Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 122–131
Food Chemistry
www.elsevier.com/locate/foodchem

Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at −1.4 and −3.6 °C

A.S. Duun *, T. Rustad

Department of Biotechnology, NTNU, NO-7491 Trondheim, Norway

Received 9 February 2007; received in revised form 16 March 2007; accepted 18 May 2007

Abstract

The most important factor for increasing shelf life is the product temperature, and since fish is more highly perishable than meat, the temperature is even more important. In the present study, portions of fillets of farmed Atlantic salmon (*Salmo salar*) were superchilled at two temperature levels, −1.4 and −3.6 °C. Texture, drip loss, liquid loss, cathepsin activities and protein extractability were investigated during storage and compared to ice chilled and frozen references. Drip loss was not a major problem in superchilled salmon. Textural hardness was significantly higher in superchilled salmon fillets stored at −3.6 °C compared to those stored at −1.4 °C, ice chilled and frozen references. Cathepsins B and B + L were not deactivated at the selected storage temperatures. The storage time of vacuum packed salmon fillets can be doubled by superchilled storage at −1.4 °C and −3.6 °C compared to ice chilled storage.

Keywords: Superchilling; Atlantic salmon; Texture; Drip loss; Shelf life; Liquid loss; Protein extractability; Cathepsin; Free amino acid

1. Introduction

Fish farming provides an opportunity to obtain a seasonally independent supply of fresh fish to the market, and the demand for fresh salmon is rapidly increasing at the expense of frozen salmon (Norwegian Seafood Export Council, 2005). Since the shelf life of fresh fish is lower than that of frozen fish, the need to develop methods for maintaining good postmortem quality of the fish on its way to the market increases. Extending the shelf life of fish will increase profitability, both because product prices in the fresh market are higher than in the frozen market and by reducing the amount of product becoming unacceptable for sale.

The most important factor for increasing shelf life is the product temperature, and since fish is more highly perishable than meat, the temperature is even more important. Superchilling is often used to describe a process, where food products are stored between the freezing point of the products and 1–2 °C below this. The surrounding temperature is set below the initial freezing point of the food, which is between −0.5 °C and −2.8 °C (Fennema, Powrie, & Marth, 1973), and depending on the method used some ice is formed in the outer few millimeters. For many food products, superchilling results in better quality when compared to conventional chilling (Einarsson, 1988).

When some of the water freezes out, the concentration of solutes in unfrozen solutions increases. This may lead to increased enzymatic activity, denaturation of the muscle proteins and structural damage of membranes, which can result in increased drip loss, loss of water holding capacity and textural changes (Foegeding, Lanier, & Hultin, 1996). The shelf life of superchilled food is far shorter than that of frozen food, but the lower amount of water frozen out will lead to less change in microstructure and a lower degree of freeze denaturation and less drip loss (Einarsson, 1988). However, freeze denaturation was found to take place in superchilled cod fillets (Duun & Rustad, in press).

* Corresponding author. Tel.: +47 73594070; fax: +47 73591283.
E-mail address: anne.sissel.duun@biotech.ntnu.no (A.S. Duun).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.051

Superchilled salmon stored at −2 °C in combination with modified atmosphere packaging (MAP) maintained good quality with negligible microbial growth for more than 24 d based on both sensory and microbial analyses. Superchilled salmon stored in air had a 21 d sensory shelf life, whereas MAP and air-stored fillets at chilled conditions spoiled after 10 d and 7 d, respectively (Sivertsvik, Rosnes, & Kleiberg, 2003). Superchilled cod showed increased shelf life, based on reduced growth of sulphide producing bacteria compared to that of ice chilled. Drip loss was lower, but liquid loss by low-speed centrifugation was higher in superchilled cod fillets compared to ice chilled. This is due to freeze denaturation of muscle proteins (Duun & Rustad, in press). In the superchilling temperature range, small variations in temperature cause large variations in ice content and large variations in product quality. A steady storage temperature is, therefore, of great importance to secure the ice content at an optimal and constant level.

Most of the studies on superchilling, have focused on microbiology, sensory analysis and spoilage indicators such as TVB-N (Olafsdottir, Lauzon, Martinsdottir, Oehlenschlager, & Kristbergsson, 2006; Sivertsvik, Jeksrud, & Rosnes, 2002; Sivertsvik et al., 2003; Zeng, Thorarinsdottir, & Olafsdottir, 2005), and have only, to a minor extent, investigated the effect of superchilling on biochemical processes and how they influence quality parameters, such as loss of juiciness and negative textural changes. The colour and texture are two of the most important quality characteristics of farmed salmon. The colour of farmed salmon is particularly important as salmonids normally accumulate astaxanthin, which provides the desirable reddish-orange flesh colour, from their natural diet (Higuera-Ciapara, Felix-Valenzuela, & Goycoolea, 2006). Astaxanthin may be lost both during freezing (Regost, Jakobsen, & Rora, 2004) and chilled storage (Gobantes, Choubert, & Gomez, 1998). The texture of fish meat is influenced by several factors. Among these are the rate and extent of postmortem muscle shortening (rigor mortis), the rate and extent of postmortem pH decline and the rate and extent of proteolysis causing myofibril breakdown (Haard, 1992). Tissue softening in salmon has been observed both during frozen (Einen, Guerin, Fjaera, & Skjervold, 2002) and ice chilled storage (Hultmann, 2003). The postmortem protein degradation in ice chilled storage, resulting in tissue softening, was caused by endogenous proteases.

The aim of this study, was to investigate the effects of a superchilling process at two different temperatures on selected quality parameters of salmon. Two superchilling temperatures were chosen in order to study differences in muscle properties caused by different levels of ice content.

2. Materials and methods

2.1. Raw materials and processing

Twenty-one Atlantic salmon (*Salmo salar*) from a fish farm in mid-Norway, were slaughtered (stunned by a blow to the head, bled and gutted) and transported on ice to the laboratory in October, 2004. After three days of ice chilled storage, the salmon were headed, filleted and trimmed. The fish had an average weight (±SD) of 4713 ± 319 g and the length was 76 ± 2 cm. Two portions of 469 ± 46 g were cut from the middle of each skin-on fillet, vacuum packed and labelled. The samples were subsequently cooled in a freezing tunnel at a temperature of −30 °C and air velocity of 2 m/s. The duration of the cooling was based on previous experiments and simulations (Hardarson, 1996). After cooling, the core temperature was approximately −1.0 °C. The samples were immediately transferred to a cold storage room at −1.4 ± 0.4 °C or −3.6 ± 0.3 °C for temperature equalization and storage for up to 34 days. The day of processing was defined as day 0. Air temperature was recorded every 2 min during the superchilled storage by loggers with internal sensor (Optic StowAway® Temp logger, Onset Computer Corporation, Mass, US). References were kept on ice for up to 17 days or frozen at −40 °C for 37 days, respectively. Prior to analysis (1–2 h depending on fillet thickness) subzero stored samples were transferred to room temperature for thawing.

