Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 1268–1281
Food Chemistry
www.elsevier.com/locate/foodchem

# Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon (*Salmo salar*) fillets

L. Gallart-Jornet [a], T. Rustad [b], J.M. Barat [a], P. Fito [a], I. Escriche [a,*]

[a] *Instituto Universitario de Ingeniería de Alimentos para el Desarrollo (IUIAD), Department of Food Technology, Universidad Politécnica de Valencia, Camino de vera s/n, E-46022 Valencia, Spain*
[b] *Department of Biotechnology, NTNU, N-7491 Trondheim, Norway*

Received 31 May 2006; received in revised form 18 July 2006; accepted 16 October 2006

**Abstract**

The aim of this work has been to evaluate the effect of superchilled storage compared with ice and frozen storage on the quality of raw material and subsequent behaviour during processing of lightly salted salmon (*Salmo salar*), as the first step of smoked salmon production. Physicochemical parameters used as quality indicators were α-glucosidase activity, protein denaturation and degradation (as changes in protein solubility, SDS–PAGE and free amino acids), texture attributes, and mass transfer phenomena during salting. The results obtained for the raw material within the storage range studied (until 16 days) allowed us to conclude that salmon superchilled for 9 days behaved as salmon stored on ice for 2 days with regard to hardness, protein solubility and free amino acids. In general, salting minimises the effect of the different storage methods. Superchilling for 9 days obtained the highest process yield, indicating that this method is a good way to preserve freshness of the raw material before processing.


*Keywords:* Atlantic salmon; Storage method; Superchilling; Freshness; Protein solubility; SDS–PAGE; Free amino acids; Salting; Yield and texture attributes

## 1. Introduction

Atlantic salmon (*Salmo salar* L.) has had a remarkable commercial growth in Norway (Elvevoll, Sørensen, Østerud, Ofstad, & Martínez, 1996). In the last decades production of farmed Atlantic salmon for fresh and cold smoked products has been developed into an important commercial enterprise with a great market demand in several Northern European countries, such as Norway, Scotland, Ireland, Faroe Islands, as well as Canada and Chile (NF, 2006). According to the Norwegian Seafood Export Council (2005), the Norwegian production of farmed Atlantic salmon was estimated to be about 423,000 t in 2000 increasing to 545,000 t in 2005. This has contributed to the growth of the smoking industry. It is estimated that 40–50% of the farmed salmon produced in Europe is consumed as cold-smoked products (Rørå et al., 1999).

Freshness is the main quality parameter in fresh fish, used either for direct consumption or as raw material for the processing industry. In addition, fat content, colour, texture, etc. are important quality attributes in salmon (Nielsen, Hyldig, & Larsen, 2002; Ólafsdóttir et al., 1997; Sigurgisladóttir, Torrissen, Lie, Thomassen, & Hafsteinsson, 1997). The development of aquaculture allows easy access to fresh salmon all year round and an efficient distribution, as long as time and temperature are controlled (Hempel, 1998, 1999; Nielsen et al., 2002; Roberts, 1998; Sigurgisladóttir et al., 1997). However, for markets further away from the main salmon producers, the relative short shelf-life either necessitates expensive air freight, frozen distribution or inclusion of more preservative factors. The time elapsing between the harvesting in Norway and the arrival at European destinations may vary from 3 up to 5 days (personal communication). Therefore, there is a

* Corresponding author. Tel.: +34 963877366 x73661; fax: +34 963877369.
*E-mail address:* iescrich@tal.upv.es (I. Escriche).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.10.040

need to optimise the freshness of salmon during transport and storage (Sivertsvik, Rosnes, & Kleiberg, 2003).

To increase shelf life and reduce the rate of microbial and biochemical degradation, different preservative methods, mainly based on low temperature, have been employed for storage and distribution. The most used include refrigerated ice storage between 0 and 4 °C, superchilled storage in the range of −1 to −4 °C, by means of slurry ice or in superchilled chambers without ice, and frozen storage at −18 to (−40) °C.

