various histological studies myofibrils are very stable during ice storage (Taylor, Fjaera et al. 2002, Ofstad, Olsen et al. 2006). However, myofibre-myofibre detachments might be associated with softening due to loss of hardness (Taylor, Fjaera et al. 2002). Each fibres and groups of fibres are surrounded by connective tissue. Fish muscles contain less collagen and have fewer cross-links than mammalian flesh (Luther, Munro et al. 1995, Johnston, Alderson et al. 2000). Collagen has significant effect on properties of fish flesh contributing tensile strength (Espe, Ruohonen et al. 2004). During ice storage, it seems that weakening of connective tissue and disintegration of collagen fibrils results in softening of fish muscle and gaping (Love 1988, Eckhoff, Aidos et al. 1998). Decreasing of solubility of collagen, especially of collagen V is associated with rapid softening of fish muscle (Espe, Ruohonen et al. 2004).

The muscle fibres in most fish can be divided into 2 major types, white fast twitch and red slow twitch fibres Two types are distinguished by histological, ultra structural and biochemical features such as isoforms of myosin, mitochondria content, myoglobin content and degree of capillarization (Hoar and Randall , Johnston 1999, Johnston 2001, Johnston, Manthri et al. 2004). The proportion of white and red muscles may change with fish size. However, white muscles comprise the main bulk of the fish, where the fibres are tightly packed (Johnston, Alderson et al. 2000, Johnston 2001). White muscles utilize anaerobic metabolism for high speed swimming. The red muscle using fatty acids and pyruvate for

energy supply through aerobic oxidation for routine activity (Johnston 1999). Red muscle rarely exceed a quarter of the total cross section of fish, in most fishes less than 10 %, and cover the axial muscle composed mainly of slow fibres (Johnston 1999, Johnston, Manthri et al. 2004). Red muscles are well supplied with capillaries and contain a high density of mitochondria (Hoar and Randall , Johnston 2001). Some species have intermediate layer of pink muscle (Johnston 1999, Johnston, Alderson et al. 2000).

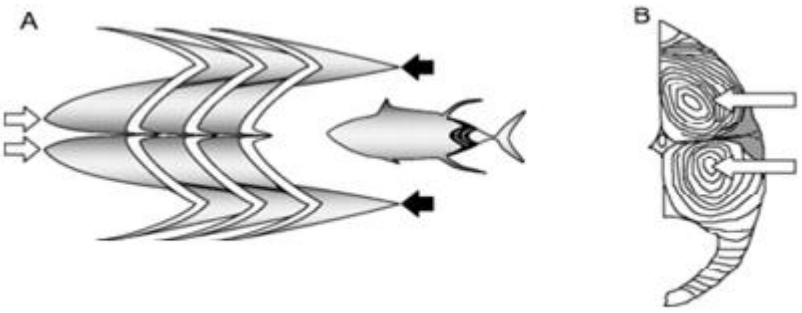


Figure 1(A) Structure of the myotomes. This diagram shows myotomes each separated by the removal of intervening myotomes. (B) Diagram of the in cross section, or steak view. The red, oxidative muscle is in a small wedge in close apposition to the skin along the mid-lateral line. The remainder of the muscle consists of white, glycolytic fibers within the cones. (Katz 2002)

1.4 The QIM

Freshness associated with quality of fish is a key quality aspect of fish products for consumer preference and acceptance. Storage of fresh fish and fish products at ambient temperature can lead to rapid spoilage and deterioration and, hence, shorter shelf life and economical losses (Ganesan, Jeyasekaran et al. 2005). Chilling method is used to extend the shelf life and allows transporting fish products to distant markets (Cyprian et al., 2008). Ice is convenient chilling medium for storage of fresh fish. Ganesan, Jeyasekaran et al. (2005) estimated that 6 hours delayed icing decreased the shelf life by 5 days due to biochemical, sensory and microbiological changes. Reduction in the storage temperature can increase the shelf life of fish. However, delayed icing is common practice during processing in industry (Ganesan, Jeyasekaran et al. 2005). The sensory assessment is important due to rapid expansion of salmonid market last decades. The Quality Index Method (QIM) is a freshness grading system and most convenient method for rapid salmon quality and freshness assessment and estimation of the remaining storage time in ice (Sveinsdottir, Martinsdottir et al. 2002). The QIM scheme is developed for various species (Sveinsdottir, Martinsdottir et al. 2002, Ganesan, Jeyasekaran et al. 2005, Cyprian, Sveinsdóttir et al. 2008).

The sensory attributes mainly include external characteristics of fish such as appearance, odor and texture changing through the storage time. The end of shelf life is marked by evident spoilage. The sum of scores evaluated according to QIM presences as the Quality Index (QI) (Sveinsdottir, Martinsdottir et al. 2002). It is estimated that average quality index (QI) score is significantly correlated with days in ice (Sveinsdottir, Martinsdottir et al. 2002). The QI score is the lowest after catch and increases with the storage time reaching maximum score at the end of shelf life (Cyprian, Sveinsdóttir et al. 2008). The QI score for Atlantic salmon goes from 0 to a maximum of 22 when the fish has reached the maximum of its shelf life and is spoiled (Sveinsdóttir et al. 2002).

1.5 Flesh quality characteristics

Flesh quality of fish is becoming more important for the consumer preference and acceptance and, hence, more interesting for the producers. High quality of products needs to expand in the market. However, requirements of quality may vary from country to country and, moreover, consumer preference for quality may also change time to time. A variety of quality schemes have been developed (Laird, Stead et al. 2002). Nevertheless, good quality begins at the farms, during processing and storage of products. Flesh quality can be determined as combination of variety of characteristics such as appearance, smell, taste, color, texture, nutritional value and hygienic quality (Cyprian et al. 2008; Fernández et al. 2009). Flesh quality varies between seasons and storage time (Espe et al. 2004). Quality attributes change during ice storage resulting in quality deterioration and spoilage of fish by the end of shelf-life.

1.5.1. Chemical composition

The chemical content of farmed Atlantic salmon is mainly water, fat and protein, and varies with size, age, maturation, season and nutrition (Haard 1992, Bahuaud, Mørkøre et al. 2008). Various reports indicated that the protein content of salmon is found between 18 and 20%, whereas the total fat content in slaughter sized fish varies from 10 to 14% (Haard 1992, Rørå and Einen 2003). Water and fat in muscle are inversely related and make up to 80% of the muscle weight (Haard 1992). The fat content decreases with increasing water content (Mørkøre, Hansen et al. 2002).

Chemical composition greatly affects the parameters of quality of fish such as texture and flavor (Katikou, Hughes et al. 2001, Robb, Kestin et al. 2002). The amount of fat as well as

water and protein level affects the quality of fish. The fat level and its distribution through the fillet affect the texture and can be associated with a soft texture and gaping (Haard 1992, Aussanasuwannakul, Kenney et al. 2011).

During ice storage, only small changes are observed in the chemical composition (Hultmann and Rustad 2004). For instance, the total protein content is very similar because of most of the muscle protein is composed of myofibrillar protein, however, minor changes might be observed due to slow protein detenaturation (Fauconneau, Alami-Durante et al. 1995, Berg and Bremset 1998, Laird, Stead et al. 2002). Fat content increases with size and age of fish compared to protein content. The fat content of farmed Atlantic salmon varies with oil content in the diet, season and maturation (Haard 1992). High content of dietary oil results in an increasing lipid level in fish muscle (Robb, Kestin et al. 2002). In respect to Berg and Bremset (1998) salmon tend to get fattier in winter compared to summer due to maturation status whereas the protein content is stable. However, farmed Atlantic salmon showed less seasonal variation in the fat level of flesh than wild fish (Haard 1992).

1.5.2 Texture

The texture of fish is considered to be an important quality parameter (Chéret, Delbarre-Ladrat et al. 2007, Godiksen, Morzel et al. 2009). Firm fish flesh muscle is more sought-after for consumers. Texture-related problems such as softening and gaping create problems during processing, impair quality of final products and hence marketability. Texture is a complex trait depending on various factors. Softening and gaping can be affected by fat content, season, handling before slaughter and storage conditions (Lund and Nielsen 2001, R.G., O. et al. 2002). The textural post-mortem changes seen in fish are results of breakdown of myofibrillar proteins, extracellular matrix and collagen fraction (Andersen, Thomassen et al. 1997).

1.5.3 Fillet gaping

Fillet gaping is the result of disconnection in myocomata due to breakdown of the sarcolemma membrane. The incidence of gaping results in an economic loss of 5 - 10 % (Skjervold, Bencze Rørå et al. 2001).

The degree of fillet gaping can be described by interaction of various factors. Muscle fibre size, muscle pH, fat level and its distribution through the fillet and are important factor affecting the development of gaping (Kestin and Warriss 2000). Gaping might be affected by

season with the highest score during spring and summer (Mørkøre, Hansen et al. 2002). Gaping is associated with degradation of collagen fibres resulting in loss of the firmness (Espe, Ruohonen et al. 2004).

According to Skjervold, Bencze Rørå et al. (2001) the pre-rigor filleted fillets have lower degree of gaping compared to post-rigor fillets. Processing of in-rigor fillets have the highest gaping scores (Skjervold, Bencze Rørå et al. 2001). Degree of gaping increases with storage time. One of the recommendations is immediately processing and chilling of fish products to avoid gaping (Skjervold, Bencze Rørå et al. 2001).

1.5.4. Color

Deep pink color of salmon flesh is an important quality characteristic that determines consumer preferences and choice. Color is associated by consumers with freshness of products. The color is significantly correlated with concentration of pigments (Bahuaud, Mørkøre et al. 2010, Yagiz, Kristinsson et al. 2010). Pigmentation of salmon flesh is determined by deposition and concentration of oxygenated carotenoids. The one of the main pigments is astaxanthin providing salmon characteristic orange color that cannot be synthesized *de novo*. Therefore, astaxanthin is absorbed from the diet. The concentration of astaxanthin varies between 4-10 mg kg^{-1} and 3-11 mg kg^{-1} in farmed and wild salmon, respectively (Johnston, Li et al. 2006). The deposition of astaxanthin depends on genetic origin, age, maturation, growth rate (Johnston, Li et al. 2006). Moreover, it was believed that number and size distribution of fibres can affect the astaxanthin concentration trough number of astaxanthin binding sites (Nickell and Bromage 1998). According to Bahuaud, Mørkøre et al. (2010) color intensity in salmon flesh is dependent on storage time and harvesting seasonal periods. Bahuaud, Mørkøre et al. (2010) reported that intensity of color was increased during first days of storage. It was also suggested that color intensity was decreased after days 9 (Bahuaud, Mørkøre et al. 2010), however, the astaxathin is very stable under different storage condition (Choubert, Brisbarre et al. 2011). In respect to Bahuaud, Mørkøre et al. (2010), flesh color is found to differ between seasons in the beginning of the ice storage. The color score is higher in April and August than in February and October because color becomes more intensive in response to an increase in photoperiod and temperature (Bahuaud, Mørkøre et al. 2010).

1.5.5 Changes in liquid loss during ice storage

The ability to hold liquid is important quality characteristic of raw fish. The loss of salable weight and significant amount of nutrients interact due to the liquid loss negatively affect the total quality of raw fish products. The loss or gain of water is not economically profitable.

About 80% of salmon muscle is composed of water and lipids (Ofstad et al. 1996). The liquid in muscle is kept by capillary within intracellular locations (Ofstad et al. 1996). After harvesting of fish, the amount of liquid and its locations in muscle change that affects the liquid-holding properties.

Liquid holding capacity of fish muscle depends on interaction effects between internal and external factors such as temperature, season, ionic strength and pH (Ofstad et al. 1996). The low post-mortem pH is associated with poor liquid-holding capacity (Ofstad et al. 1996). According to Ofstad R et al. (1989) liquid loss varied with season due to different pH value, however, differences are not significant.

Ofstad R et al. (1996) reported that liquid loss is influenced by fibril contraction and distribution of intracellular and extracellular fluid. Moreover, post mortem ultra-structural changes result in a higher liquid loss. Shrinkage of the myofibrils due to rigor contractions results in more liquid released from fish flesh.

1.6 Post-mortem changes

Fish is an easily perishable product. Food spoilage can be defined as any quality changes during storage which lead to unacceptability of fish or fish products for human consumption and, hence, economic losses and legal consequences (Huis in't Veld 1996). Spoilage is related to the quality assessment and negatively affects fish products reducing its shelf-life. Food spoilage is interaction of *post-mortem* microbiological and biochemical activity. It is believed that one-fourth of the world's food is lost is due to microbiological problems (Huis in't Veld 1996). Microbial growth leads to spoilage of fish by the end of shelf-life. When bacterial counts excess the spoilage level the fish is no longer fit for human consumption (Gram and Huss 1996; Huis in't Veld 1996). Initial quality deterioration is caused by the action of autolytic changes in fresh fish flesh, which later a followed by microbiological activity (Gram and Huss 1996). Since *post-mortem* pH decreases immediately after dearth, enzymes are responsible for sensorial changes of fish flesh during the first days of storage (Hultmann and Rustad 2004, Bahuaud, Mørkøre et al. 2008). Endogenous fish enzymes are highly active

during ice storage (Bahuaud, Mørkøre et al. 2008). Protein degradation is one of the factors that lead to the fish muscle softening. Among various proteases involved in degradation of fish muscle lysosomal cathepsins are more studied (Haard and Simpson 2000).

1.6.1 Microbiological growth and TVN

Microbiological problems associated with bacteria can cause spoilage of foodstuff that leads to great economical losses (Kestin and Warriss 2000). Fresh and light processed salmon are popular food. These processes commonly do not inactivate contaminating bacteria flora. During ice storage up to 3 weeks, these products are liable to microbial spoilage. Spoilage of fish can be obvious, for instance, visible growth or production of off-odors and off–flavor caused by bacterial growth and its metabolism (Gram and Huss 1996, Huis in't Veld 1996). Gram and Huss (1996) suggested that 10^8-10^9 colony forming units per g (CFU/g) of fish is required level of spoilage bacteria to cause spoilage of iced fish. Bacterial microflora may also change after slaughter and during storage due to the differing environmental conditions (Gram and Huss 1996). Raw fish are initially contaminated with microorganisms that are found on the outer and inner surface of the fish. Raw fish products possess a high protein quality and moisture content, optimal pH and, therefore, high *post-mortem* microbiological activity. Muscle of live fish is sterile, nonetheless, after slaughter microorganisms are able to grow deeper in muscle due to break down of the barriers to microbiological invasion. Microflora may adapt to the chilled conditions and grow faster after slaughter. Therefore, storage at low temperature may not prevent spoilage (Huis in't Veld 1996). However, during harvesting, ice storage can be used to minimize level of bacterial multiplication and, hence, contamination of fish. Chilling with ice immediately post-slaughter may reduce spoilage of fish and avoid loss of product quality, especially during summer and autumn periods when water temperature is raised (Kestin and Warriss 2000).

Microbiological growth may develop in different ways and depends on intrinsic, extrinsic factors, the variety of processing practice and preservation and implicit parameters (Huis in't Veld 1996). Implicit parameters include the synergetic or antagonistic effects on the microbial activity of other microoraganisms leading to growth of a certain group of microorganisms and, as consequences, the availability of essential nutrients. Minor changes in processing can lead to great changes in type of spoilage association (Gram and Huss 1996). Huss suggested that among intrinsic factors high *post-mortem* pH compared with land animals and the presence of large amount of non-protein-nitrogen fraction including trimethylamine oxide

(TMAO) are important factors affecting the spoilage association. Non-protein-nitrogen fraction is substrate for bacterial growth. Decomposition of non-protein-nitrogen fraction by bacteria causes the formation of volatile compounds in iced fish stored aerobically. Some specific spoilage bacteria are able to produce hydrogen sulphide from sulphur containing aminoacids - cystein and, as a consequence, production of rotten, sulphydryl odor and flavor in raw food. Among various genera of spoilage bacteria, representatives of gram-negative psychotrophic bacteria *Aeromonas, Pseudomonas spp.*, *Shevanella spp.* and *Vibrionaceae* are most common spoilage organisms in aerobically stored fish (Gram and Huss 1996, Koutsoumanis and Nychas 2000). These spoilage microorganisms are able to utilize TMAO resulting in formation of trimethylamine (TMA) and produce H_2S from the L-cysteine (Gram and Huss 1996). TMA and total volatile nitrogen (TVN) are compounds responsible for the spoilage (Koutsoumanis and Nychas 2000). Koutsoumanis and Nychas (2000) reported that these compounds increased significantly during storage of fish products. Besides these, some specific spoilage bacteria are able to produce volatile compounds such as aldehydes, ketones, ester, sulphides and hypoxanthine utilizing various substrates (Gram and Huss 1996). *Pseudomonas spp*. spoils fish stored in ice. *Vibrio spp.* becomes responsible for spoilage at higher temperature (Huis in't Veld 1996).