Calorimetric quantification of ice were performed immediately after superchilling. Simple calorimeters (1 m^3) based on an insulated and waterproof storage chamber, instrumented with 10 thermocouples (±0.1 °C) to measure exact product and water temperatures, were used for ice fraction measurements. The superchilled samples were submerged in the centre of the chamber (27 l) with a defined volume of water. Sample and water temperatures were measured continuously as the samples were tempered and the water was cooled. The temperature changes were, together with information about the samples thermal and physical properties, used to estimate the amount of ice in the samples. Analyses were performed on three samples for each storage temperature.

2.2. Sampling

Superchilled samples at −1.4 °C were analyzed on day 2, 6, 9, 16, 20, 23, 27, 30 and 34, while superchilled samples at −3.6 °C were analyzed on day 2, 7, 10, 16, 20, 23, 27, 30 and 34. Ice stored references were analyzed on day 0, 6, 9, 14, 17 and 21 and frozen references stored at −40 °C were analyzed on day 37. Two samples were analyzed on each of the sampling days, except at day 0 and 37, where four samples were analyzed.

For quantification of the drip loss, the sample was removed from the vacuum bag and the liquid left in the bag was weighed. Mean values were calculated from two replicates. Water content in the drip liquid was determined by drying sample of ~2 g at 105 °C for 24 h in duplicate.

A commercial visual test (ColiFAST, Colifast AS, Norway) was used for a rapid detection of growth of sulphide producing bacteria (SPB) (Skjerdal, Lorentzen, Tryland, & Berg, 2004). During growth on a medium containing

ferricitrate and cysteine, SPB forms H_2S resulting in a black precipitate of FeS. The growth of SPB can thus be enumerated by counting black colonies on iron agar (Gram, Trolle, & Huss, 1987) or by observation of colour change in liquid medium from yellow to black at different times, depending on the amount of viable SPB (Skjerdal et al., 2004). Two muscle samples (1 × 1 × 1 cm), were aseptically cut from the surface (location A on Fig. 1) of the fillet after opening the vacuum bag and each sample were transferred to a medium vial. Samples were incubated at 30 °C and observed after 5, 8, 10, 12 and 14 h to detect the time of colour change. The analysis was performed only at selected points at day 1, 9, 10, 17, 20 and 31.

Textural analyses of the fillets were performed by the method of Einen and Thomassen (1998) on a TA.XT2 Texture Analyzer (Stable Micro Systems, UK) equipped with a load cell of 5 kg and a flat-ended cylindrical plunger (12 mm diameter) (Hultmann & Rustad, 2002). The plunger was pressed downwards twice at a constant speed of 1 mm/s into the fillets until it reached 60% of the sample height with a holding time of 5 s. Texture profile analysis (TPA) was performed, recording the resistance force of the fillets starting at 1 g for calculating textural parameters (fracturability = force at first breaking point and hardness = maximum force at first depression) as described by Bourne (1978) using the Texture Expert Exceed Application (Stable Micro Systems, UK). Six measurements were run on each fillet just above the mid-line (location C on Fig. 1).

Colour was determined by measuring lightness (L^*), redness (a^*) and yellowness (b^*) by the method of CIE (1979) using a Spectrophotometer 948 (X-Rite, USA). The instrument was calibrated against a white standard at the same light conditions and temperature (4 °C). The analysis was performed four times on each fillet surface.

Samples of white muscle for analyses of pH, water content and liquid loss (LL) were cut from one area above the mid-line (location B on Fig. 1) of each fish sample. The sample was minced for 10 s in a small food mill and kept on ice.

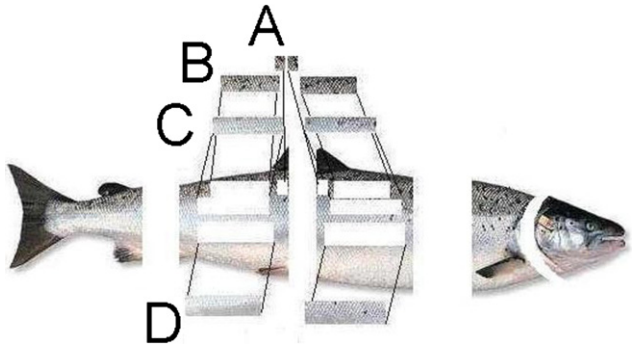

Fig. 1. Sampling locations. A: sulphide producing bacteria, B: water holding capacity, protein and moisture content, C: texture analysis and D: protein extractability and enzymatic activity. All analyses were performed on ventral side of fillet.

Determination of pH was carried out on approximately 2 g of minced sample, mixed with an equal amount of 0.15 M KCl. Mean values were calculated from two replicates.

Liquid loss (LL) was determined on minced white muscle by low-speed centrifugation as described by the water holding capacity method of Eide, Borresen, and Strom (1982). A centrifugal force of 210*g* was used instead of 1500*g* (Hultmann & Rustad, 2002). The LL is expressed as the percentage of weight of the mince lost during centrifugation of ~2g of sample for 5 min. The analyses were run in quadruplicate. Water content in the mince was determined by drying minced sample of ~2g at 105 °C for 24 h. The analyses were run in duplicate.

Total N was determined by a *C*/*N* elemental analyzer (NA 1500, Carlo Erba Instruments, Italy) on lyophilized muscle sample (Kirsten, 1979) and protein was estimated by multiplying total N by a conversion factor of 6.25. Measurements were performed in quadruplicate.

The extractions of proteolytic enzymes were performed at 4 °C, as described by Hultmann, Rora, Steinsland, Skara, and Rustad (2004). White muscle (10 g) (location D on Fig. 1) was homogenized in 15 ml cold distilled water using an Ultra Turrax homogenizer. After centrifugation (20 min at 9700*g*, 4 °C), the supernatant was filtered through glass wool and the volume was made up to 25 ml with distilled water. The extraction procedure was carried out once on each fillet and the extracts were frozen and stored at −80 °C until analysis.