1.6.2 Lysosomal cathepsins

Cathepsins are endogenous proteolytic acid enzymes. Several studies indicate that lysosomal cathepsins are involved in post-mortem softening of fish muscle (Aoki and Ueno 1997). In respect to various reports, myofibre-myofibre detachments and myofibre-myocommata detachments are positively correlated with cathepsin activity (Chéret, Delbarre-Ladrat et al. 2007, Bahuaud, Mørkøre et al. 2010). Godiksen, Morzel et al. (2009) suggested that an increase in cathepsin activity results in a higher muscle degradation.

The proteolytic activity of these enzymes varies with temperature, pH of the fish muscle, harvesting season and hence controlled by specific inhibitors. Bahuaud, Mørkøre et al. (2010) reported that level of cathepsin activity is positively correlated with a low pH value. After slaughter of fish, the muscle pH decreases from 7.4 to 6.3 (Erikson, Misimi et al. 2011). After rigor mortis, the pH value is more or less stable and close to 6.2-6.5 (Erikson, Misimi et al. 2011). Low muscle pH results in release of cathepsin and an increase of their activity.

There are four classes of cathepsins involved in muscle degradation: cysteine proteinases (cathepsins B, H and L), aspartic, serine and metalloproteinases. Among 13 cathepsins

involved in muscle degradation, cathepsin B, L, D and H are more studied (Haard and Simpson 2000).

Cathepsin B and L are responsible of deterioration and tenderization processes, respectively. According to (Ladrat, Verrez-Bagnis et al. 2003) the muscle softening process in salmon mostly is caused by cathepsin L. Optimal pH of cathepsin B and L is 6,5 and 7 that corresponded to post-mortem muscle pH after death and during rigor mortis period (Haard and Simpson 2000). Cathepsins lose their activity at pH that is not optimal. Therefore, cathepsin B and L have higher active in *post-mortem* fish muscle. Cathepsins are inactive in living fish. However, after death of fish, lysosome disruption results in release of cathepsins in cytosol and contact with their substrates. Cathepsin B participates in hydrolyzation of peptides, myofibrillar proteins, myosin and actin (Haard and Simpson 2000).

The aim of this study was to investigate the biochemical, microbiological and flesh quality changes taking place in Atlantic salmon being stored on ice from 0 to 22 days at two different seasons (winter and autumn) 2013. The aim was also to explore any effect on these parameters of early and late loading from the well boat and into the slaughter house.

It is hypothesized that there is a difference in biochemical, microbiological and flesh quality in Atlantic salmon between winter and autumn. It is also hypothesized that late loading has a stress effect on the fish due to longer handling and crowding in the well boat, which will have impact on the flesh quality.

2. Material and Methods

2.1 Fish material

The Atlantic salmon used in this experiment were obtained from Mainstream, Norway. A total of 120 salmon was sampled from the slaughterhouse of Mainstream, Skutvik Norway. The sixty fish slaughtered on January 28th 2013 had an average weight of 3.9±0.8 kg and had been set into seawater as 1-yearling smolt in August 12th 2011 (66 gram). The sixty fish slaughtered on October 16th had an average weight of 4.6±0.3 kg and had been put into seawater as 0-yearling smolt in May 28th 2012 (79 gram). All fish was of the AquaGen origin. Both groups had been farmed at similar locations and cage type in Steigen, Norway. The annual seawater-temperature varied between 4 and 13°C . The sea temperature at slaughter was 4.4°C in January and 10.5°C in October. In the seawater phase the fish had been fed with feed from Ewos (Norway), pellet size and fat level increasing with increasing fish size. The feed the last two months before slaughter was Opal 120 2500, 12 mm, containing 34 protein, 36 % fat and 30 mg astaxanthin. The fish was fasted for 4 weeks before slaughter in winter and 2 weeks in autumn. The fish was transported to the slaughter house with a well boat, transport time 0,5 hours. The fish was kept in the well boat under oxygenation for 11 hours before loading from the boat to the slaughter house was started, and the loading took six hours in total. Thirty fish was sampled from the transport boat at the start of the loading (early), and thirty fish was sampled at the end of the loading when the boat was nearly empty (late).
The fish was anaestezised, bled, gutted, washed and packed in Styrofoam boxes before transported to Faculty of Biosciences and Aquaculture (FBA), University of Nordland for ice storage experiment University of Nordland.

2.2 Biological measurements and experimental design

At arrival FBA the same day as slaughtered 60 salmon were tagged and coded with a random number unrelated to storage time. Each fish was measured for total length and gutted weight. Thereafter the fish was stored on ice in Styrofoam boxes in a cold store (4ºC) for a total of 22 days. At day 1, 4, 7, 10, 16 and 22 days of ice storage 10 fish were sampled, 5 from the early loading and 5 from the late loading of the transport boat (Table 1). The fish was analyzed for freshness using the quality index method (QIM, Table 2), and the Norwegian Quality Cut was cut out for histology analysis (Fig. 2). Thereafter the fish was filleted. The left fillet was used

for quality assessment; the right fillet was used for microbiology and TPA texture analysis. Specific spoilage bacteria were grown on Iron agar and Long&Hammer Agar during autumn sampling only. The skin, belly flap and pin bones was removed from the left fillet after texture and color measurements. Fish muscles were grounded into a homogenous mass for 2 x 10 seconds using food processor to analyze chemical composition and TVN.

Table 1:Sampling days and analysis.

Analysis	Number of days on ice																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
QIM,	WA			W	A		W	A		W		A			A	W					W	A
Color, texture, fillet gaping,	WA			W	A		W	A		W		A			A	W					W	A
Histology	WA			W	A		W	A		W		A			A	W					W	A
Microbiology TBC	WA			W	A		W	A		W		A									W	A
Microbiology Iron agar												A										A
Microbiology Long-Hammer												A										A
Proximate composition	WA			W	A		W	A		W		A			A	W					W	A
Liquid loss	WA			W	A		W	A		W		A			A	W					W	A
Cathepsin B+L/H	WA			W	A		W	A		W		A			A	W					W	A

2.3 QIM evaluation

Two persons participated in the freshness evaluation of salmon each time. The fish were removed from the ice and observations were conducted immediately under standardized conditions at room temperature. The appearance of skin, eyes, gills, the odour of gills and abdomen, and the texture was evaluated using the QIM scheme developed by Sveinsdottir et al., 2003. For each parameter a score between 0 and 3 was given, the score 0 is given when fish was the highest sensory quality, and 3 for the lowest score (Table 2). The quality index (QI) for each fish were established by cumulating the resulting scores.

Tabell 2: The QIM scheme developed for farmed salmon according to Sveinsdottir, Hyldig et al. (2003)

Quality parameters	Description	Point
Skin		
Colour/appearance	Pearl-shiny all over the skin	0
	The head is still pearl-shiny, but the restless, perhaps yellow	1
Mucus	Clear and not clotted	0
	Milky and clotted	1
	Yellow and clotted	2
Odour	Fresh seaweedy, cucumber	0
	Neutral to metal, dry grass, corn	1
	Sour	2
	Rotten	3
Eyes		
Pupils	Clear and black, metal shiny	0
	Dark grey	1
	Mat, grey	2
Form	Flat	0
	Little sunken	1
	Sunken	2
Abdomen		
Blood in abdomen	Blood light red/not present	0
	Blood more brown	1
Odour	Neutral	0
	Corn	1
	Sour	2
	Rotten/rotten kale	3
Gills		
Colour/appearance	Red/dark brown	0
	Light red/brown	1
	Grey-brown, grey, green	2
Mucus	Transparent	0
	Yellow, clotted	1
	Brown	2
Odour	Fresh, seaweed	0
	Metal	1
	Sour	2
	Rotten	3

2.4 Sample preparation for histology

A steak (5 mm thick) was prepared at 70% of the total fish length for muscle histology from 3+3 fish per sampling day (3 from early loading and 3 from late loading sampling). A total of three muscle blocks (5x5x5 mm) were cut from the Norwegian Quality Cut (NQC, Fig. 2) and mounted on cork using Cryomatrix (Bergman As, Oslo). Muscle blocks were frozen in 2-methyl butane (Isopentane, C_6H_{12}) cooled to near its freezing point (-159^oC) in liquid nitrogen, wrapped in aluminum foil and stored at – 80^oC prior to analysis.

Before cryo-sectioning the muscle blocks were acclimated to -24^o C for approximately 20 minutes and cut in 7µm thick slices using a cryostat (HM 550 Micromet, Micromet). The slices were mounted on poly-L-lysine treated slides and air dried. All sections were stained with Harris Haemotoxyline (Sigma Aldrich, Steinheim, Germany) solution during 10 minutes and mounted using Glyserol Gelatin (Sigma Aldrich, Steinheim, Germany). The histology samples were studied and digitized using a light microscope equipped with a digital camera (Axioskop 2 mot plus, Carl Zeiss, Germany). All images were analyzed using software (Axio Vision Rel. 4.2, Carl Zeiss). The percentage of myofiber-myofibre detachment was evaluated by counting all muscle fibres in each pictures (at 10x magnification) by light microscopy. Thereafter all myofibres in each picture that were detached from other myofibres with at least 25% of the outer cell surface were counted as detached.

Percentage of detachment was calculated as:

$$\frac{\text{number of cells}}{\text{number of the detachment}} * 100$$

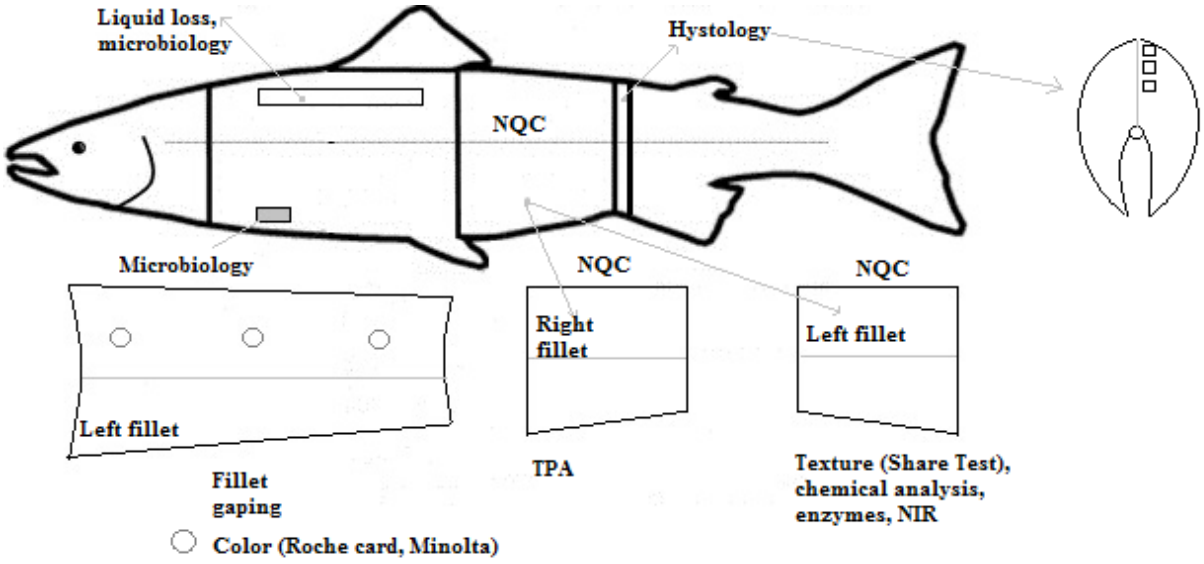


Figure 2: Sample sites for determination of flesh characteristics in the experiment.

2.5 General flesh quality parameters

2.5.1 Color measurements

The color was measured on the dorsal part of the left fillet (Fig. 2) with both Roche color fan under standardized conditions in a light cabinet and with a Minolta Chroma meter CR00 (Konica Minolta, Osaka Japan) calibrated against a white and a black standard. Measurements were taken at the three locations on the fillet oriented in an anterior-posterior direction above to the lateral line (Fig. 1). The tristimulus L*a*b* measurement mode was used to define color. The L* value represents lightness from black to white (0-100 point scale), positive a*values represent red and positive b* values represent yellow.

2.5.2 Gaping score assessment

The gaping score was visually assessed on the dorsal and ventral side of left fillet from head to tail direction. The gaping scores were determined according to a categorical scale; 0 is no gaping, 1 is minor gaping, 2 is moderate gaping, 3 and 4 is extreme gaping (see appendix).

2.5.3. Instrumental texture measurement

The texture parameters were performed using a texture profile analyser, model TA-XT2 (Stable Micro Systems, Haslemere, England). Two different attachments were applied for a shear test on the left fillet and a TPA test on the right fillet, respectively; a steel knife and a puncture probe. The graphs were recorded by a computer and analyzed. Texture analysis using a steel knife blade was performed by cutting two muscle blocks (5x5x5 cm) closed to the NQC cut on the left fillet (Fig. 2). A steel knife blade was pressed downwards 120% of the fillet thickness with a constant speed of 1 mm/sec the cross sectional on the muscle fibres. The maximum shear force (measured in Newton) and the total work corresponded to area under curve (measured in Newton) were registered. Analyses using TPA test was performed close to the NQC cut on the right fillet of the fish. A puncture probe was pressed down twice at a constant speed 2 mm/sec to achieve 30% compression depth of the fish thickness. The maximum force at the first compression is recorded as hardness. The total works needed at the first and second compression are corresponding to the area under the first and second curves respectively (measured in gram*seconds). Relationship between the work at the first and second compression is defined as cohesiveness and explains extent how fish goes back to its original form after the compression.

$$Cohesivenes = \frac{Total\ area\ at\ second\ compression}{Total\ area\ at\ first\ compression} * 100$$

Gumminess is defined as:

$$Gumminess = Hardness * \frac{Total\ area\ at\ second\ compression}{total\ area\ at\ first\ compression}$$

2.5.4. Liquid loss

Liquid loss (measured in %) during storage was measured on the dorsal part of left fish fillet (Fig. 2). One piece (180x30 mm) was cut out, weighed and placed in a plastic bag on ice in a cooling room (+4^{o}C) for 7 days. After 7 days the muscle piece was removed from the plastic bag, wiped off with a paper towel and weighed. The liquid loss was calculated as

$$100\text{x}\frac{Initial\ weight - final\ weight\ of\ the\ piece}{Initial\ weight\ of\ the\ piece}$$

.

2.5.5. pH measurement

A pH electrode (pH 2401, Radio Metro, Copenhagen, Denmark) calibrated against the acidic buffer (pH 4) and a neutral buffer (pH 7) was used to measure pH in the minced fish muscle. A pH electrode was inserted into fish sample and results were recorded.

2.5.6. Total volatile nitrogen (TVN)

Total volatile nitrogen was measured in duplicates using Kjeltec auto 2300 analyser (Foss Tecator AB, Hoganas, Sweeden). Homogenized 10 g of fish muscle was mixed with 50 ml distilled water, 3 ml antifoam in a TVN glass tube. Thereafter, 1 g of MgO was added into the TVN glass tubes and the volume of 2M HCl was recorded. The mg TVN/100g sample was calculated as:

$$100 \times \frac{ml\ titrated\ HCl\ \times\ M\ \times\ 14.01}{weight\ of\ sample}$$

where *M* is molarity of the used HCl solution

2.5.7 Chemical analysis

All chemical analyses were performed in duplicates. Protein content was calculated assuming a conversion factor of 6,25. Total fat was analyzed after extraction with ethyl acetate.

2.5.7.1 Protein content

The Kjeltec method was used to determine crude protein content. Approximately 1 g of the minced fish muscle sample was weighed into a nitrogen free paper (Whatman, GmbH, Germany) and transferred to Kjeltec tubes containing 15 ml of concentrated sulfuric acid and 2 Kjeltabs. Samples were heated to 420^0 C for 45 minutes and, thereafter, cooled to room temperature and analyzed by means of Kjeltec auto 2300 analyser (Foss Tecator AB, Hoganas, Sweeden)

2.5.7.2 Analyses of fat content

Fillet fat content was determined in the NQC (Fig. 2) using duplicates10 ±0.5 g of minced fish samples extracted in ethyl acetate. Homogenized fish samples and 20 g of water free sodium sulphate were mixed with 50 ml etyacetat in the 100 ml glass bottles and extracted out on the shaking table for an hour. Working in a fume hood, 20 ml of the sample was filtrated

through a filter (31 – 50 µm, Schleicher & Scül) and evaporated in a water bath. The sample was dried at 105 – 110 ^{o}C for 15 – 20 minutes. Fat amount was determined by weighing the cooled down to room temperature evaporated aliquots of the solution and calculated as

$$\frac{10300 \times fat(g)}{\left(40 - 2.17 \times fat(g)\right) \times sampleweight(g)}$$

2.5.7.3 Water content

From the mince 5±0.5 g of muscle was weighted into a aluminum cup and rubbed up along the walls of the cup. Samples were dried at 105^{o} C overnight (16 hours), cooled down to room temperature in a dessicator. The water content was determined as weight loss,

$$100 \times \frac{initial\ weight\ (g) - dry\ weight(g)}{initial\ weight(g)}$$

2.6 Microbiology

Pieces of white muscle was aniceptically cut out underneath the dorsal fin of the fish. First the skin was removed using sterilized scalpel and forceps, thereafter was approximately 8 gram of fish muscle was cut out into Stomacher bags with peptone water (1:9) and homogenized for 60 seconds in a Stomacher 400 blender (Sewar Stomacher 400, England). The fish-peptone dilution was further diluted according to increasing number of bacteria during the storage experiment. Dilution (1ml) was pipette on petri dishes containing Standard Plate Count Agar medium (Merck KGaA, Darmstadt, Germany) and incubated at 22 ^{o}C for 48 hours for detection of total bacteria colonies. Hydrogen sulphide producing bacteria colonies and psychrotolerant, heat labile micro-organisms were grown in petri dishes containing Iron Agar and Long & Hammer agar, respectively. Plates with Iron Agar were incubated at 20 – 25 ^{o}C for 72 hours, and . Long & Hammer Agar plates was incubated at 15 ^{o}C for 5 – 7 days.