After thawing the extracts were centrifuged (7840*g*, 4 °C for 10 min). Samples were diluted with distilled water. The activities of cathepsin B and cathepsins B + L were measured against synthetic fluorogenic substrates, N_α-carbobenzoxy-L-argininyl-L-arginine-7-amido-4-methylcoumarin and N_α-carbobenzoxy-L-phenylalaninyl-L-arginine-7-amido-4-methylcoumarin (Sigma, St. Louis, MO, USA), respectively (Barrett & Kirschke, 1981). Enzyme extract (0.15 ml, suitably diluted) was incubated with 0.15 ml substrate (0.0625 mM in 105.26 mM bis-Tris, 21.05 mM EDTA, 4.21 mM dithiothreitol, pH 6.5) at 4 °C. The reaction was arrested after 10 min by adding 3 ml 1% SDS in 50 mM bis-Tris, pH 7.0. A blank was prepared by adding distilled water instead of enzyme extract to the reaction mixture. When the enzymes cleave the synthetic substrate, 7-amino-4-methylcoumarin (AMC) is liberated. Fluorescence of AMC was measured at 440 nm (5 nm slits) after excitation at 370 nm (10 nm slits) (Perkin–Elmer 3000 Fluorescence Spectrometer, Perkin–Elmer Inc., Buckinghamshire, UK). Increase in fluorescence intensity was used to calculate the activity, given as the increase in fluorescence per g wet weight × min during incubation. The analysis were run in triplicate.

Extractions for investigation of protein solubility were performed in two steps resulting in a water soluble and a salt soluble fraction by a modification of the methods of Anderson and Ravesi (1968) and Licciardello et al. (1982). Approximately 4 g of minced white muscle was

homogenized in 80 ml of buffer 1 (50 mM KH_2PO_4, 0.5% triton X-100, pH 7.0) at 4 °C using an Ultra Turrax and centrifuged (20 min, 9700*g*, 4 °C). The supernatant was decanted through glass wool and the volume was made up to 100 ml with buffer 1. This was the water soluble fraction. The sediment was re-homogenized in 80 ml of buffer 2 (50 mM KH_2PO_4, 0.5% triton X-100, 0.6 M KCl, pH 7.0) and re-centrifuged. The supernatant was decanted through glass wool and the volume was made up to 100 ml with buffer 2. This was the salt soluble fraction. The procedure was conducted once for each fillet sample. The amount of protein in the extracts was determined by BioRad protein assay, using bovine serum albumin as a standard (Bradford, 1976). The analyses were run in triplicate.

The amount of free amino acids was determined in the water soluble protein extracts by the procedure of Osnes and Mohr (1985) and analyzed with reverse phase high performance liquid chromatography (RP-HPLC) using the method of Lindroth and Mopper (1979) as modified by Flynn (1988). Glycine/arginine and methionine/tryptophane were determined together, as their peaks merged. The analysis was performed in duplicate on each extract. Total amounts of free amino acids were calculated as mg/g wet weight.

The composition of the salt soluble protein fractions was investigated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970) using PhastGel gradient 4–15 gels, SDS buffer strips, high and low molecular weight standards. The gels were stained with Coomassie Brilliant Blue. All equipment for electrophoresis was delivered from Amersham Biosciences (Uppsala, Sweden). The analyses were carried out according to the instructions from the manufacturer.

2.3. Data analysis

Microsoft Excel was used for data processing and Minitab for statistical analysis. The significance level was set at 95% ($p < 0.05$).

3. Results and discussion

3.1. Estimates of ice content of superchilled samples

Immediately after superchilling, the amount of ice in the samples to be stored at temperatures of −1.4 °C and −3.6 °C, was $32 \pm 2\%$ and $49 \pm 5\%$ (mean ± SEM), respectively. The high ice content of the samples to be stored at −1.4 °C, may be explained by a slightly too long duration of the cool down procedure, since the ice fraction curve is very steep in this temperature area. The fillets stored at −3.6 °C had a firm, frozen appearance.

3.2. Drip loss

The drip loss was found to be larger, and to vary more in samples stored at −1.4 °C than in the other storage groups (Fig. 2). The highest recorded value was 1.6% of sample weight after 34 days of superchilled storage at −1.4 °C. However, values of 1–2% drip loss cannot be regarded as high (Dalgaard, Gram, & Huss, 1993; Einen et al., 2002) and therefore cannot be considered as a major problem in superchilled salmon. Samples stored at −3.6 °C and ice chilled or frozen references all lost less than 0.3% of the weight during storage. The drip loss in superchilled salmon at −1.4 °C was comparable to the drip loss on superchilled cod at −2.2 °C. The drip loss of ice chilled cod was higher than that of superchilled salmon fillets (Duun & Rustad, in press). In earlier studies of Atlantic cod stored at 0 or −3 °C, it has been found that free drip increased during storage, and the increase was largest for partial frozen samples (Simpson & Haard, 1987). Their samples were superchilled at −3 °C, which also resulted in some degree of freeze denaturation. Drip loss contains both water and substances leaking from cells as these are ruptured during processes such as cooling, storage and thawing. Superchilled samples at −1.4 °C were found to have the lowest amount (14.6–20.8%) of dry matter in the drip loss and those at −3.6 °C had the highest (22.1–35.3%). Ice chilled and frozen samples had from 22.6% to 24.8% and 21.5% of dry matter in the drip loss, respectively. The proportion of dry matter in the drip, decreased with increasing drip loss. The drip loss was correlated to pH, liquid loss and extractability of salt soluble proteins ($p \leqslant 0.01$) and hardness ($p \leqslant 0.05$).

3.3. Muscle pH and liquid loss

During storage the pH in the fillets increased slightly from the initial 6.13–6.20 to 6.28–6.30 (Table 1), which is in agreement with results from other studies (Sigholt et al., 1997). This increase was delayed in superchilled (day 16–20) compared to ice chilled (day 9–17) fillets.