2.7 Proteolytic enzymes (Cathepsins)

Cathepsin B+L and H activities were measured according to the method Barret and Kirschke (1981). 1±0.05 g of fish sample was homogenized in 5 ml of cold enzyme extraction buffer (50 mM NaOAc, 1 mM Na_2EDTA, 100mM NaCl and 0.2% Tween 20 (pH 5.0,Sigma, Oslo, Norway) for 60 s at 22,000 rpm, using polytron (mod. PT 1200 CL, Kinematica AG/Anders Phil AS, Dale I Sunnfjord, Norway). The extract was kept on ice for 10 min and 1,5 ml was transferred into a Eppendorf tubes and centrifuged at 20,000×g (rcf) for 30 min at 4^{o}C. Liquid debris was removed by vaccum. The supernatant was transferred into a new Eppendorf tube, and the samples were stored at - 80^{o}C before further analysis.

Cathepsin B+L and H activity was determined in duplicates at 30^{o}C, using a fluorescent spectrophotometer (Eclips, Varian/Holger, Oslo, Norway). 25 µl of enzyme extract pre-incubated with 975µl of assay buffer. For cathepsin B+L and H, the assay buffer (pH 6.0 and pH 6.6) contained 200 mM NaOAc , 2mM EDTA, 0.05% (v/v) Tween 20, freshly made 4 mM DTT for 2 min and 200 mM Na2PO4 was used instead 200 mM NaOAc, respectively. The substrates (for cathepsin B+L and H, Z-Phe-Arg-7-amido-4-methylcourmarin and Arg-7-amido-4- methylcourmarin respectively) were added freshly (Sigma, Oslo, Norway). The measurement was performed with the excitation (λ_{ex}) and emission (λ_{em}) wavelength set to 380 and 460nm respectively. The amount that hydrolyzed 1 mmole substrate per minute at 30 ^{o}C was defined as one unit of enzyme activity.

2.8 Statistical analysis

Statistical analysis was performed using the SPSS statistics (IBM SPSS Statistics 20, SPSS Inc. US). All the data was checked for normality prior to analysis using Shapiro – Wilk W rest and visual assessment of histograms. One – way ANOVA were applied to find any effect of season on the biochemical and quality parameters. The effect of ice storage and early/late loading were tested by two-way ANOVA. Data were expressed as the mean ± standard deviation. The statistical significance was set normally set at <0.05.

The principal component analysis was performed using Unscrambler (Ver. 10X, Camo A/S). PCA analysis gives data description as graphical overview of the relationships between the analysed variables as well as the main factors of importance the quality variation between in the material (Esbensen, Guyot et al. 2000, Perera 2011).

3. Results

3.1 Biological data

The average weights of farmed Atlantic salmon used in this study were 3948±847g and 4563±282g in winter and autumn sample periods, respectively (Table 3). Condition factors were about 1.2±0.13 (winter) and 1.2±0.09 (autumn). The total lengths were 68.9±4.26 (winter) and 72.9±2.41 (autumn).

Tabell 3: Mean ± standard deviation of biological parameters of the farmed Atlantic salmon harvested in winter (n=60) and autumn (n=60).

	Total length (cm)	Gutted weight (g)	K-factor
Winter (February)	68.9±4.26	3948±847	1.2±0,13
Autumn (October)	72.9±2.41	4563±282	1.2±0,09

3.2 Changes in the QI score in accordance to ice storage time

The QI calculated for each of the 6 storage days (0, 4, 7, 10, 16, 22) showed a high correlation (r^2=0.968 and r^2=0.939) between storage time in ice and the QI score and with a slope of 0.800 and 0.795 for both sampling, winter and autumn, respectively (Fig. 3). The slope was similar to the slope observed by Sveinsdottir, Martinsdottir et al. (2002).

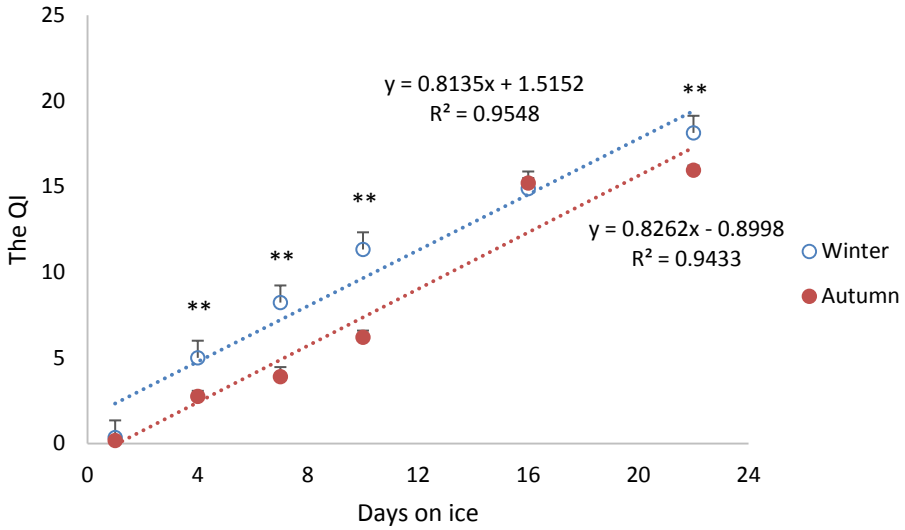


Figure 3: Average QI score for Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (=p<0.01).**

The QI increased significantly with storage days (p<0.001,Fig. 3). The average scores for day 1 was the lowest (0.4±0.2 and 0.2±0.1) and highest scores (18.1±0.4 and 16.0±0.3) were found for day 22 for both sample periods. The QI scores wre significantly higher in winter than in autumn at day 4, 7, 10 and 22.

3.3 Changes in proximate composition with storage time in ice

The water content was stable throughout the experiment and did not change significantly ($p>0.05$) with storage time (Table 4, Fig. 4A). The water content varied from 64.3±0.7% to 66.4±0.8% in winter harvested fish and from 63.4±0.2% to 64.6±0.5% in autumn harvested fish. However, water content were found significantly higher in fish harvested in winter than in autumn at 16 and 22 days of ice storage ($p<0.05$).

There was no significant differences ($p>0.05$) in fat content with respect to the time of ice storage (Table 4, Fig. 4B). The average values of fat content were similar throughout ice storage experiment and varied from 13.8±0.3% to 15.5±0.8% (winter) and from 14±0.8% to 16.6±0.4% (autumn). There was no significant differences between winter and autumn harvested fish, accept day 22 ($p<0.05$) when fat content was higher in autumn than in winter.

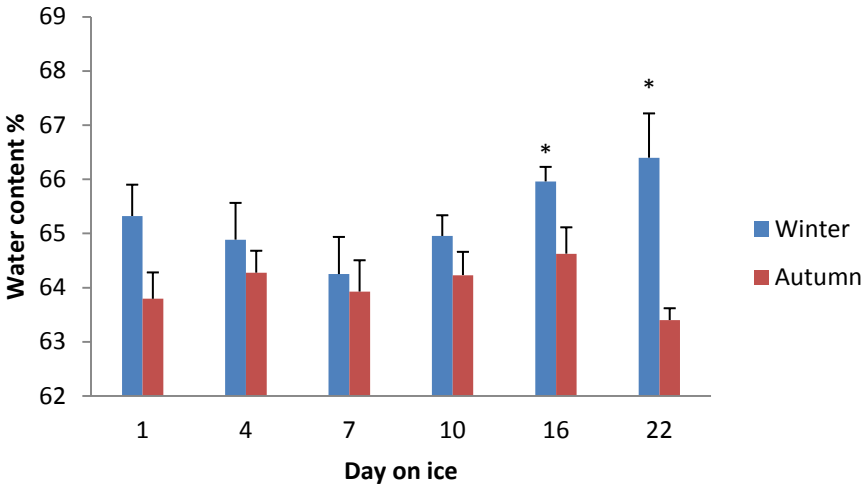
The protein value decreased significantly with respect to storage time ($p<0.05$, Table 4, Fig. 4C) from 20.1±0.5% to 19.8±0.2% and 20.5±0.1% to 19.6±0.2% in winter and autumn harvested fish respectively. The average protein value was found significantly higher ($p<0.05$) in autumn harvested fish than in winter harvested fish at day 0 whereas the remaining sample points (4 – 22 days) were not significant difference between both sample periods.

No effect for early and late loading was observed in relation to proximate composition (Table 4).

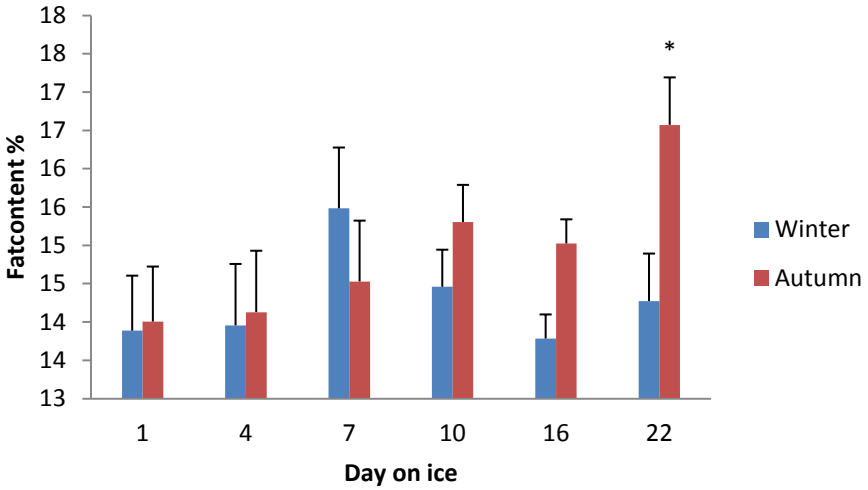
Table 4:Effect of loading (early and late) and days on ice (from 0 to 22 days) on water, fat and protein contentin Atlantic salmon harvested in both seasonal periods.

Two-way ANOVA	Effect of loading	Effect of days on ice
Water content	ns	ns
Fat content	ns	ns
Protein content	ns	0,001[b]

a,b and c: significant at 5% and 1% and 0,1% respectively.



A.



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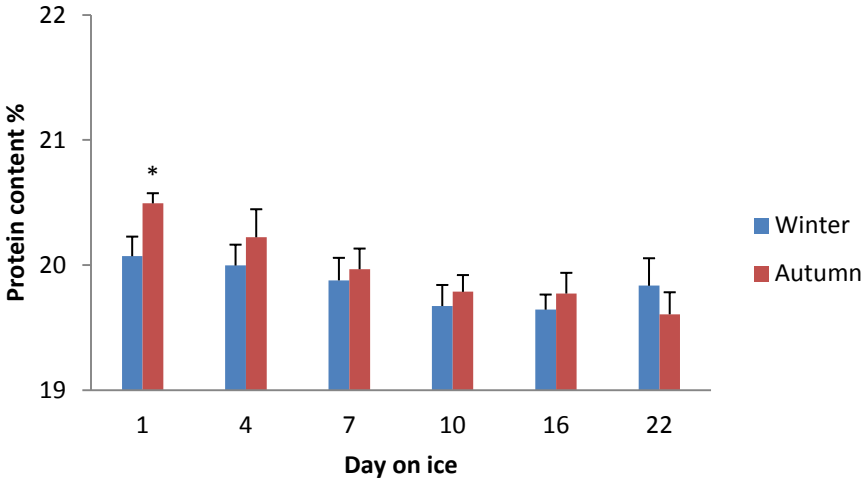


C.

Figure 4:The percentage of water (A), fat (B) and protein (C) content in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*=p<0.05).

3.4 Changes in liquid loss with storage time in ice

The liquid loss increased significantly with storage time up to 10 days in autumn and 16 days in winter, when liquid loss started to decreased in both sample periods ($p<0.05$). There was the lowest value of released liquid at day 0 in both sample points ($p<0.05$, Fig. 5). The highest value of liquid loss was observed at day 16 (5.33%±0.80) in winter and at day 22 (4.92%±0.39) in autumn. At day 7, 10, 16 and 22 the liquid loss was significant different between winter and autumn harvested fish ($p<0.05$). However, clear trend was not observed. Stress (loading) significantly affected liquid loss ($p<0.01$, Table 5). Fish from late loading showed higher liquid loss compared to fish from early loading.

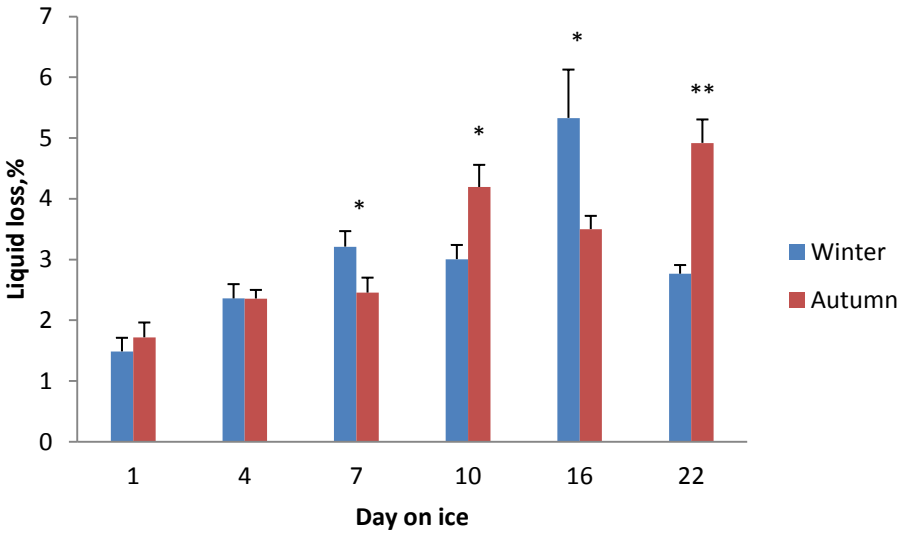


Figure 5: Liquid loss (%) in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (=p<0.01; *=p<0.05).**

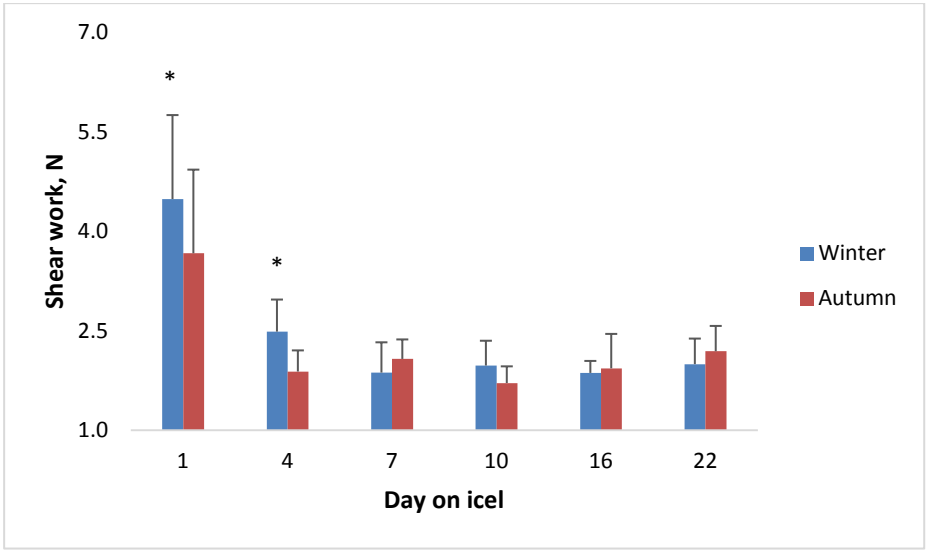
3.5 Changes in texture with storage in ice

The average value of shear work was plotted against days in ice (Fig. 6A). Fish muscle softened during ice storage. The highest value 4.5±1.3 N (winter) and 3.6±1.3 N (autumn) was obtained at day 0 of ice storage when the fish was still in rigor mortis. From day 4 onwards were not significant differences between days were observed ($p>0.05$). The lowest value recorded shear work was at day 16 in winter (1.9±0.2 N) and at day 10 in autumn (1.7±0.3 N). A significant differences between seasons was only found at 0 and 4 ($p<0.05$), where winter was higher that autumn.

The fillet hardness was significantly reduced during storage ($p<0.05$) in winter whereas hardness recorded in autumn was constant over storage time, accept at day 1 ($p<0.05$, Fig. 6B). The hardness was 1.8±0.1 N (winter) and 2±0.1 (autumn) the highest at day 0 while the lowest value 1.2±0.1N and 1.3±0.1 N was recorded in winter and autumn harvested fish respectively. There was no significant differences between sample periods, accept at 4 and 16 day ($p<0.05$). The hardness is significantly affected by loading time (Table 5).

A cohesiveness and gumminess showed the same significant trend as a hardness ($p<0.05$). The highest value of cohesiveness recorded was 1.7±0.1 N (winter) and 1.9±0.1 N (autumn) at day 0 whereas the lowest was 0.9±0.04 N and 1.12±0.07 N recorded at day 16 and 22 in winter and autumn harvested fish. The same significant group trend was shown for gumminess (Fig.6C). The highest and lowest values of gumminess are shown at Table 13 (see appendix). There was no significant differences between sample periods, accept 16 day (cohesiveness) and 4 day (gumminess, $p<0.05$).

A.



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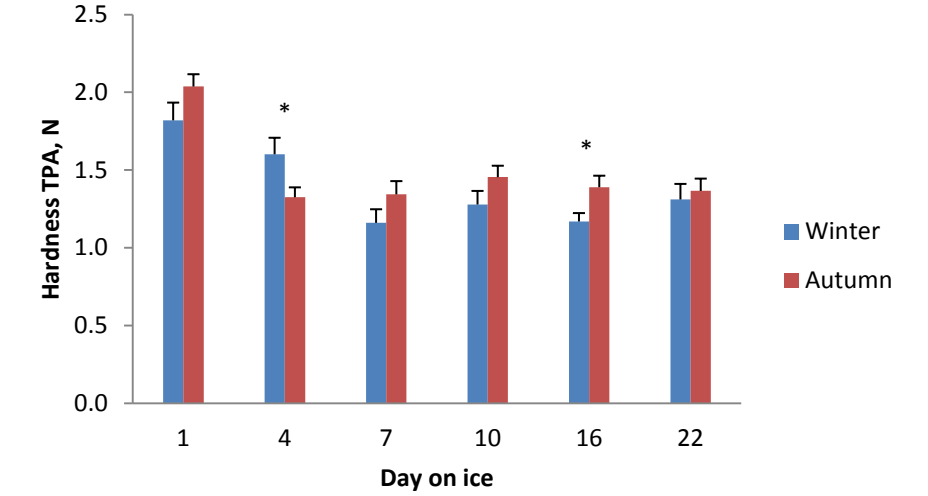


C.

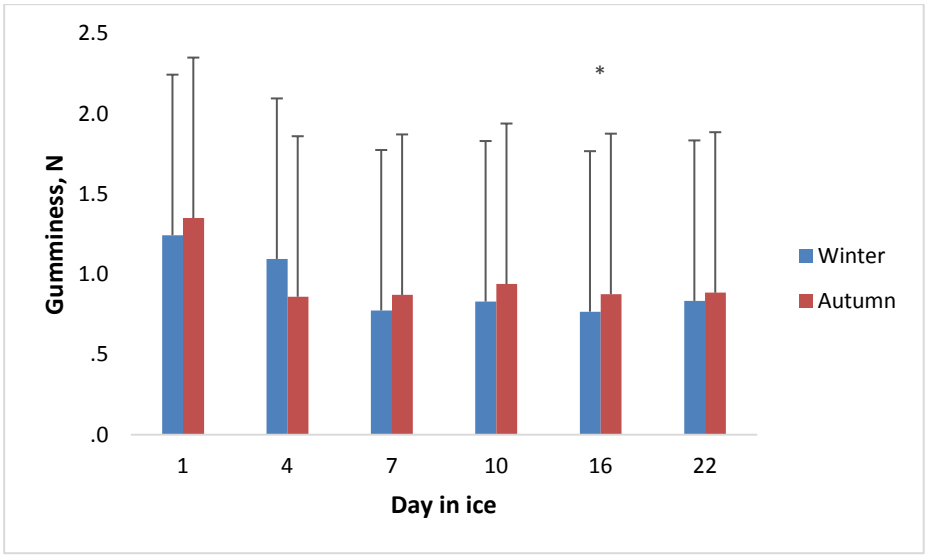


Figure 6: Shear work (A), hardness (N) (B) and gumminess (C) in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (*=p<0.05).

3.6 Changes in fillet gaping in respect to storage time

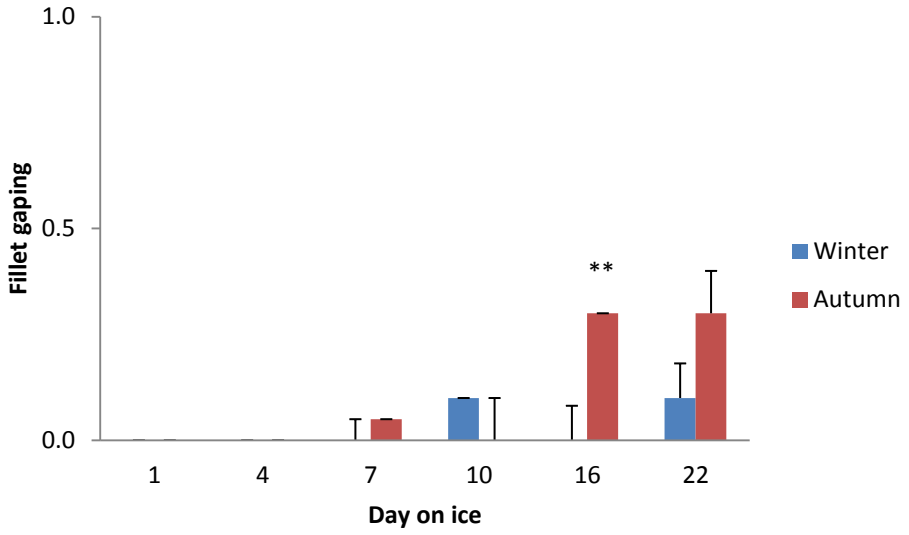
The fillet gaping was low (<0.5) at both sample points and during ice storage with reaching highest score by the end of storage experiment. No gaping was detected up to 10 days in winter and 7 days in autumn harvested fish, but thereafter there was an increase in fillet gaping with storage time ($p<0.05$, Fig 7.) reaching the highest score by the end of storage experiment. There was a tendency towards higher score in autumn compared with winter significant only at day 16 ($p<0.05$).

There was no significant effect of loading time on the fillet gaping (Table 5)

Table 5:Effect of loading (early and late) and days on ice (from 0 to 22 days) on liquid loss, texture, and fillet gaping in Atlantic salmon harvested in both seasonal periods.

Two-way ANOVA	Effect of loading	Effect of days on ice
Liquid loss	0,01[b]	ns
Texture (Shear work)	ns	<0,001[c]
Texture (TPA)	0,03	<0,001[c]
Fillet gaping	ns	0,02[a]

a,b and c: significant at 5% and 1% and 0,1% respectively.



Figur 7: Changes in fillet gaping in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (*=p<0.05).

3.7 Changes in color throughout the ice storage experiment

The visual color of farmed salmon fillet evaluated by Salmo Fan (Roche cards) showed no significant differences in respect to storage time and between sample periods, accept at days 4, 7 and 10 ($p<0.05$, Fig. 8), where higher value was observed in winter harvested fish.

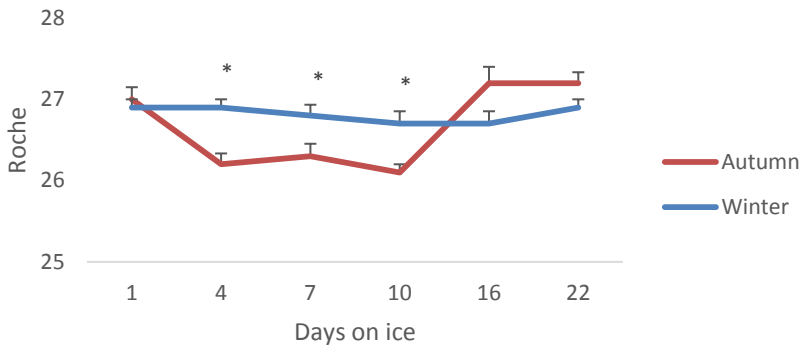


Figure 8: Changes of visual color in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (*=p<0.05).

Instrumental color analysis revealed that the color of the farmed salmon fillet (L, a* and b*) varied with storage time in both sample periods ($p<0.05$, Fig. 9). No differences were found in lightness (L value) between winter and autumn samples, accept at day 22 ($p<0.05$). However, lightness increased significantly by the end of storage experiment ($p<0.05$).

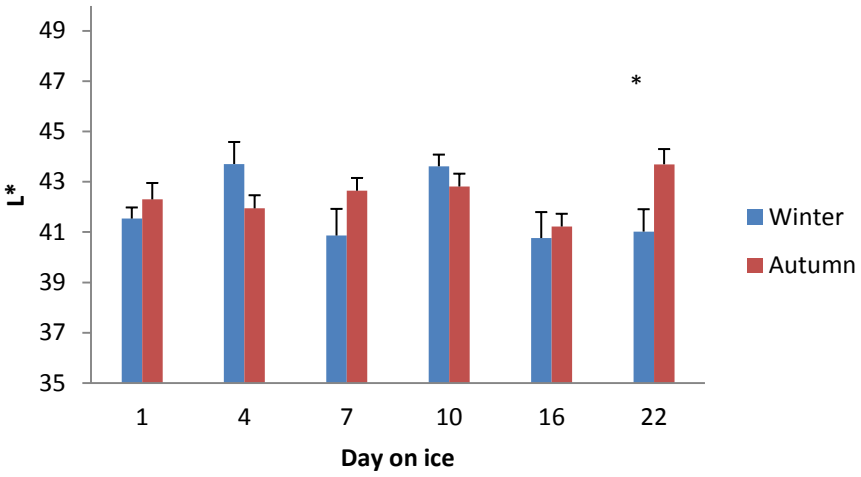
At day 22 in autumn, salmon fillet had the highest redness (a* value) and yellowness (b* value, $p<0.05$, Fig. 9B and 9C) while there was no significant difference in a* value and b* value in respect to storage time in winter ($p>0.05$). The a* value and b*value at day 0, 10 and 22 differed between sample periods ($p<0.05$).

Color (L, a* and b* value) was not significantly affected by loading time (Table 6).

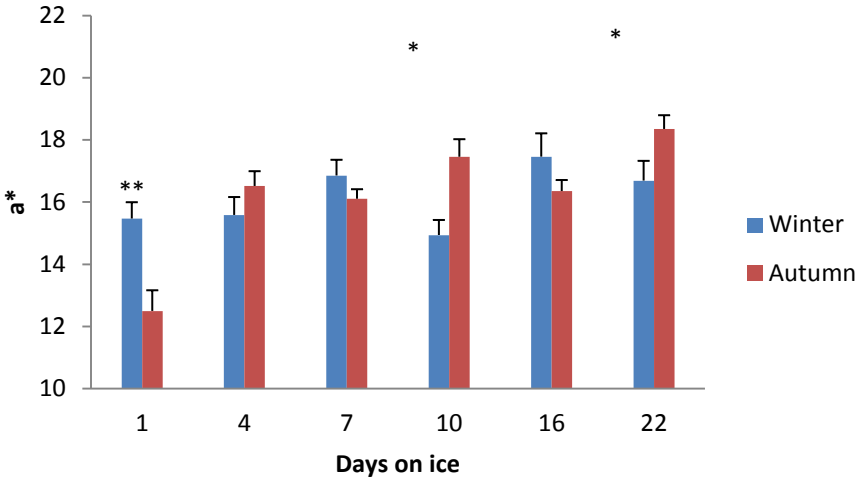
Table 6: Effect of loading (early and late) and days on ice (from 0 to 22 days) on lightness (L), redness (a*) and yellowness (b*) in Atlantic salmon harvested in both seasonal periods.

Two-way ANOVA	Effect of loading	Day on ice
L value	ns	ns
a* value	ns	$<0,05^{c}$
b* value	ns	$<0,05^{c}$

a,b and c: significant at 5% and 1% and 0,1% respectively



A.



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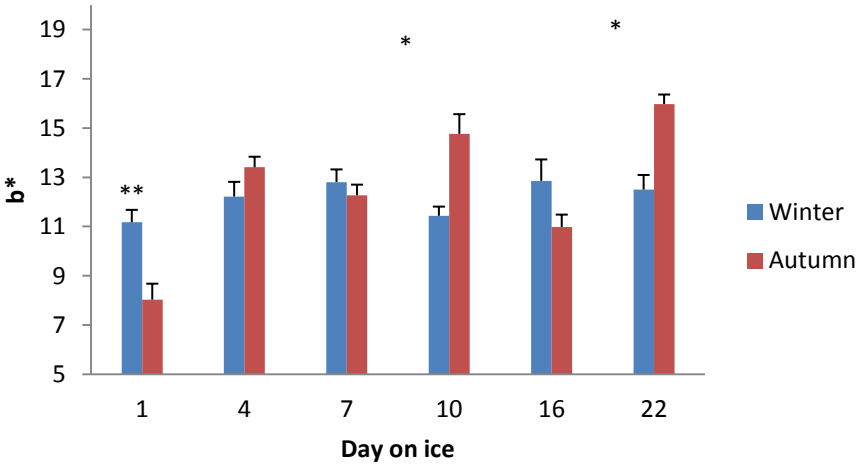


C.

Figure 9:Changes of (A). Lightness (L value), (B) redness (a*) and (C) yellowness (b*) o in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (*=p<0.05).

3.8 Changes in pH in respect to storage time

The muscle pH decreased significantly ($p<0.05$, Fig. 10) in respect to storage time up to 16 days when pH increased again in autumn harvested fish (results from winter harvested fish are not shown because of technical problems, accept 16 and 22 days). At day 16, the muscle pH was significantly higher in winter harvested fish than in autumn harvested fish ($p<0.01$).
No effect of loading time was seen on the muscle pH (Table 7).

Table 7: Effect of loading (early and late) and days on ice (from 0 to 22 days) on pH, TVN and Cathepsin B+L and H activity in Atlantic salmon harvested in both seasonal periods.

	Loading	Day on ice
pH	ns	<0,001[c]
TVN	ns	<0,001[c]
Cathepsin B+L activity	0,06	ns
Cathepsin H actvity	ns	ns

a,b and c: significant at 5% and 1% and 0,1% respectively

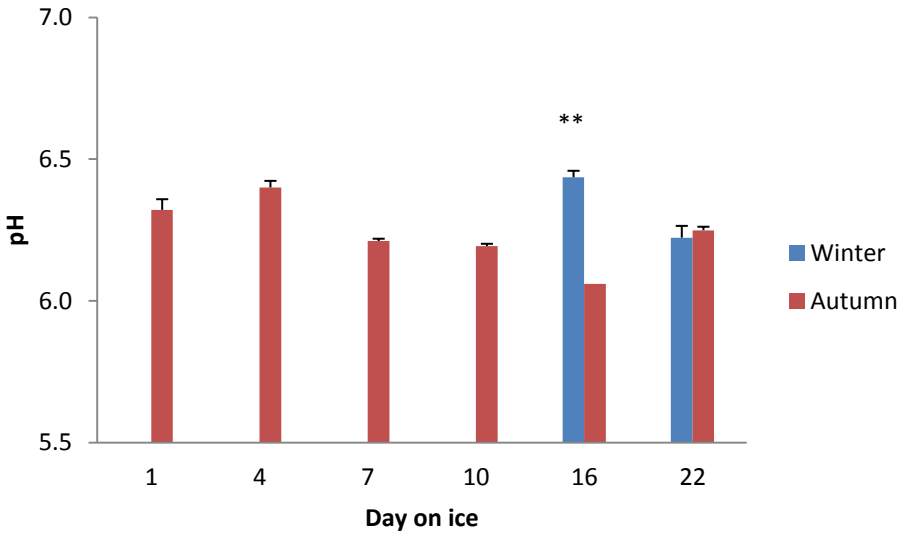


Figure 10:pH in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (=p<0.01).**

3.9 Change in the TVN value

The TVN value did not change significantly throughout the experiment ($p>0.05$) in both sample periods (Fig 11). The amount of TVN varied from 17±0.9 to 16.5±1.1 (winter) and from 21±2.5 to 17.1±1.2 (autumn). There was no significant difference between two seasons, accept 0 day of ice storage ($p<0.01$).