At day 0, the liquid loss (mean ± SEM) was $7.2 \pm 1.4\%$ of wet weight (Fig. 3). In superchilled samples stored at

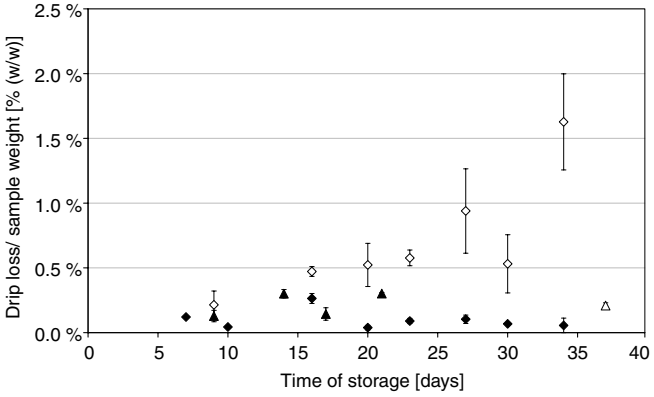

Fig. 2. Relative quantity of drip loss in farmed salmon during storage. Iced and frozen references and superchilled samples at −1.4 and −3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Values (percentage of initial sample weight) are given as mean ± SEM. $N = 2$.

Table 1
Changes in dry matter content of drip loss and muscle, and muscle pH during storage

Storage time (days after processing)	Iced* or frozen# references			Superchilled at −1.3 °C			Superchilled at −3.6 °C		
	Dry matter in drip loss (%(w/w))	Dry matter in muscle (%(w/w))	pH	Dry matter in drip loss (%(w/w))	Dry matter in muscle (%(w/w))	pH	Dry matter in drip loss (%(w/w))	Dry matter in muscle (%(w/w))	pH
0	–	*32.3 ± 0.6	*6.17 ± 0.01						
2				–	32.2 ± 0.6	6.18 ± 0.01	–	31.5 ± 0.2	6.18 ± 0.01
6	–	*32.1 ± 1.2	*6.20	–	33.9 ± 0.0	6.20 ± 0.06			
7							31.7	32.5 ± 1.2	6.16 ± 0.01
9	*24.8 ± 2.5	*33.5 ± 0.6	*6.19 ± 0.01	21.0 ± 3.7	34.5 ± 0.1	6.18 ± 0.02			
10							35.3 ± 5.0	33.9 ± 0.6	6.13 ± 0.01
14	*24.3 ±1.7	*33.6 ± 0.5	*6.24 ± 0.05						
16				18.6 ± 1.7	34.8 ± 0.5	6.14 ± 0.02	22.1 ± 1.3	30.7 ± 0.1	6.16 ± 0.00
17	*24.5 ± 1.5	*32.4 ± 0.9	*6.31 ± 0.02						
20				17.1 ± 0.4	33.6 ± 0.4	6.30 ± 0.03	33.2 ± 7.0	33.1 ± 1.1	6.28 ± 0.02
21	*22.6 ± 1.5	*32.0 ± 0.1	*6.30 ± 0.01						
23				15.9 ± 0.1	33.2 ± 0.2	6.29 ± 0.02	23.0 ± 0.9	34.1 ± 0.4	6.23 ± 0.01
27				14.6 ± 0.4	33.1 ± 0.6	6.34 ± 0.04	23.8 ± 1.9	33.4 ± 0.3	6.23 ± 0.02
30				17.2 ± 0.8	35.4 ± 0.3	6.28 ± 0.05	26.6 ± 1.4	34.3 ± 0.2	6.17
34				20.8 ± 6.4	34.5 ±0.1	6.24 ± 0.02	25.5	33.7 ± 0.2	6.19 ± 0.01
37	#21.5 ± 0.8	#33.2 ± 0.7	#6.24 ± 0.01						

Values are given as mean ± SEM.

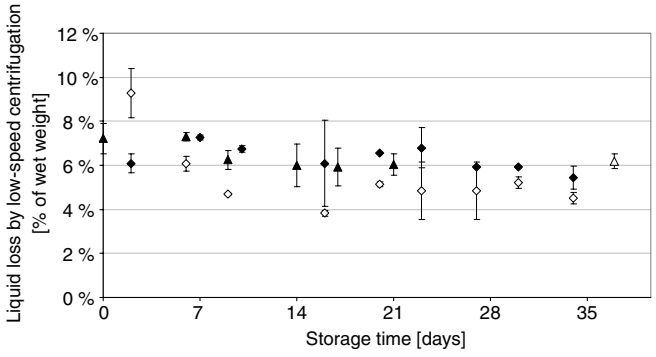

Fig. 3. Changes in liquid loss in farmed salmon during storage. Iced and frozen references and superchilled samples at −1.4 and −3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Values (percentage of sample weight) are given as mean ± SEM. $N = 2$.

−1.4 °C the amount of liquid loss decreased during the first two weeks and was at its lowest at 3.8 ± 0.1% after 16 days of storage. During the remaining storage period, the liquid loss in samples stored at −3.6 °C, was at least 0.7% higher compared to those stored at −1.4 °C. The water content in muscle (mean ± SD) was 66.8 ± 1.3% and the protein content was 19.9 ± 2.0%, and no significant differences were observed between the groups. Therefore, the differences in liquid loss cannot be explained by differences in water content. Fish with a higher water content, has a higher proportion of loosely bound water which can result in higher liquid loss (Duun & Rustad, in press).

In ice chilled salmon fillets, the liquid loss (5.9–7.3%) was lower than that of cod fillets (8.6–14.5%). In superchilled salmon fillets, the liquid loss was generally much lower (3.8–9.3% at −1.4 °C and 5.4–7.3% at −3.6 °C) than in cod fillets (20.5–29.8% at −2.2 °C) (Duun & Rustad, in press). These results are supported by Ofstad, Kidman, Myklebust, and Hermansson (1993), who found the liquid loss in coarsely chopped cod muscle to be more than twice as high as for salmon muscle.

3.4. Bacterial status

The bacterial status measured by the rapid test, gave results of the classifications "very good", "good", "marginal" and "unacceptable" corresponded to a load of $<1 \times 10^3$, 1×10^3–5×10^5, 5×10^5–5×10^6 and $>5 \times 10^6$ sulphide producing bacteria per g of sample, respectively. Our results (Table 2) confirm low growth of spoilage bacteria during superchilled storage (Duun & Rustad, in press; Sivertsvik et al., 2003). Both ice chilled and superchilled fillets stored at −1.4 °C held good or very good quality with respect to growth of sulphide producing bacteria. Sulphide producing bacteria were not detected in superchilled fillets stored at −3.6 °C or in frozen fillets. This confirms that the raw material was of high microbial quality.