There was no effect of early and late loading on the TVN value (Table 7).

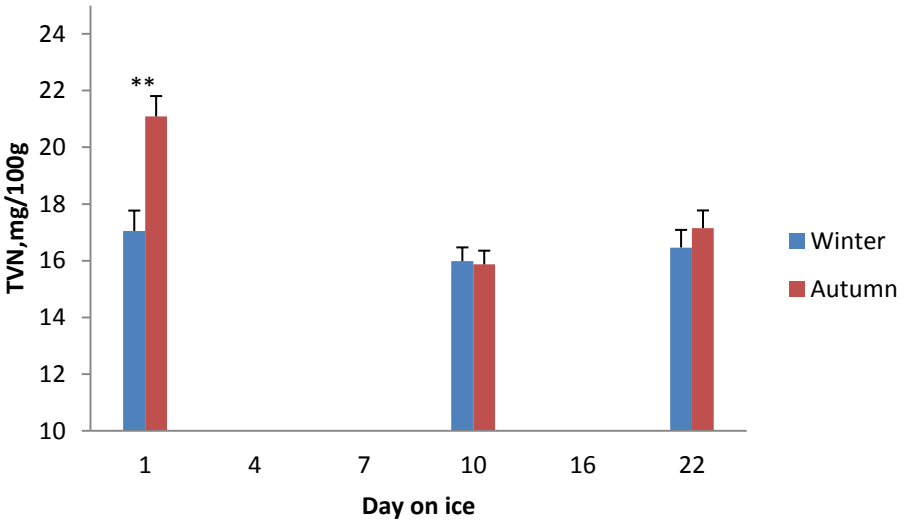
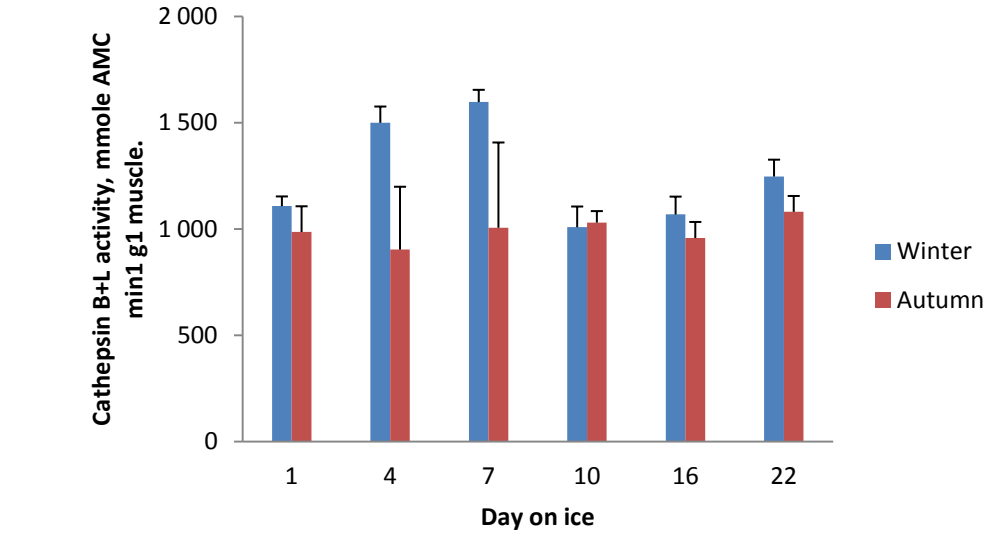


Figure 11:Total volatile nitrogen (TVN) in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (=p<0.01).**

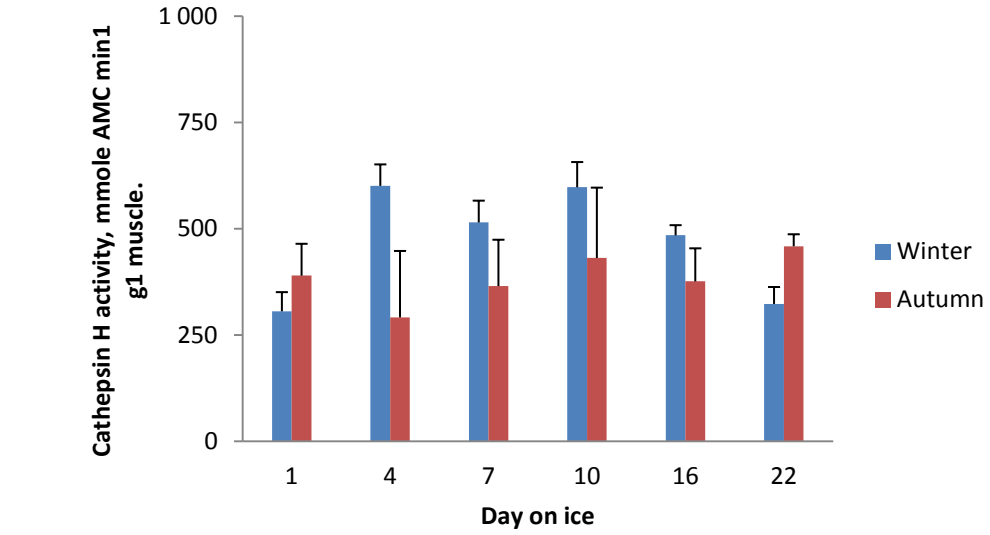
3.10 Cathepsin activity

Figure 12 shows the cathepsin activity during ice storage. Cathepsin B+L and H activity did not vary significantly with storage time in either winter or autumn sample periods. The cathepsin B+L activities varied from 1108±46 at day 0 to 1247±79 at day 22 in winter harvested fish and from 986±121 at day 0 to 1081±75 at day 22 in autumn harvested fish. There was no significant difference between seasons ($p>0.05$) for both cathepsin B+L and H activities.

Significant effect of loading was found only for cathepsin B+L activity, where higher activity was observed during early loading compared with late loading (Table 7).



A.



B.

Figure 12: Activity of Cathepsin B+L (A) and Cathepsin H (B) in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation.

3.11 Changes in total bacteria count (TBC) and specific spoilage bacteria (SSO)

Summing up the microbiology data, it was clear that the total viable counts increased significantly with the storage. Fig. 13A shows the mean growth pattern of total bacteria counts (TBC) during ice storage. No bacteria were found at day 1. TBC increased significantly ($p<0.001$) after 10 days of ice storage and reached maximum levels at 22 days of ice storage, with final counts varying between 2139±1054 (winter) and 1061±143 (autumn). Number of bacteria colonies was significant higher in winter harvested fish than in autumn harvested fish at 10 and 22 days ($p<0.0001$).

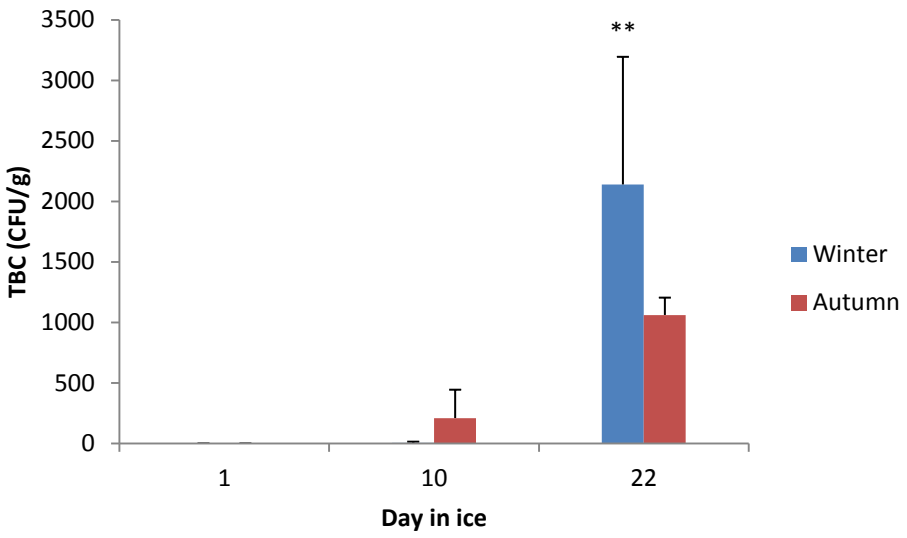
The similar trend was found in spoilage bacteria. The highest value of bacteria growing on Iron agar (1562±601) and Long&Hammer (1036±386) was observed at 22 days on ice in autumn harvested fish ($p<0.05$, Fig. 13B).

There is no significant effect of loading time on the TBC and specific spoilage bacteria, accept bacteria grown on Iron Agar ($p<0.001$). Number of spoilage bacteria grown on Iron agar was higher during early loading compared to late loading ($p<0.0001$).

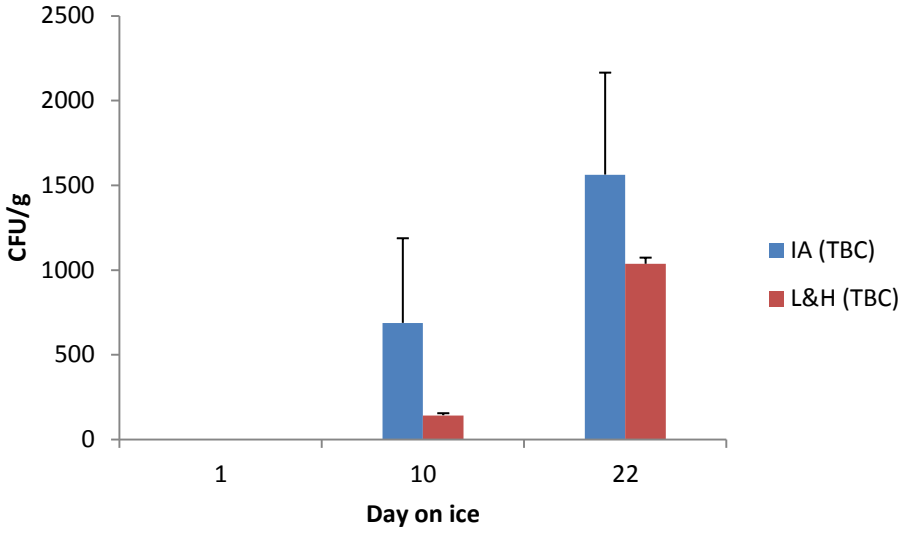
Table 8: Effect of loading (early and late) and days on ice (from 0 to 22 days) on TBC and specific spoilage organisms in Atlantic salmon harvested in both seasonal periods

	Loading	Day on ice
TBC	ns	<0,001[c]
Iron Agar (TBC)	<0,001[c]	<0,001[c]
Long&Hammer Agar (TBC)	ns	<0,001[c]
Black colonies (Iron Agar)	ns	<0,05[a]
Luminescent bacteria (Long&Hammer Agar)	ns	<0,05[a]

a,b and c: significant at 5% and 1% and 0,1% respectively



A.



B.

Figure 13: Total bacteria count (A) and specific spoilage bacteria (B) in Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days. Bars indicate mean value with standard deviation. The level of significant between seasons is shown by asterisks (*). (=p<0.05).**

3.12 Histology

Images of muscle fibre cross-section of fish white muscle tissue from the two different seasons and storage time are shown in Table 10. At 1 day *post mortem*, cross-sections of muscle tissue showed intact muscle fibres and well-preserved connective tissue of myocommata in both samples periods. But when counting myofibre-myofibre detachment approximately 9 % of the fibres are detached from each other at day 1, increasing to 21% at day 22 (Fig. 14, $p<0.05$). There was no significant differences between season, accept at 1, 4 and 7 days on ice ($p<0.05$, Table 9) when percentage of detachment increase in autumn compared to winter. Due to few good histology images, the effect of loading is not analyzed.

Table 9:Percentage of myofibre-myofibre detachments in respect to the ice storage in Atlantic salmon stored on ice from 0 to 22 days.

Day on ice	Detachment in percentage			
	Winter	Autumn	Effect of season	
			p<	Test
Day 1	3	14	0.003	ANOVA
Day 4	9	10	0.003	ANOVA
Day 7	4	18	0.005	ANOVA
Day 10	18	12	0.280	ANOVA
Day 16	21	13	0.274	ANOVA
Day 22	18	19	0.282	ANOVA

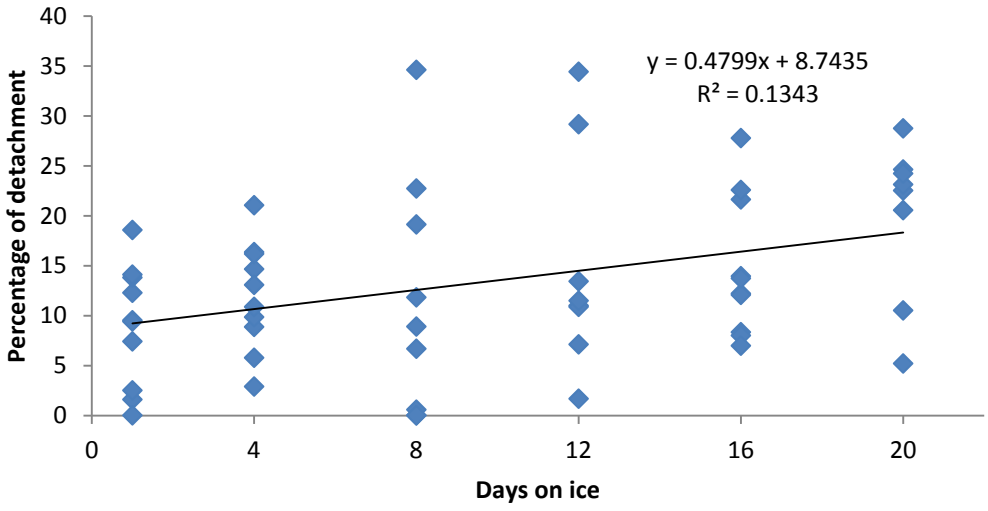
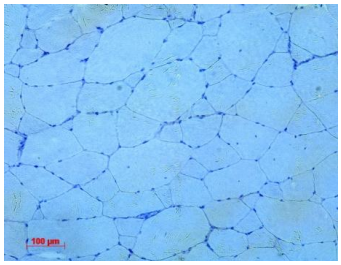
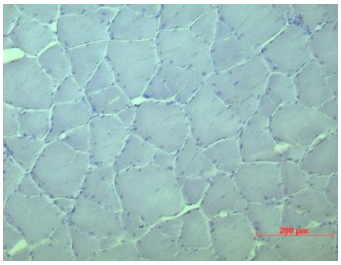
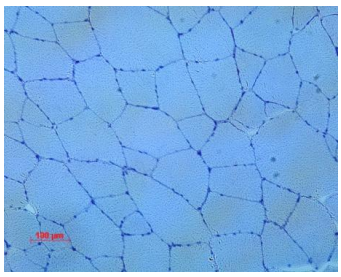
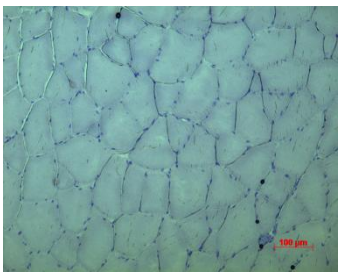
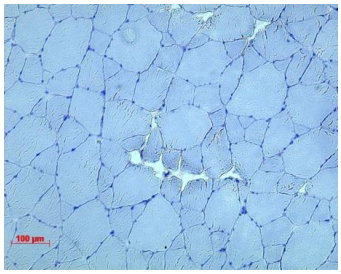
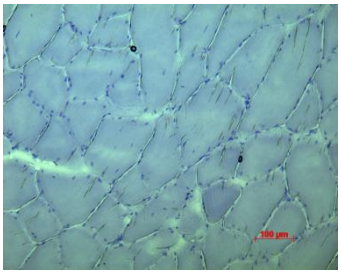
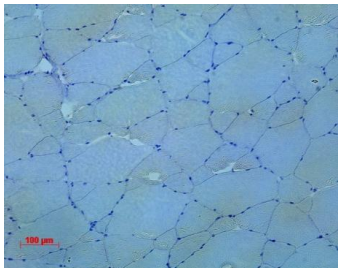
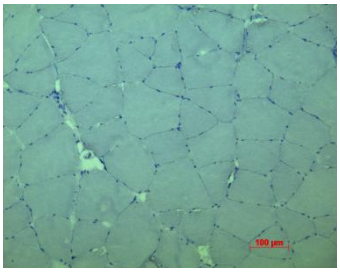
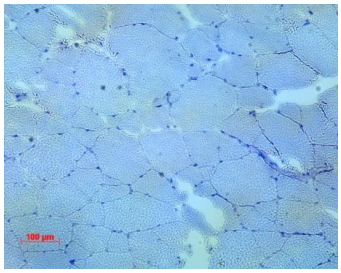
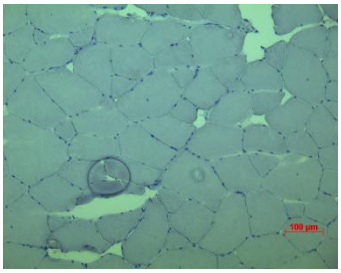
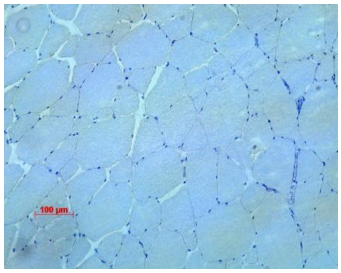
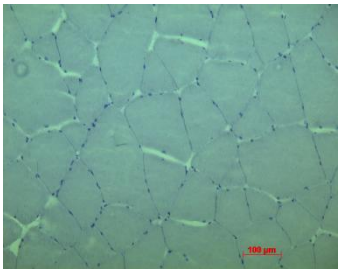


Figure 14: Percentage of detachment with storage time In Atlantic salmon harvested either winter or autumn and stored on ice from 0 to 22 days.

Table 10:Images obtained by light microscope of cross-sectioned muscle tissue of winter and autumn harvested salmon stained with hematoxyline, from the earlier and late loading of the transport boats.

	Fish harvested in winter	Fish harvested in autumn
Day 1		
Day 4		
Day 7		
Day 10		
Day 16		
Day 22		

3.13 Correlation between quality attributes

Table 11 shows the correlations between various parameters in salmon harvested in both sample periods. The best correlation was found between fat and water contents (-0.76, $p<0.01$), and QIM and liquid loss (0.56, $p<0.01$).

Tabell 11: Correlation coefficients of some of the quality attributes for all samples from both harvested periods.

	Correlation coefficient
Fat/water	-0.76**
Fat/gaping	0.295*
Protein/TVN	0.38*
Protein/gaping	-0.32**
QIM/protein	-0.44**
Liquid loss/TVN	-0.33*
QIM/TVN	-0.398**
TVN/Shear work	0.67**
QIM/gaping	0.36**
QIM/liquid loss	0.56**
Liquid loss/color (a* and b* value)	0.39**
Hardness/color (a* and b* value)	-0.35**
Hardness/liquid loss	-0.39**

*,**: significant at 5% and 1% respectively.

3.14 Principal component analysis (PCA)

The PCA score plot (Fig. 15A) revealed that 83% of variation in the data is explained by PC 1 whereas PC 2 explains 13%. Figure 15A shows that that the fish sampled at day 1from early and late loading have exposed high variation in PC1 from the remaining days of ice storage. The data of the first day of storage was excluded as it improved the resolution of the PCA score plot. Changes from 4 to 22 days on ice are shown on Figure 15B, where PC1 explains 72 % of the variation among the remaining sample points. The PC2 related to the differences in fish from early and late loading explains another 23 % of the variance.

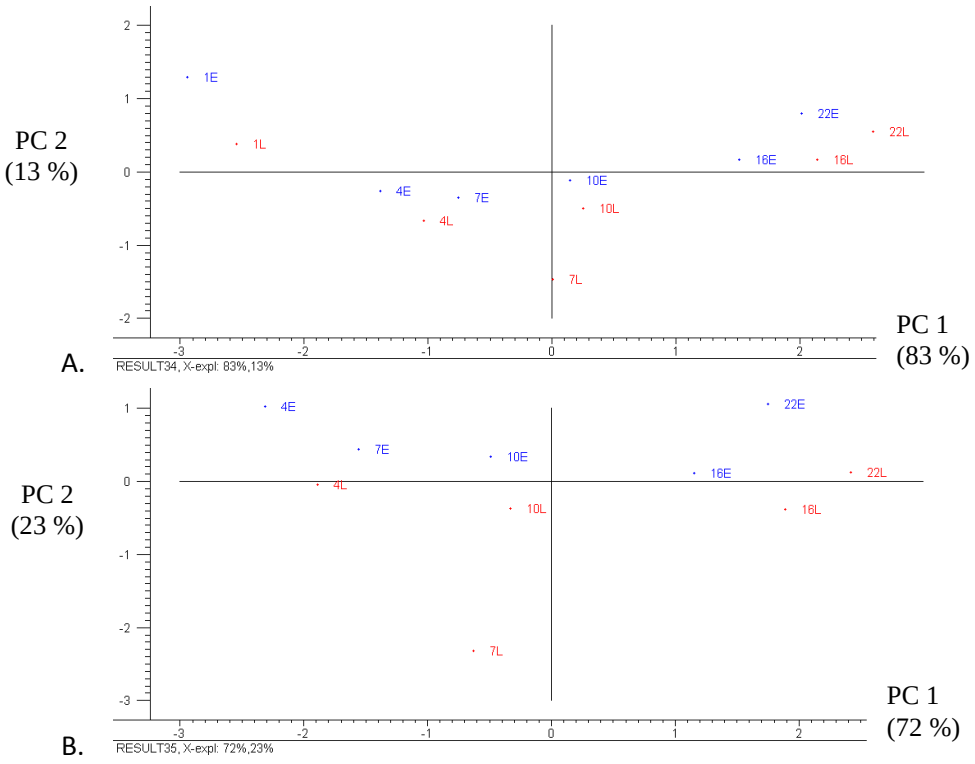


Figure 15: Score plot including day 1data (A) showing variation in PC1 and PC2. E and L indicate fish from early and late loading respectively. The numbers from 1 to 22 indicate the sample points.

However, the fish harvested in autumn and winter did not show clear distinct grouping in PC 2 even so the data of the first day was omitted (PC1 and PC2 explained 93% of total variation, Fig. 16).

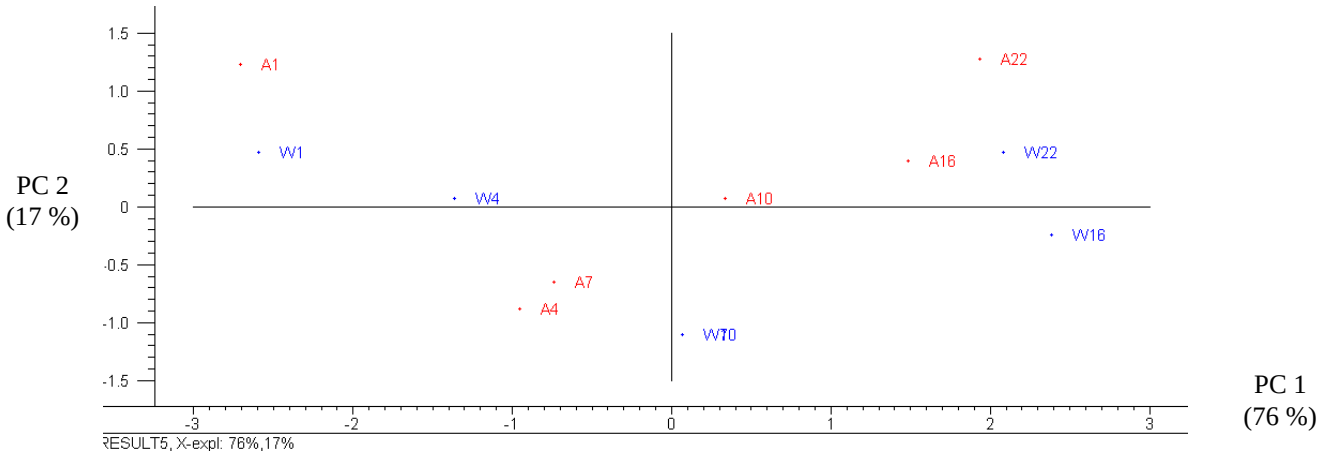


Figure 16: Score plot showing variation in PC1 and PC2 where W and A indicate winter and autumn respectively. The numbers from 1 to 22 indicate the sample points.

Most of variation in the data is contributed by variables lying between the two circles. The inner circle explains 50% whereas the outer circle explains 100% of the variation. To improve the resolution of the PCA plot some quality attributes were excluded. Figure 17 clearly shows that most of the variation along PC1 is explained by texture, liquid loss and QIM. The correlation between variables can also be evaluated by PCA correlation loading plot. This plot reveals that liquid loss and the QI scores are located close to each other and positively correlated while texture (hardness and shear work) is negatively correlated to days of storage.

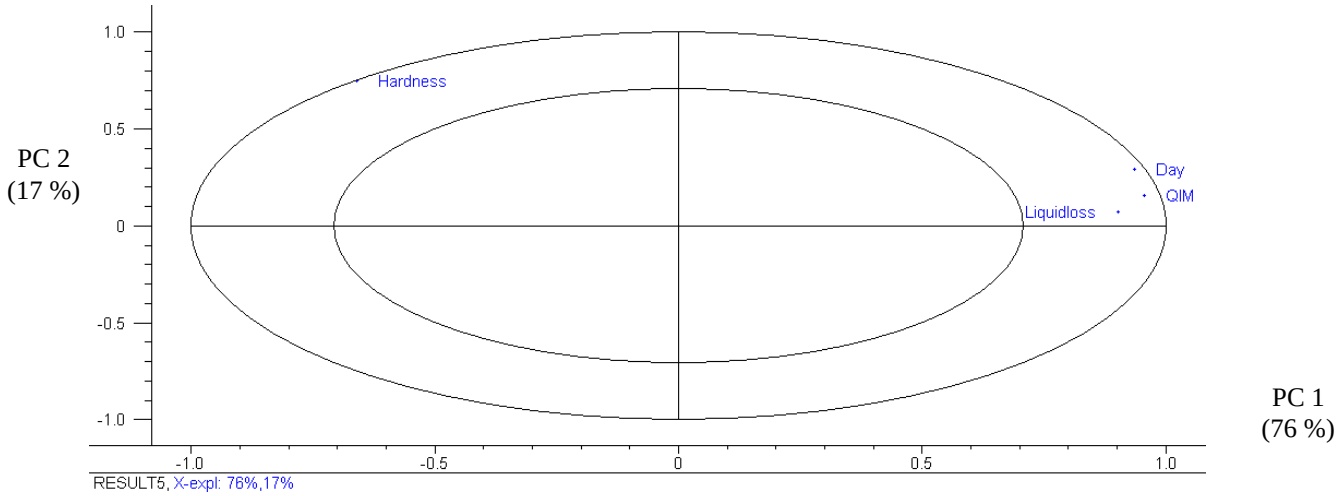


Figure 17: Loading plot showing only some of quality parameters explain 93 % of total variation.

4. Discussion

Quality of fish and fish products is of high importance for consumers and, hence, producers. Complains regarding quality deterioration especially gaping and softens under different storage conditions have increased. In spite of numerous investigations have placed much emphasis on quality deterioration, little is known about biochemical mechanisms leading to quality losses in Atlantic salmon stored on ice. The main goal of the present work was to study the changes in quality of Atlantic salmon during the whole shelf life period at two different seasons. To ensure, fish was selected from the same farm for both experimental periods. Fish was similar in respect to the feed, feeding regime, age, weight and the same slaughter procedure. One exception is the time of starvation before slaughter, which were 2 weeks in autumn and 4 weeks in winter. But taking the sea water temperature into account the number of day degrees during the starvation period is not very different. Approximately 140 day degrees for both seasons. Thus, influence of these factors should be the same. Salmon harvested during different seasons (winter and autumn) and from different loading of the boat (late and early) was compared to see whether seasonal differences and time of loading contribute the deterioration of salmon quality stored on ice. The principal component analysis (PCA) indicates that increasing storage time on ice have impact on several quality parameters, whereas loading time have minor effect on quality. Season only affected the quality index. Our results discovered in this Master thesis provide new knowledge to existing concerning impact on biochemical, microbiological and quality changes during ice storage of Atlantic salmon with respect to season and loading stress.

4.1 Change in the QI score with ice storage

As was mentioned in the introduction, spoilage of fish is a combined effect of autolytic and bacterial activity. Generally, maximum shelf-life of salmon is 24 days on ice (Sveinsdottir, Martinsdottir et al. 2002).When the QI is above 20, the spoilage of fish becomes evident (K. Sveinsdottir et al., 2002). In present study, since, the average QI scores were less 20 and the H_2S producing bacteria loads were low by the end of storage period, the fish spoilage did not become evident. We can therefore conclude in agreement with Sveinsdottir et al. (2002) that salmon is safe for human consumption for more than 22 days when stored on ice.

When considering the seasonal effect in present study, there was a trend that winter harvested fish had a higher QI scores for freshness compared to autumn. Data on seasonal effect on development of the QI scores in salmon is limited. The present findings are in accordance

with earlier study (Wünnenberg and Oehlenschläger 2008), who found that rainbow trout scored lower in autumn while winter harvested fish received inferior scores. It is tempting to suggest that the seasonal effect might be due to the water temperature at the time of slaughter. Since bacterial flora plays key role in the loss of sensory quality, fish harvested in winter created a more suitable environment for bacteria growth due to smaller temperature drop between seawater and ice compared with autumn. Thus, the acclimation to new environmental conditions was going faster and the results also showed a tendency towards higher bacterial growth in winter sampling even though not significant.

4.2 Changes in color during ice storage

The red color in farmed Atlantic salmon is an important quality characteristic and is due to the oxygenated carotenoids, mainly astaxanthin being stored in the muscle (Christiansen, Struksnæs et al. 1995, Bahuaud, Mørkøre et al. 2008). In agreement with previous reports (Choubert, Mendes-Pinto et al. 2006, Erikson, Misimi et al. 2011) it was found that storage time have minor effect on the fillet color. This is associated with a good stability of astaxanthin under ice storage of whole fish (Yagiz, Kristinsson et al. 2010, Choubert, Brisbarre et al. 2011). Small *post-mortem* changes in redness (a* value) and yellowness (b* value) were observed in accordance to *post-mortem* aging, basically corresponding to previous study (Erikson, Misimi et al. 2011). The stable activities of cathepsins observed in the present experiment indicates a slow protein denaturation going on during ice storage leading to slowly change the fillet surface. This may explain a small increase in a* value during ice storage.

4.3 Changes in the proximate composition with ice storage

Water and fat content measured in a present study is in respect with numerous studies (Nordgarden, Ørnsrud et al. 2003, Løje and Løje 2007, Bahuaud, Mørkøre et al. 2010). Fat content in Atlantic salmon is reported to increase with increasing fish weight and season (Løje and Løje 2007, Mørkøre, Rødbotten et al. 2010). In our experiment the fish size was the larger in autumn probably explaining the higher fat content. A small decrease in protein content is probably associated with protein degradation during ice storage due to relatively stable cathepsin activity (Hultmann and Rustad 2004).

4.4 Liquid loss

Liquid loss is important quality parameter and high liquid loss results in a negative effect on consumer acceptance (Mørkøre, Hansen et al. 2002). Liquid-holding capacity varies with interaction of different factors. A low *post mortem* pH affects the liquid-holding capacity resulting in a higher liquid loss (Ofstad, Egelandsdal et al. 1996). However, changes in liquid loss cannot solely be explained by the pH value due to no significant changes during the storage and sample periods. Maturation and nutritional status can also results in alterations in liquid holding capacity (Ofstad, Egelandsdal et al. 1996). Post -spawned and re-fed fish showed a higher drip loss. Ofstad, Egelandsdal et al. (1996) showed a small variation in liquid holding capacity between seasons. However, it might be explained by the different pH values in matured fish. In our case no clear differences were observed in liquid loss between seasons, probably due to the fish not being mature and not differing in muscle pH. Mørkøre, Vallet et al. (2001) also suggested that fat fish is more susceptible to water loss. In agreement with previous investigations (Mørkøre, Hansen et al. 2002, Rørå and Einen 2003, Bahuaud, Mørkøre et al. 2008), liquid loss increased with storage time in both seasons. It might be explained by muscle protein denaturation during ice storage. Among various tests to determine liquid loss of raw salmon, leakage test seemed to be more suitable. Due to the centrifugation test and the filter press method are expensive and time-consuming. Thus, in present work, it was decided to use liquid leakage test (Mørkøre, 2002) as described in Material and Methods. This method is preferred to be performed quick, easy and cheap.

4.5 Changes in pH in ice storage

As was mentioned in the introduction rapid *post-mortem* changes in pH values can lead to negative effects on quality of fish muscle such as high liquid loss, rapid degradation of tissue, increased gaping and soft texture (Ofstad, Egelandsdal et al. 1996, Taylor, Fjaera et al. 2002, Hultmann and Rustad 2004, Bahuaud, Mørkøre et al. 2010). In the present case, the muscle pH value did not vary significantly during storage period which are in agreement with previous storage investigations (Love 1988, Ofstad, Egelandsdal et al. 1996). The decrease in pH is due to the production of lactic acid *post mortem* (Foegeding et al 1996) An increase of the ultimate pH value can be explained by high microbiological activity that was recorded at the end of storage period. For Atlantic halibut, the lowest pH is observed in summer months when feeding activity was higher (Olsson, Olsen et al. 2003). In contrast, Espe, Ruohonen et

al. (2004) reported opposite patterns of the *post-mortem* development of pH values for Atlantic salmon. The highest value was observed in June compared to winter months (February) when the pH value was the lowest. However, the pH value did not significantly vary with season (Love 1988). In the present work, the pH value was not measured in winter sample period because of technical problems. Thus, it is impossible to compare the pH value in winter and autumn sample periods.