3.5. Fillet colour

The colour of Atlantic salmon is influenced by its storage, with the concentration of astaxanthin decreasing with frozen (Regost et al., 2004) and chilled storage (Gobantes et al., 1998). In the present study, no differences in red or yellow colour were found between samples stored at different temperatures (results not shown). However, in superchilled fillets, white spots emerged after equalization of temperature (e.g. first few days of storage). Superchilled fillets stored at −3.6 °C showed more intense spots than superchilled fillets stored at −1.4 °C. Lightness values of fillets stored at −3.6 °C were generally higher than of those stored at −1.4 °C (Fig. 4). The higher values might be

Table 2
Visual colour change due to sulphide precipitation caused by sulphide producing bacteria (SPB)

Storage time (days)	Ice chilled storage	Superchilled storage at −1.3 °C	Superchilled storage at −3.6 °C	Frozen storage at −40 °C
0	Very good	–	–	–
9	Good	Very good	–	–
10	–	–	Very good	–
17	Good	–	–	–
20	–	Good	Very good	–
21	Very good	–	–	–
30	–	Very good	Very good	–
34	–	Very good	Very good	Very good

Analysis performed by the method of Skjerdal et al. (2004) with a commercial ColiFAST® test kit. $N = 2$.

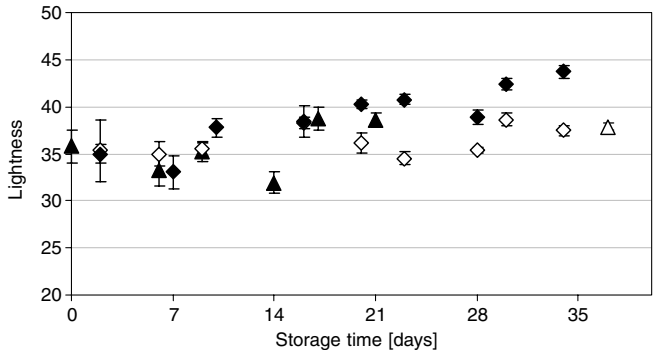

Fig. 4. Colour changes on surface of farmed salmon fillets during storage. Iced and frozen references and superchilled samples at −1.4 and −3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Lightness is given as mean CIE L-values of four measurements on each fish ± SEM. $N = 2$.

due to the more intense white spots of fillets stored at −3.6 °C. The origin of similar white spots on cod fillet surface may be caused by drip channels (Duun & Rustad, in press).

3.6. Textural profile analyses

The texture of fish is an important quality characteristic, and soft fillets are a problem for the fish industry (Haard, 1992; Hultmann & Rustad, 2004; Sigholt et al., 1997). The hardness values were significantly higher in superchilled fillets stored at −3.6 °C compared to superchilled fillets stored at −1.4 °C, ice chilled fillets and frozen fillets (Fig. 5). Results showed that the hardness values of salmon superchilled at −3.6 °C, decreased during the first two weeks of storage. During the entire storage period, the hardness values of superchilled fillets stored at −1.4 °C were significantly higher than that of the ice chilled or frozen fillets. Textural hardness values of ice chilled and frozen salmon were in the same range or higher than found in earlier studies (Hultmann & Rustad, 2004; Morkore, Hansen, Unander, & Einen, 2002; Skjervold et al., 2001). An increase in the maximum penetration force in ice stored post rigor filleted salmon, has also been observed (Skjervold et al., 2001), which agrees with the present results. After three weeks of superchilled storage, the textural hardness values stabilized. At the end of storage, the hardness values of the frozen references were lower compared to 0 days, while the values of superchilled samples were higher.

3.7. Protein stability

The muscle proteins are important for quality characteristics such as textural properties. Differences were not sig-

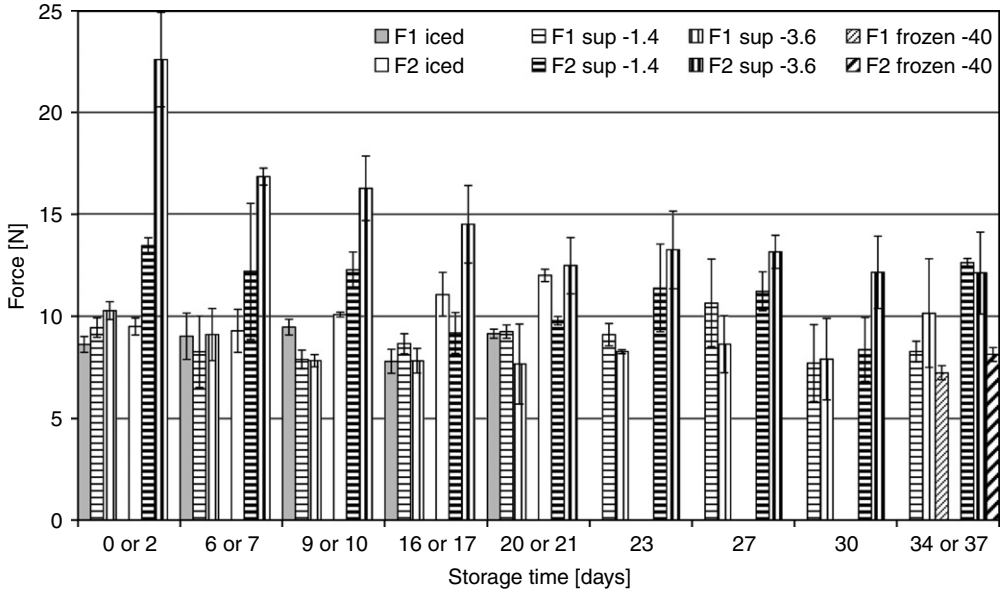

Fig. 5. Changes in fillet fracturability (F1) and hardness (F2) in farmed salmon during storage. Values are given as mean force (N) ± SEM. $N = 2$.