4.6 Changes in fillet texture

Texture is a complex parameter affected by interaction of various factors. Texture is showed to vary with storage time, pH, fat content, size of fish and stress (Andersen, Thomassen et al. 1997, GoMez-GuillÉN, Montero et al. 2000, Mørkøre, Vallet et al. 2001). However, little is still known about reason of softening. Since proteolysis and breakage of fibre had little effect on textural changes, fish muscle softens probably due to denaturation of proteins and connective tissue changes (Taylor, Fjaera et al. 2002). Moreover Taylor, Fjaera et al. (2002) also suggested that slow resolution of rigor affects a rapid softening during the first days of ice storage. A decrease in hardness (shear force) and TPA measured instrumentally for the first 4 days on ice is in agreement with previous reports (Andersen, Thomassen et al. 1997, Mørkøre, Hansen et al. 2002, Taylor, Fjaera et al. 2002) where texture changed significantly during a period of 24 hours with additional changes during the first 5 days, and therafter was unchanged.

Since instrumental texture measurements are more precise and minimize potential errors among measurements, the present study used two instrumental techniques including shear test and TPA to assess fillet texture. It was reported (Mørkøre and Einen 2003) that hardness TPA is more suitable technique due to more strongly correlation with sensory assessment of firmness, which is also supported by our PCA-analysis.

4.7 Cathepsin activity

When considering the effect of storage time, no significant effects on the cathepsin activities were seen. These results are in accordance with previous works (Duun and Rustad 2008, Gaarder, Bahuaud et al. 2012), where cathepsin activities increased early and remained relatively active and stable throughout the storage period. Gaarder, Bahuaud et al. (2012) reported that cathepsin B+L activity did not undergo any changes after 6 days of storage

period when the highest peak was observed. It seems that the cathepsins are released during the storage period due to naturally damage of lysosomal membrane. As mentioned in the introduction, enzyme activities are determined by drop in pH that, in turn, may explain the liberation of cathepsins during the storage. The stability of cathepsins activity could be related to the textural and sensory changes of the salmon during the whole storage period, even though no significant correlation between cathepsin activity and texture/gaping was found.
In present study, it appears that the cathepsin activities were not affected by seasonal periods. However, Gómez-Guillén and Batista (1997) showed a significantly higher activity of cathepsin D-like in sardine muscle during autumn compared to winter probably due to maximum spawning activity.

4.8 Microbiology

The flesh of newly slaughtered salmon contained very low total viable counts because the immune system prevents from the bacteria growth. The immune system collapses after dearth and due to the nutrient richness of fish muscle microbial growth increases rapidly (Gram and Huss 1996, Sveinsdottir, Martinsdottir et al. 2002). According to various researches (Sveinsdottir, Martinsdottir et al. 2002, Lougovois, Kyranas et al. 2003, Bahuaud, Mørkøre et al. 2008) the spoilage of fish is characterized by bacterial growth to high numbers (more than $10^6 – 10^7$ CFU/g). In present cases, the bacterial counts was less than 10^5 CFU/g (Stannard 1997) by the end of storage experiment indicating a good quality salmon throughout the storage. It is suggested that counts of specific spoilage bacteria can be used as indicators of iced fish spoilage (Sveinsdottir, Martinsdottir et al. 2002, Lougovois, Kyranas et al. 2003). Sensory quality of salmon is correlated with bacterial counts. Sveinsdottir, Martinsdottir et al. (2002) showed that the decrease of the sensory quality of fish (high scores in QIM) is associated with growth of microflora, in particular, H_2S-producing bacteria. H_2S-producing bacteria reported to be specific spoilage bacteria (Gram and Huss 1996), were analyzed only after 10 days of storage. The present results agree with previous investigations (Bronstein, Price et al. 1985, Sveinsdottir, Hyldig et al. 2003). The study support that early loss of the sensory quality is caused by the autolytic reactions (Lougovois, Kyranas et al. 2003) since in present work the cathepsin activities are consistent over the storage period, whereas bacterial growth very low at day 1 and increasing in day 10 and 22.

4.9 Changes in the TVN value with storage time

TVN (total volatile nitrogen) includes the measurements of trimethylamine (TMA), dimethilamine (DMA), ammonia and other volatile nitrogen compounds (Koutsoumanis and Nychas 2000). Production of total volatile nitrogen (TVN) and trimethyl amine (TMA) are caused mainly by microbial activity and, therefore, are used as indexes for spoilage (Joffraud, Cardinal et al. 2006). Since total bacteria counts increased by the end of storage time, an increasing of the TVN and TMA were also expected as reported previously (Özogul, Polat et al. 2004, Kilinc and Cakli 2005, Hozbor, Saiz et al. 2006, Joffraud, Cardinal et al. 2006, Sallam 2007). Salmon is reported to have lower value of TMA and TVN compared to other species due to a low level of TMAO (Sallam 2007). Depletion of TMAO is coincident with the increase of TVN and TMA (Kilinc and Cakli 2005). However, TVN was measured instead of TMA in the present study. It was found that TVN amount did not changed significantly throughout the storage experiment in the present study. The TVN value was low and not in excess of 30 mg N/100 mg that indicated by technical standard (Fernández, Aspe et al. 2009). The low formation of TVN during ice storage of salmon also reported previously (Sallam 2007, Fernández, Aspe et al. 2009) is probably due to low level of TMAO used by bacteria for growth. According to present study, farmed Atlantic salmon is still fit for human consumption after 21 days in ice.

4.10 Histological alteration during ice storage

As well-known, storage affects the microstructure of fish muscle resulting in an increase in the myofibre-myofibre detachments (Taylor, Fjaeras et al. 2002). Changes in microstructure are associated with reduced connective tissue strength and denaturation of proteins due to low *post-mortem* pH (Love 1988). In present study, observations of muscle fibre cross-section showed only small increase in myofibre-myofibre detachments that agrees with Kaale and Eikevik (2013) who found that increasing detachment in microstructure became evident after 20 days of ice storage. In contrast, Bahuaud, Mørkøre et al. (2008) showed more rapid acceleration of myofibre-myofibre detachments. Taylor, Fjaera et al. (2002) also found that fibre were detached from each other after the 5 days of storage. It was supported by several studies (Taylor, Fjaera et al. 2002) that myofibre-myofibre detachments as well as myofibre-myocommata lead to muscle softens. It is interesting to note that small increase of percentage of detachments in muscle might be associated with loss of hardness during first day of ice

storage as reported by Taylor, Fjaera et al. (2002). The loss of fibre-fibre attachment might be due to disconnection of cell cytoskelton from the sarcomeres to the endomysium (Taylor, Fjaera et al. (2002). The seasonal effect on the histological alterations did not show clear differences and might be due to similar cathepsin activity throughout the storage time in both winter and autumn harvested fish.

4.11 PCA

Principal component analysis is valuable method for assessment of the difference between winter and autumn harvested Atlantic salmon, and moreover, differences between late and early loading. The loading plots (Fig. 17) shows that the texture is inversely correlated to days on ice, while the liquid loss increased with storage time. This is in an agreement with previous studies where soften is associated with storage time (Taylor, Fjaera et al. 2002; Mørkøre, Hansen et al. 2002, Rørå and Einen 2003, Bahuaud, Mørkøre et al. 2008).We also observed that cathepsin activity had tendency to inversely correlate to the texture of fish (not shown). Perera 2011 found the same trend when a high cathepsin activity results in soften of the texture.

4.12 Effect of loading

Several studies have shown a negative effect of stress during transport and harvesting procedures on textural characteristics of different species (Bagni, Civitareale et al. 2007, Mørkøre, Mazo T et al. 2008, Matos, Gonçalves et al. 2010). However, mechanisms relating to the softening of fish remain unclear. If the fish from the late loading from the boat were more stressed than from the early loading, we could expect more soften fillet texture, which also was the case. In addition we found late loading to have higher liquid loss, which also indicate a more rapid drop in pH *post mortem*. Rapid decrease of pH results in the denaturation of protein affecting the liquid-holding capacity and higher liquid loss during ice storage (Mørkøre, Mazo T et al. 2008). It is interesting to note that cathepsin B+L activity is lower in the late loading also compared with early loading which agrees with previous study (Hultmann, Phu et al. 2012) cathepsin B+L activity reduced in stressed cod although not significant. Rapid drop of pH immediately after death of fish due to stress involves a *post-mortem* damage of fish muscle, especially of texture and liquid loss (Love 1988, Mørkøre, Hansen et al. 2002, Bagni, Civitareale et al. 2007, Bahuaud, Mørkøre et al. 2008). It is well-

known that short-term stress leads to a faster drop of pH value and a lower final pH compared to stress of long duration (Skjervold, Fjæra et al. 2001). In our case, we can speculate that differences in texture and liquid loss of fish from different loadings of the boat might be due to decrease in the pH drop during first 24 hours.

5. Conclusion

The present study demonstrates that ice storage of Atlantic salmon (Salmo salar) during 22 days leads to decreased hardness and shear work, and an increase in microbiological growth, liquid loss, fillet gaping and the QI scores while the other quality parameters measured remain stable in respect to storage time in both seasons. Only small changes were observed in protein content and, flesh color a* and b* values, and myofibre-myofibre detachments. After 22 days of ice storage the total bacteria count in Atlantic salmon is still below 10^5 CFU/g, indicating that the product is still safe to eat.

Winter sampled fish have higher QI scores compared with autumn, whereas the other parameters measured was not affected by season.

Loading time in this study had only a small effect on flesh quality, late loadingresulting in slightly softer texture, increased liquid loss and decreasing cathepsin activity.

Season had not a significant effect on parameters measured with exception of the QI score ($p<0.05$). A higher QI score during winter is believed due to water temperature at the time of slaughter.

During late loading, only a slight effects were observed, resulting in soften, increasing liquid loss and decreasing of cathepsin activity.

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Appendix

Appendix 1: Test results of influence of storage time for the quality parameters measured

Tabell 12:Test results of influence of storage time for the parameters measured in Atlantic salmon harvested in winter (n=60) and autumn (n=60).

Quality parameter	P value		Test
	Winter	Autumn	
Water	0.05	0.484	ANOVA, Tukeys test
Protein	0.438	0.006	ANOVA, Tukeys test
Fat	0.09	0.078	ANOVA, Tukeys test
TVN	0.063	0.218	ANOVA, Tukeys test
Fillet gaping	0.540	0.0001	Kruskal-Wallis test
pH	0.001	0.000.	Kruskal-Wallis test
Liquid loss	0.0001	0.0001	Kruskal-Wallis test
QIM	0.0001	0.000.	ANOVA, Tukeys test
Roche	0.673	0.0001	Kruskal-Wallis test
L	0.023	0.058	ANOVA, Tukeys test
a*	0.046	0.0001	ANOVA, Tukeys test
b*	0.0001	0.0001	ANOVA, Tukeys test
Shear work	0.0001	0.0001	Kruskal-Wallis test
Hardness	0.690	0.0001	ANOVA, Tukeys test
Cohesiveness	0.453	0.0001	ANOVA, Tukeys test
Gumminess	0.576	0.0001	ANOVA, Tukeys test
Cathepsin B+L	0.145	0.660	ANOVA, Tukeys test
Cathepsin H	0.013	0.187	ANOVA, Tukeys test
TBC	0.546	0.0001	Kruskal-Wallis test
IA (TBC)	not measured	0.0001	Kruskal-Wallis test
Long&Hammer Agar (TBC)	not measured	0.0001	Kruskal-Wallis test

- Appendix 2-

Appendix 2: Test results between winter and autumn

Tabell 13:Mean value, standard deviations and test results between winter and autumn.

Parameter	Days on ice	Winter			Autumn			p	Test
		Mean	SD	#	Mean	SD	#		
Water (%)	Day 1	65.32	1.41	6	63.79	1.19	6	0.071	ANOVA
Water (%)	Day 4	64.88	1.66	6	64.27	0.99	6	0.458	ANOVA
Water (%)	Day 7	64.24	1.67	6	63.92	1.41	6	0.72	ANOVA
Water (%)	Day 10	64.95	0.93	6	64.23	1.05	6	0.237	ANOVA
Water (%)	Day 16	65.96	0.65	6	64.62	1.19	6	0.037	ANOVA
Water (%)	Day 22	66.39	2.00	6	63.40	0.53	6	0.458	ANOVA
Protein (%)	Day 1	20.07	0.38	6	20.49	0.19	6	0.038	ANOVA
Protein (%)	Day 4	19.99	0.40	6	20.22	0.54	6	0.438	ANOVA
Protein (%)	Day 7	19.87	0.44	6	19.96	0.40	6	0.724	ANOVA
Protein (%)	Day 10	19.67	0.41	6	19.78	0.32	6	0.610	ANOVA
Protein (%)	Day 16	19.64	0.29	6	19.77	0.40	6	0.543	ANOVA
Protein (%)	Day 22	19.83	0.53	6	19.60	0.43	6	0.438	ANOVA
Fat (%)	Day 1	13.88	1.75	6	14.00	1.83	6	0.952	ANOVA
Fat (%)	Day 4	13.95	1.96	6	14.12	0.81	6	0.060	ANOVA
Fat (%)	Day 7	15.48	1.94	6	14.52	2.55	6	0.706	ANOVA
Fat (%)	Day 10	14.45	1.18	6	15.30	1.17	6	0.685	ANOVA
Fat (%)	Day 16	13.78	0.76	6	15.02	1.38	6	0.219	ANOVA
Fat (%)	Day 22	14.27	1.51	6	16.57	0.87	6	0.076	ANOVA
TVN	Day 1	21.08	2.53	10	17.04	0.94	10	0.02	Mann-Whitney U test
TVN	Day 10	15.86	0.46	10	15.98	0.86	10	ns	Mann-Whitney U test
TVN	Day 22	17.14	1.24	10	16.46	1.05	10	0.218	Mann-Whitney U test
Fillet gaping	Day 1	0.00	0.00	10	0.00	0.00	10	ns	Mann-Whitney U test
Fillet gaping	Day 4	0.00	0.00	10	0.00	0.00	10	ns	Mann-Whitney U test
Fillet gaping	Day 7	0.00	0.0	10	0.05	0.15	10	0.739	Mann-Whitney U test
Fillet gaping	Day 10	0.10	0.31	10	0.00	0.00	10	0.739	Mann-Whitney U test
Fillet gaping	Day 16	0.00	0.00	10	0.30	0.25	10	0.023	Mann-Whitney U test
Fillet gaping	Day 22	.010	0.31	10	0.30	0.25	10	0.105	Mann-Whitney U test
pH	Day 1			0	6.32	0.11	10		ANOVA
pH	Day 4			0	6.40	0.07	10		ANOVA
pH	Day 7			0	6.21	0.02	10		ANOVA
pH	Day 10			0	6.19	0.02	10		ANOVA
pH	Day 16	6.4	0.07	10	6.06		1	0.01	ANOVA
pH	Day 22	6.22	0.13	10	6.25	0.03	10	0.555	ANOVA
QIM	Day 1	0.35	0.47	10	0.17	0.37		0.393	Mann-Whitney U test
QIM	Day 4	5.00	0.47	10	2.75	1.02	10	0.393	Mann-Whitney U test
QIM	Day 7	8.22	0.96	10	3.90	1.77	10	0.001	Mann-Whitney U test
QIM	Day 10	11.32	0.75	10	6.20	1.23	10	0.001	Mann-Whitney U test
QIM	Day 16	14.48	1.06	10	15.20	0.92	10	0.315	Mann-Whitney U test
QIM	Day 22	18.12	1.13	10	15.95	0.87	10	0.001	Mann-Whitney U test
Liquid loss	Day 1	1.09	0.26	10	1.44	0.62	10	0.368	Mann-Whitney U test
Liquid loss	Day 4	1.62	0.46	10	2.01	.05	10	0.368	Mann-Whitney U test
Liquid loss	Day 7	2.19	0.37	10	1.88	0.61	10	0.247	Mann-Whitney U test
Liquid loss	Day 10	2.21	0.57	10	3.32	0.93	10	0.05	Mann-Whitney U test
Liquid loss	Day 16	4.14	2.06	10	3.06	0.68	10	0.165	Mann-Whitney U test
Liquid loss	Day 22	2.71	0.52	10	4.39	1.34	10	0.007	Mann-Whitney U test
Roche	Day 1	26.90	0.31	10	27.00	0.47	10	0.739	Mann-Whitney U test
Roche	Day 4	26.90	0.31	10	26.20	0.42	10	0.739	Mann-Whitney U test
Roche	Day 7	26.80	0.42	10	26.30	0.48	10	0.063	Mann-Whitney U test
Roche	Day 10	26.70	0.48	10	26.10	0.31	10	0.023	Mann-Whitney U test
Roche	Day 16	26.70	0.48	10	27.20	0.63	10	0.123	Mann-Whitney U test
Roche	Day 22	26.90	0.31	10	27.20	0.42	10	0.315	Mann-Whitney U test

Appendix 2

Table 13: Cntd..

Parameter	Days on ice	Winter			Autumn			p	Test
		Mean	SD	#	Mean	SD	#		
L	Day 1	41.53	1.40	10	42.29	2.06	10	0.247	Mann-Whitney U test
L	Day 4	43.70	2.77	10	41.94	1.65	10	0.247	Mann-Whitney U test
L	Day 7	40.86	3.35	10	42.64	1.60	10	0.123	Mann-Whitney U test
L	Day 10	43.61	1.46	10	42.81	1.61	10	0.436	Mann-Whitney U test
L	Day 16	40.76	3.27	10	41.22	1.60	10	0.971	Mann-Whitney U test
L	Day 22	41.01	2.81	10	43.69	1.91	10	0.029	Mann-Whitney U test
a*	Day 1	15.46	1.65	10	12.49	2.09	10	0.05	Mann-Whitney U test
a*	Day 4	15.58	1.82	10	16.51	1.50	10	0.05	Mann-Whitney U test
a*	Day 7	16.85	1.59	10	16.10	0.96	10	0.280	Mann-Whitney U test
a*	Day 10	14.93	1.52	10	17.45	1.78	10	0.003	Mann-Whitney U test
a*	Day 16	17.45	2.37	10	16.35	1.12	10	0.280	Mann-Whitney U test
a*	Day 22	16.68	2.00	10	18.34	1.39	10	0.035	Mann-Whitney U test
b*	Day 1	11.17	1.58	10	8.02	2.05	10	0.01	Mann-Whitney U test
b*	Day 4	12.21	1.88	10	13.40	1.35	10	0.01	Mann-Whitney U test
b*	Day 7	12.80	1.62	10	12.26	1.37	10	0.247	Mann-Whitney U test
b*	Day 10	11.43	1.17	10	14.76	2.52	10	0.004	Mann-Whitney U test
b*	Day 16	12.85	2.74	10	10.98	1.58	10	0.143	Mann-Whitney U test
b*	Day 22	12.49	1.87	10	15.97	1.22	10	0.001	Mann-Whitney U test
Shear work	Day 1	4.48	1.26	10	3.66	1.26	10	0.166	ANOVA
Shear work	Day 4	2.48	0.48	10	1.88	0.31	10	0.004	ANOVA
Shear work	Day 7	1.86	0.45	10	2.07	0.29	10	0.246	ANOVA
Shear work	Day 10	1.97	0.37	10	1.71	0.25	10	0.081	ANOVA
Shear work	Day 16	1.86	0.18	10	1.93	0.51	10	0.696	ANOVA
Shear work	Day 22	1.99	0.38	10	2.19	0.38	10	0.271	ANOVA
Hardness	Day 1	1.81	0.36	10	2.03	0.29	10	0.159	ANOVA
Hardness	Day 4	1.60	0.31	10	1.32	0.22	10	0.043	ANOVA
Hardness	Day 7	1.16	0.27	10	1.34	0.30	10	0.176	ANOVA
Hardness	Day 10	1.27	0.27	10	1.45	0.26	10	0.165	ANOVA
Hardness	Day 16	1.16	0.17	10	1.38	0.27	10	0.043	ANOVA
Hardness	Day 22	1.31	0.31	10	1.36	0.28	10	0.690	ANOVA
Cohesivenes s	Day 1	1.65	0.40	10	1.86	0.33	10	0.232	ANOVA
Cohesivenes s	Day 4	1.25	0.22	10	1.14	0.25	10	0.348	ANOVA
Cohesivenes s	Day 7	0.97	0.30	10	1.19	0.30	10	0.116	ANOVA
Cohesivenes s	Day 10	1.00	0.25	10	1.20	0.20	10	0.081	ANOVA
Cohesivenes s	Day 16	0.92	0.13	10	1.16	0.25	10	0.018	ANOVA
Cohesivenes s	Day 22	1.02	0.33	10	1.12	0.24	10	0.453	ANOVA
Gumminess	Day 1	1.24	0.25	10	1.35	0.20	10	0.320	ANOVA
Gumminess	Day 4	1.09	0.20	10	0.86	0.16	10	0.013	ANOVA
Gumminess	Day 7	0.77	0.20	10	0.87	0.20	10	0.300	ANOVA
Gumminess	Day 10	0.83	0.18	10	0.93	0.19	10	0.212	ANOVA
Gumminess	Day 16	0.76	0.11	10	0.87	0.18	10	0.130	ANOVA
Gumminess	Day 22	0.83	0.20	10	0.88	0.19	10	0.576	ANOVA

Appendix 2

Table 13: Cntd…

Parameter	Days on ice	Winter			Autumn			p	Test	
		Mean	SD	#	Mean	SD	#			
Cathepsin B+L	Day 7	1597.33	1267.69	10	1006.22	181.38	10	0.393	Mann-Whitney test	
Cathepsin B+L	Day 10	1008.47	171.33	10	1030.20	308.45	10	0.971	Mann-Whitney test	
Cathepsin B+L	Day 16	1068.32	239.24	10	957.78	266.57	10	0.123	Mann-Whitney test	
Cathepsin B+L	Day 22	1246.91	235.78	10	1081.01	250.93	10	0.280	Mann-Whitney test	
Cathepsin B+L	Day 7	1597.33	1267.69	10	1006.22	181.38	10	0.393	Mann-Whitney test	
Cathepsin B+L	Day 10	1008.47	171.33	10	1030.20	308.45	10	0.971	Mann-Whitney test	
Cathepsin H	Day 1	305.95	237.15	10	389.71	142.77	10	0.089	Mann-Whitney test	U
Cathepsin H	Day 4	600.94	494.14		291.63	159.53	10	0.089	Mann-Whitney test	U
Cathepsin H	Day 7	514.82	344.72	10	291.63	159.52	10	0.529	Mann-Whitney test	U
Cathepsin H	Day 10	597.55	523.17	10	365.27	162.32	10	0.631	Mann-Whitney test	U
Cathepsin H	Day 16	484.44	246.41	10	431.36	187.83	10	0.739	Mann-Whitney test	U
Cathepsin H	Day 22	323.21	89.53	10	376.14	76.24	10	0.009	Mann-Whitney test	U
TBC	Day 1	0	0	10	0	0	10	ns	Mann-Whitney test	U
TBC	Day 10	5.50	8.99	10	209.60	234.29	10	0.001	Mann-Whitney test	U
TBC	Day 22	2139.50	1054.12	10	1061.30	143.13	10	0.001	Mann-Whitney test	U
IA (TBC)	Day 10			0	7.90	12.10	10	0.001	Mann-Whitney test	U
IA (TBC)	Day 22			0	49.70	36.24	10	0.001	Mann-Whitney test	U
Blackcolonies in IA	Day 10			0	142.60	96.56	10	0.001	Mann-Whitney test	U
Blackcolonies in IA	Day 22			0	1036.50	386.77	10	0.001	Mann-Whitney test	U
L&H Agar (TBC)	Day 10			0	142.60	96.56	10	0.001	Mann-Whitney test	U
L&H Agar (TBC)	Day 22			0	1036.50	386.77	10	0.001	Mann-Whitney test	U
luminous bacteria (L&H)	Day 10			0	24.00	32.42	10	0.001	Mann-Whitney test	U
luminous bacteria (L&H)	Day 22			0	1.00	2.10	10	0.001	Mann-Whitney test	U

- Appendix 3

Appendix 3: Test results between late and early loadings

Table 14: Mean value, standard deviation of the fish from the late and early loadings

Parameter	Days on ice	Early loading			Late loading		
		Mean value	SD	#	Mean value	SD	#
Water	Day 1	64.70	1.31	6	64.41	1.74	6
Water	Day 4	65.17	1.75	6	63.98	1.07	6
Water	Day 7	63.57	1.31	6	64.60	1.24	6
Water	Day 10	64.99	1.75	6	64.19	0.89	6
Water	Day 16	65.16	1.31	6	65.42	0.59	6
Water	Day 22	65.51	1.75	6	64.28	1.16	6
Protein	Day 1	20.20	0.47	6	20,37	0.24	6
Protein	Day 4	19.95	0.26	6	20.22	0.63	6
Protein	Day 7	19.63	0.38	6	20.16	0.26	6
Protein	Day 10	19.91	0.34	6	19.55	0.29	6
Protein	Day 16	19.56	0.38	6	19.86	0.23	6
Protein	Day 22	19.56	0.53	6	19.88	0.39	6
Fat	Day 1	13.57	1.86	6	14.32	1.63	6
Fat	Day 4	13.36	1.15	6	14.72	1.45	6
Fat	Day 7	15.34	2.97	6	14.67	1.32	6
Fat	Day 10	14.17	1.08	6	15.60	0.91	6
Fat	Day 16	14.58	1.72	10	14.23	0.64	10
Fat	Day 22	14.98	1.80	10	15.87	1.58	10
TVN	Day 1	18.22	2.25	10	19.92	3.12	10
TVN	Day 10	15.86	0.93	10	15.99	0.30	10
TVN	Day 22	16.81	1.50	10	16.80	0.81	10
Gaping	Day 1	0.00	0.00	10	0.00	0.00	10
Gaping	Day 4	0.00	0.00	10	0.00	0.00	10
Gaping	Day 7	0.05	0.16	10	0.00	0.00	10
Gaping	Day 10	0.00	0.00	10	0.10	0.32	10
Gaping	Day 16	0.15	0.24	10	0.15	0.24	10
Gaping	Day 22	0.15	0.24	10	0.25	0.35	10
pH	Day 1	6.31	0.168	5	6.33	0.058	5
pH	Day 4	6.38	0.096	5	6.42	0.038	5
pH	Day 7	6.23	0.028	5	6.20	0.013	5
pH	Day 10	6.19	0.029	5	6.20	0.025	5
pH	Day 16	6.41	0.175	10	6.40	0.073	10
pH	Day 22	6.28	0.071	10	6.19	0.099	10
Liquid loss	Day 1	1.32	0.49	10	1.31	0.63	10
Liquid loss	Day 4	1.64	0.46	10	1.99	0.59	10
Liquid loss	Day 7	1.86	0.56	10	2.22	0.43	10
Liquid loss	Day 10	2.62	0.54	10	2.93	1.24	10
Liquid loss	Day 16	3.07	1.07	10	4.14	1.91	10
Liquid loss	Day 22	3.14	0.81	10	3.97	1.62	10
QIM	Day 1	.35	.53	10	.18	.29	10
QIM	Day 4	4.15	1.18	10	3.60	1.59	10
QIM	Day 7	5.95	2.41	10	6.18	2.94	10
QIM	Day 10	8.55	2.57	10	8.98	3.16	10
QIM	Day 16	15.25	.90	10	14.83	1.07	10
QIM	Day 22	17.35	1.86	10	16.73	1.00	10

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Table 14: Cntd…

Parameter	Days on ice	Early loading			Late loading		
		Mean value	SD	#	Mean value	SD	#
Roche	Day 1	27.00	0.47	10	26.90	0.32	10
Roche	Day 4	26.70	0.48	10	26.40	0.52	10
Roche	Day 7	26.60	0.52	10	26.50	0.53	10
Roche	Day 10	26.40	0.52	10	26.40	0.52	10
Roche	Day 16	27.10	0.57	10	26.80	0.63	10
Roche	Day 22	27.10	0.32	10	27.00	0.47	10
L	Day 1	42.33	1.63	10	41.51	1.88	10
L	Day 4	43.22	2.50	10	42.43	2.36	10
L	Day 7	41.12	3.12	10	42.39	2.23	10
L	Day 10	42.80	1.76	10	43.63	1.28	10
L	Day 16	42.33	1.92	10	39.65	2.39	10
L	Day 22	43.10	1.51	10	41.62	3.48	10
a*	Day 1	13.94	2.06	10	14.02	2.79	10
a*	Day 4	15.78	2.04	10	16.32	1.31	10
a*	Day 7	16.32	1.17	10	16.63	1.54	10
a*	Day 10	15.85	2.56	10	16.54	1.49	10
a*	Day 16	15.92	1.29	10	17.88	1.94	10
a*	Day 22	17.36	2.37	10	17.67	1.36	10
b*	Day 1	10.11	2.51	10	9.09	2.31	10
b*	Day 4	13.11	1.75	10	12.51	1.71	10
b*	Day 7	12.93	1.29	10	12.14	1.64	10
b*	Day 10	13.50	2.44	10	12.71	2.76	10
b*	Day 16	10.90	1.13	10	12.94	2.90	10
b*	Day 22	13.69	2.98	10	14.79	1.47	10
Shear work	Day 1	3.63	0.11	10	4.52	1.77	10
Shear work	Day 4	2.22	0.40	10	2.15	0.61	10
Shear work	Day 7	2.07	0.36	10	1.87	0.41	10
Shear work	Day 10	1.73	0.20	10	1.96	0.42	10
Shear work	Day 16	1.88	0.37	10	1.92	0.41	10
Shear work	Day 22	2.05	0.34	10	2.14	0.44	10
Hardness	Day 1	2.06	0.34	10	1.80	0.30	10
Hardness	Day 4	1.52	0.31	10	1.39	0.29	10
Hardness	Day 7	1.43	0.27	10	1.08	0.22	10
Hardness	Day 10	1.39	0.27	10	1.34	0.30	10
Hardness	Day 16	1.31	0.30	10	1.25	0.19	10
Hardness	Day 22	1.40	0.24	10	1.28	0.34	10
Cohesiveness	Day 1	1.83	0.41	10	1.68	0.35	10
Cohesiveness	Day 4	1.24	0.26	10	1.16	0.23	10
Cohesiveness	Day 7	1.27	0.30	10	0.90	0.21	10
Cohesiveness	Day 10	1.15	0.23	10	1.06	0.27	10
Cohesiveness	Day 16	1.10	0.26	10	0.99	0.20	10
Cohesiveness	Day 22	1.13	0.23	10	1.03	0.34	10
Gumminess	Day 1	1.39	0.24	10	1.21	0.20	10
Gumminess	Day 4	1.02	0.22	10	0.92	0.22	10
Gumminess	Day 7	0.95	0.18	10	0.70	0.15	10
Gumminess	Day 10	0.91	0.20	10	0.86	0.19	10
Gumminess	Day 16	0.84	0.19	10	0.80	0.13	10
Gumminess	Day 22	0.90	0.17	10	0.82	0.22	10

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Table 14: Cntd…

Parameter	Days on ice	Early loading			Late loading		
		Mean value	SD	#	Mean value	SD	#
Cathepsin B+L	Day 1	1108.27	389.11	10	985.35	127.21	10
Cathepsin B+L	Day 4	1490.99	950.90	10	911.67	200.66	10
Cathepsin B+L	Day 7	1648.52	1237.76	10	955.03	186.61	10
Cathepsin B+L	Day 10	1026.84	202.47	10	1011.83	289.20	10
Cathepsin B+L	Day 16	995.01	267.12	10	1031.10	251.02	10
Cathepsin B+L	Day 22	1251.42	322.58	10	1076.51	113.12	10
Cathepsin H	Day 1	459.10	193.00	10	236.56	125.56	10
Cathepsin H	Day 4	389.85	204.00	10	502.71	523.53	10
Cathepsin H	Day 7	421.29	331.20	10	458.81	217.09	10
Cathepsin H	Day 10	695.88	452.46	10	333.04	215.57	10
Cathepsin H	Day 16	386.41	161.58	10	474.17	206.54	10
Cathepsin H	Day 22	413.57	53.87	10	368.43	173.54	10
TBC	Day 1	0.00	0.000	10	0.00	0.000	10
TBC	Day 10	48.80	62.073	10	166.30	258.055	10
TBC	Day 22	1576.00	785.134	10	1624.80	1076.915	10
IA (TBC)	Day 10	1036.80	455.313	10	338.80	221.465	10
IA (TBC)	Day 22	2016.40	534.723	10	1109.40	117.470	10
Black colonies on IA	Day 10	11.00	17.190	10	4.80	3.194	10
Black colonies on IA	Day 22	53.20	47.230	10	46.20	26.348	10
L&H (TBC)	Day 10	199.20	103.212	10	86.00	48.172	10
L&H (TBC)	Day 22	1185.60	507.554	10	887.40	152.993	10
Luminous bacteria	Day 10	11.80	23.690	10	36.20	37.844	10
Luminous bacteria	Day 22	1.00	2.236	10	1.00	2.236	10

- Appendix 4

Appendix 4: Fillet gaping assessment in according to FHF.no

	Score 0: no gaping
	Score 1: Slight gaping
	Score 2: moderate gaping
	Score 3: serious gaping
	Score 4: fillet falling apart