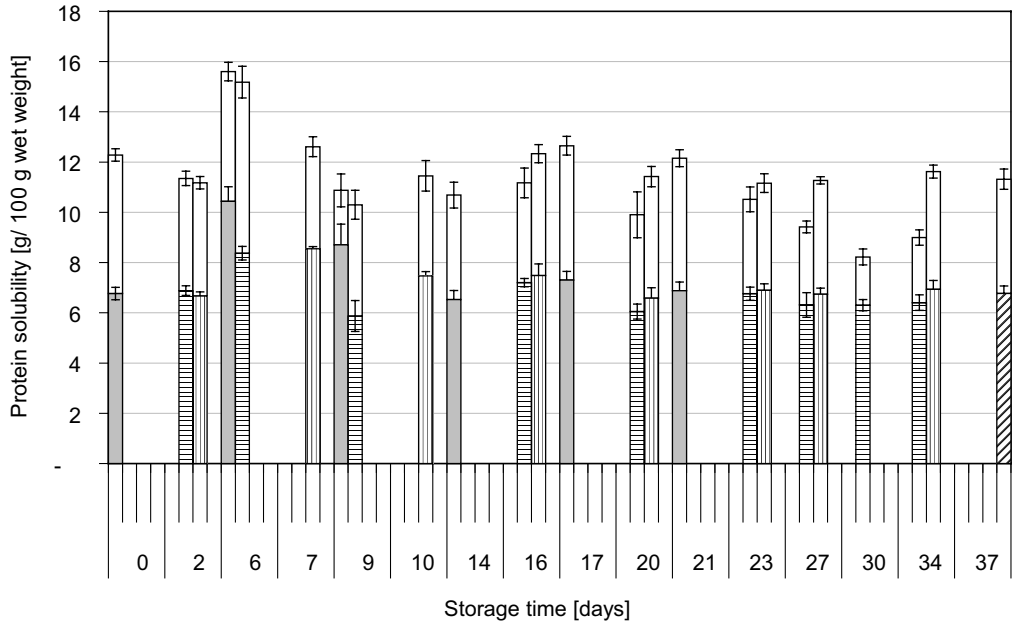

Fig. 6. Changes in extractability of water soluble and salt soluble protein in farmed salmon at 4 °C during storage are shown as lower (shaded) and upper bars (white), respectively. Iced and frozen references and superchilled samples stored at −1.4 °C or −3.6 °C are represented with gray bars, bars with diagonal, horizontal and vertical lines, respectively. Values (percentage of sample wet weight) are given as mean ± SEM. $N = 2$.

nificant between groups with respect to the water soluble proteins (Fig. 6). Extractability of salt soluble proteins from samples stored at −1.4 °C declined during storage compared to the other groups. This indicates a higher degree of protein denaturation in these samples. A higher degree of freeze denaturation can also explain the correlation with drip loss ($p \leqslant 0.01$) and was also observed in superchilled cod fillets (Duun & Rustad, in press). The extractabilities of water and salt soluble proteins were also correlated ($p \leqslant 0.05$) to the liquid loss. Previous reports have proposed that stabilization of myofibril proteins is directly related to better fish quality (Martinez, Friis, & Careche, 2001; Rodriguez, Barros-Velazquez, Pineiro, Gallardo, & Aubourg, 2006). Results from SDS–PAGE of salt soluble protein fraction (Fig. 7) verify a higher degree of protein denaturation in samples stored at −1.4 °C. The band of the myosin heavy chain is weaker in this storage group compared to the corresponding samples stored at −3.6 °C for 20 days and ice chilled (0 days) and frozen (37 days) references.

3.8. Cathepsin activities

Cathepsin B is a lysosomal cysteine protease that hydrolyses a wide range of proteins and has an important role in the hydrolysis of tissue proteins (Barrett & Kirschke, 1981). Cathepsin B + L activity is the combined activity of cathepsin B and cathepsin L. Activities of cathepsins B and B + L at 5 °C and, 20 °C were relatively stable during the storage period (Fig. 8) in all storage groups. This means that these enzymes are not deactivated at the selected storage temperatures and may, therefore, lead to softening during subsequent chilled storage. As expected, the activity values of cathepsins B + L was higher than that of cathepsin B, and higher at 20 °C than at 5 °C. The cathepsin B activities of ice chilled samples were similar to those of Hultmann and Rustad (2004).

3.9. Amount of free amino acids

In the iced or frozen reference fillets and for the first two weeks of superchilled storage, the content of free amino

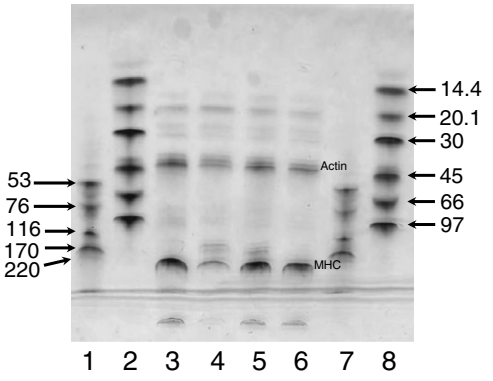

Fig. 7. Composition of salt soluble proteins in farmed salmon during storage using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Samples of ice chilled (0 days), superchilled at −1.4 °C (20 days) and −3.6 °C (20 days) and frozen (37 days) salmon fillet are represented in well 3–6, respectively. Molecular weights of standard proteins are given in kDa of high (well 1 and 7) and low molecular weight standards (well 2 and 8). Ac indicates actin and MHC indicates myosin heavy chain.

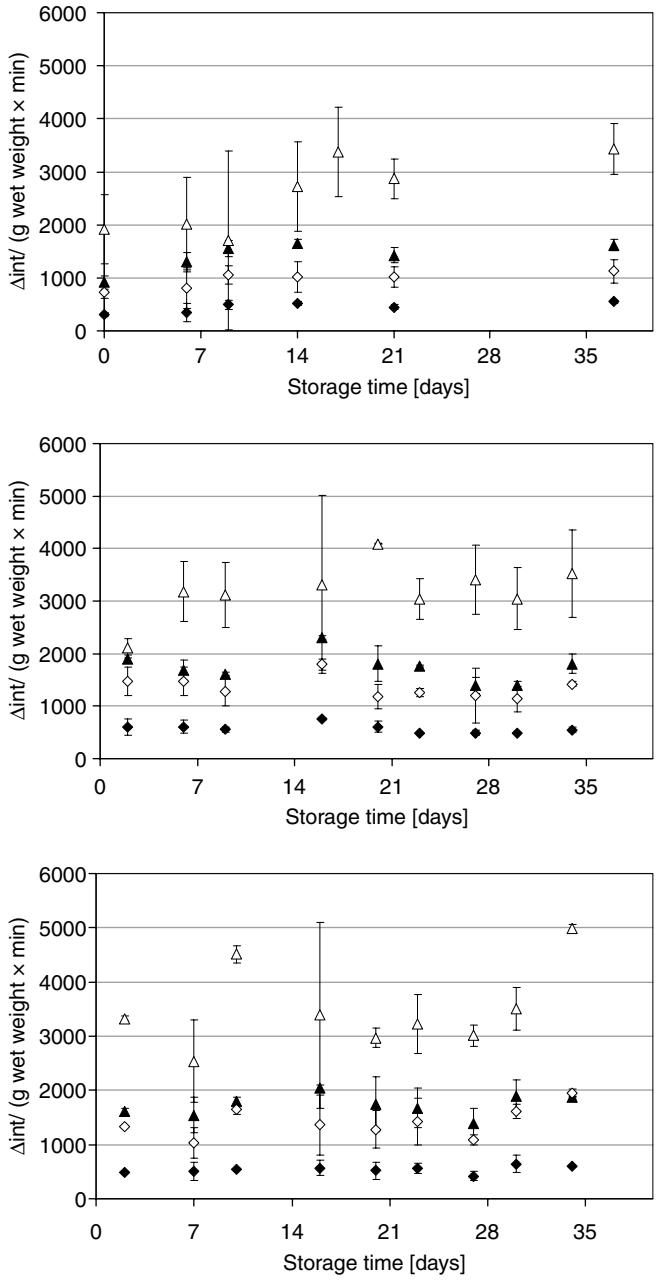

Fig. 8. Cathepsin B and B + L activities in farmed salmon during storage. The panels display activities in iced or frozen references (day 0–21 or day 37, respectively) (upper), superchilled at −1.4 °C (middle) and superchilled at −3.6 °C (lower). Cathepsin B + L activity at 20 °C and 5 °C and cathepsin B activity at 20 °C and 5 °C are represented by open and filled triangles and open and filled diamonds, respectively. Values are given as mean ± SEM of (increase in fluorescence intensity)/(g wet weight × min). $N = 2$.

acids varied between 1.2 and 1.8 mg/g wet weight (Fig. 9). This is in accordance with the findings in iced salmon (Hultmann & Rustad, 2004) and lower than for superchilled cod (Duun & Rustad, in press). Between two weeks and one month, the values increased by up to 2.4–2.5 mg/g wet weight for the superchilled samples. Increases in the content of free amino acids, indicate exoproteolytic activity

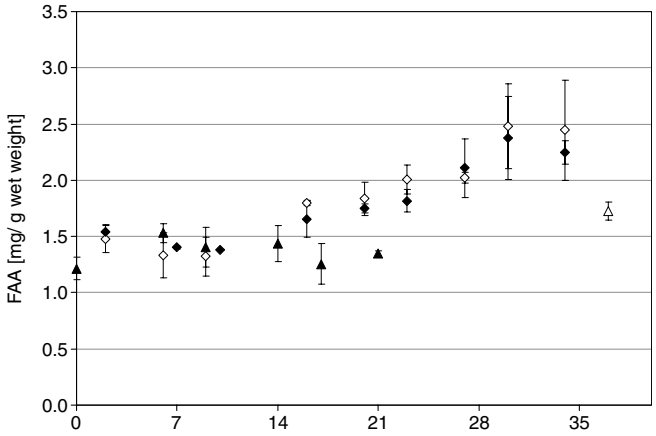

Fig. 9. Changes in the amount of free amino acids in farmed salmon during storage. Iced and frozen references and superchilled samples at −1.4 and −3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Values are given as mean ± SEM of mg/g wet weight. $N = 2$.

during storage, which can be due to increased enzyme concentrations when part of the water is frozen.

4. Conclusion

Ice chilled reference fillets from farmed salmon of premium grade, maintained good quality up to 17–21 days. The storage time of vacuum packed salmon fillets can be doubled by superchilled storage at −1.4 °C and −3.6 °C compared to ice chilled storage.

Drip loss cannot be regarded as a major problem in superchilled salmon and the liquid loss was generally much lower than in cod fillets.

Textural hardness was significantly higher in superchilled salmon fillets stored at −3.6 °C compared to those stored at −1.4 °C, ice chilled and frozen references. The extractability of salt soluble proteins from samples stored at −1.4 °C decreased during storage compared to the other groups, indicating a higher degree of protein denaturation in these samples which was supported by a higher degree of myosin denaturation in samples stored at −1.4 °C.

Activities of cathepsins B and B + L at 5 °C and 20 °C were relatively stable during the storage period in all storage groups. These enzymes may therefore lead to softening during subsequent chilled storage.

Based on the present findings, it is recommended that vacuum packed superchilled salmon fillets should be stored at a superchilling temperature somewhere between −1.4 and −3.6 °C. Salmon is considered better suited for superchilling than cod.

Acknowledgements

This work is a part of the project Superchilling of Food (140642/130), financed by the Research Council of Norway. The authors also thank apprentice P.H. Bjorsvik for laboratory assistance, engineer K.B. Anthonsen for

analysis of amino acids, engineer R. Utgard for analysis of total proteins and Sintef Energy Research for temperature management.

References

Anderson, M. L., & Ravesi, E. M. (1968). Relation between protein extractability and free fatty acid production in cod muscle aged in ice. *Journal of the Fisheries Research Board of Canada, 25*(10), 2059–2069.

Barrett, A. J., & Kirschke, H. (1981). Cathepsin-B, cathepsin-H, and cathepsin-L. *Methods in Enzymology, 80*, 535–561.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*(7), 62–72.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

CIE (1979). Absolute methods for reflection measurements. Paris, International Commission on Illumination. 44, p. 55.

Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology, 19*(4), 283–294.

Duun, A.S., Rustad, T., (in press). Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chemistry*, doi:10.1016/j.foodchem.2007.05.020.

Eide, O., Borresen, T., & Strom, T. (1982). Minced fish production from capelin (*Mallotus-villosus*) – a new method for gutting, skinning and removal of fat from small fatty fish species. *Journal of Food Science, 47*(2), 347–349, 354.

Einarsson, H. (1988). Deep chilling (superchilling, partial freezing) A literature survey Ed. SIKs Service serie. Gothenburg, Sweden, SIK – The Swedish Food Institute, Chalmers University of Technology. 784, p. 30.

Einen, O., Guerin, T., Fjaera, S. O., & Skjervold, P. O. (2002). Freezing of pre-rigor fillets of Atlantic salmon. *Aquaculture, 212*(1–4), 129–140.

Einen, O., & Thomassen, M. S. (1998). Starvation prior to slaughter in Atlantic salmon (*Salmo salar*) II White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. *Aquaculture, 169*(1–2), 37–53.

Fennema, O. R., Powrie, W. D., & Marth, E. H. (1973). *Low-temperature preservation of foods and living matter*. New York: Dekker.

Flynn, K. J. (1988). Some practical aspects of measurements of dissolved free amino acids in natural waters and within microalgae by the use of HPLC. *Chemistry and Ecology, 3*, 269–293.

Foegeding, E. A., Lanier, T. C., & Hultin, H. O. (1996). Characteristics of edible muscle tissues. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 879–942). New York: Marcel Dekker, Inc.

Gobantes, I., Choubert, G., & Gomez, R. (1998). Quality of pigmented (astaxanthin and canthaxanthin) rainbow trout (*Oncorhynchus mykiss*) fillets stored under vacuum packaging during chilled storage. *Journal of Agricultural and Food Chemistry, 46*(10), 4358–4362.

Gram, L., Trolle, G., & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. *International Journal of Food Microbiology, 4*(1), 65–72.

Haard, N. F. (1992). Control of chemical-composition and food quality attributes of cultured fish. *Food Research International, 25*(4), 289–307.

Hardarson, V. (1996). *Matvarers termofysiske egenskaper og deres betydning ved dimensjonering av frysetunneler* Faculty of Engineering Science and Technology, Department of Energy and Process Engineering (pp. XIV, 141, [120]). Trondheim: University of Trondheim, NTH.

Higuera-Ciapara, I., Felix-Valenzuela, L., & Goycoolea, F. M. (2006). Astaxanthin: A review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition, 46*(2), 185–196.

Hultmann, L. (2003). *Endogenous proteolytic enzymes – Studies of their impact on fish muscle proteins and texture* Faculty of Natural Sciences and Technology, Department of Biotechnology (pp. V, 38, [65]). Trondheim: Norwegian University of Science and Technology (NTNU).

Hultmann, L., Rora, A. M. B., Steinsland, I., Skara, T., & Rustad, T. (2004). Proteolytic activity and properties of proteins in smoked salmon (*Salmo salar*) – effects of smoking temperature. *Food Chemistry, 85*(3), 377–387.

Hultmann, L., & Rustad, T. (2002). Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology, 11*(3/4), 105–123.

Hultmann, L., & Rustad, T. (2004). Iced storage of Atlantic salmon (*Salmo salar*) – effects on endogenous enzymes and their impact on muscle proteins and texture. *Food Chemistry, 87*(1), 31–41.

Kirsten, W. J. (1979). Automatic methods for the simultaneous determination of carbon, hydrogen, nitrogen, and sulfur, and for sulfur alone in organic and inorganic materials. *Analytical Chemistry, 51*(8), 1173–1179.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of head of bacteriophage-T4. *Nature, 227*(5259), 680–685.

Licciardello, J. J., Ravesi, E. M., Lundstrom, R. C., Wilhelm, K. A., Correia, F. F., & Allsup, M. G. (1982). Time–temperature tolerance and physical–chemical quality tests for frozen red hake. *Journal of Food Quality, 5*, 215–234.

Lindroth, P., & Mopper, K. (1979). High-performance liquid-chromatographic determination of subpicomole amounts of amino-acids by precolumn fluorescence derivatization with ortho-phthaldialdehyde. *Analytical Chemistry, 51*(11), 1667–1674.

Martinez, I., Friis, T. J., & Careche, M. (2001). Postmortem muscle protein degradation during ice-storage of Arctic (*Pandalus borealis*) and tropical (*Penaeus japonicus* and *Penaeus monodon*) shrimps: A comparative electrophoretic and immunological study. *Journal of the Science of Food and Agriculture, 81*(12), 1199–1208.

Morkore, T., Hansen, A. A., Unander, E., & Einen, O. (2002). Composition, liquid leakage, and mechanical properties of farmed rainbow trout: Variation between fillet sections and the impact of ice and frozen storage. *Journal of Food Science, 67*(5), 1933–1938.

Norwegian Seafood Export Council (2005). Norwegian seafood in numbers 2003. Retrieved November 2006, from <http://www.seafood.no/>.

Ofstad, R., Kidman, S., Myklebust, R., & Hermansson, A. M. (1993). Liquid holding capacity and structural-changes during heating of fish muscle – cod (*Gadus-morhua* L.) and salmon (*Salmo-salar*). *Food Structure, 2*(2), 163–174.

Olafsdottir, G., Lauzon, H. L., Martinsdottir, E., Oehlenschlager, J., & Kristbergsson, K. (2006). Evaluation of shelf life of superchilled cod (*Gadus morhua*) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. *Journal of Food Science, 71*(2), S97–S109.

Osnes, K. K., & Mohr, V. (1985). Peptide-hydrolases of Antarctic krill. *Euphausia-superba. Comparative Biochemistry and Physiology B – Biochemistry & Molecular Biology, 82*(4), 599–606.

Regost, C., Jakobsen, J. V., & Rora, A. M. B. (2004). Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Research International, 37*(3), 259–271.

Rodriguez, O., Barros-Velazquez, J., Pineiro, C., Gallardo, J. M., & Aubourg, S. P. (2006). Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chemistry, 95*(2), 270–278.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S., & Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science, 62*(4), 898–905.

Simpson, M. V., & Haard, N. F. (1987). Temperature acclimation of Atlantic cod (*Gadus morhua*) and its influence on freezing point and biochemical damage of postmortem muscle during storage at 0° and −3 °C. *Journal of Food Biochemistry, 11*(1), 69–93.

Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of fish and fishery products –

significance of microbial growth, activities and safety. *International Journal of Food Science and Technology, 37*(2), 107–127.

Sivertsvik, M., Rosnes, J. T., & Kleiberg, G. H. (2003). Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (*Salmo salar*) fillets. *Journal of Food Science, 68*(4), 1467–1472.

Skjerdal, O. T., Lorentzen, G., Tryland, I., & Berg, J. D. (2004). New method for rapid and sensitive quantification of sulphide-producing bacteria in fish from Arctic and temperate waters. *International Journal of Food Microbiology, 93*(3), 325–333.

Skjervold, P. O., Fjaera, S. O., Ostby, P. B., Isaksson, T., Einen, O., & Taylor, R. (2001). Properties of salmon flesh from different locations on pre- and post-rigor fillets. *Aquaculture, 201*(1–2), 91–106.

Zeng, Q. Z., Thorarinsdottir, K. A., & Olafsdottir, G. (2005). Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. *Journal of Food Science, 70*(7), S459–S466.