The use of ice has been the most used system to cool fresh fish. The ice ensures a high refrigeration capacity and efficient heat transfer in contact with the fish (Magnussen, Nordtvedt, & Hardarson, 2001). A box with 20–24 kg of whole gutted fish has an additional weight of 20–30% of ice (4–6 kg), which will increase the transport costs during distribution. In addition, a reduced ice volume and refrigeration capacity, due to melting, often results in increased temperatures during distribution, resulting in a loss of fish quality (Nordtvedt et al., 1998). The shelf-life in ice for a lean fish, such as cod, is from 7 up to 13 days (Sivertsvik, Jeksrud, & Rosnes, 2002), while for iced whole salmon it is about 20 days (Sveinsdottir, Martinsdottir, Hyldig, Jorgensen, & Kristbergsson, 2002) and for salmon steaks or fillets, the shelf-life in modified atmosphere packaging (MAP) at chilled temperatures (2–4 °C) can be from 14 to 21 days (Sivertsvik et al., 2003).

Superchilling, also known as "partial freezing" and "deep chilling" is used to describe a process where foodstuffs are stored between 1 and 2 °C below the initial freezing point and it is one of the potential preserving methods to extend the period of prime quality in fish (Haard, 1992). This method is used for preservation of the catch onboard fishing vessels, by means of a slurry ice system immersion (made by addition of salts, and other compounds to the ice–water mixture) at subzero temperatures (−0.5 to −3 °C) (Alemán, Kakuda, & Uchiyama, 1982; Kato, Umemoto, & Uchiyama, 1974; Losada, Piñeiro, Barros-Velázquez, & Aubourg, 2004, 2005; Piñeiro et al., 2005; Zeng, 2003). Superchilling is also used for preservation through the distribution chain (storage, distribution and delivery for direct consumption), by using chambers with controlled temperatures at −1 to −3 °C without ice (Aune & Nordtvedt, 1999; Chang, Chang, Shiau, & Pan, 1998; Huss, 1998; Magnussen et al., 2001; Nordtvedt et al., 1998).

In the superchilled temperature range, approximately 20–50% of the water in the fish is frozen, forming an internal ice reservoir, making it an alternative to the transportation on ice, avoiding melting ice and the extra weight in the distribution (Nordtvedt et al., 1998; Nowlan, Dyer, & Keith, 1974). Aune and Nordtvedt (1999) found that salmon that was superchilled and transported had a satisfactory quality upon arrival. However, better control of air temperature in the trucks was needed. For some fish species such as cod, a negative effect on freshness prime quality has been observed due to formation of ice crystals and protein denaturation (Nowlan et al., 1974). All in all, intrinsic autolytic changes will be delayed compared to refrigerated ice storage. Lower energy consumption, less thawing problems and less structural damage are observed compared to frozen storage. Einarsson (1998) concluded that the shelf life of superchilled food increases at least 1.5 – 4 times, compared to chilled food, making superchilling an attractive compromise between freezing and conventional chilling.

Several studies have reported promising results in microbiology, nucleotide degradation, total volatile basic-nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), free amino acids, eletrophoretic profiles, and sensory evaluation, when comparing superchilling storage with the traditional storage methods for direct consumption. A better product quality, improving the shelf-life with little negative side effect, was observed in fish species such as hake (Losada et al., 2004), sardine (Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005), horse mackerel (Losada, Piñeiro, Barros-Velázquez, & Aubourg, 2005), farmed turbot (Piñeiro et al., 2005), mullet (Lee & Toledo, 1984), sea bass (Kato et al., 1974; Chang et al., 1998), mackerel (Alemán et al., 1982), salmon (Aune & Nordtvedt, 1999; Sivertsvik et al., 2003), and northern shrimp (Zeng, 2003). A few studies have been done in order to evaluate the effect of superchilling with slurry ice after processing (Losada, Rodríguez, Ortiz, & Aubourg, submitted for publication). However, the effect of superchilled salmon without ice prior to processing and on the behaviour during the salting step have not been reported in the literature.

The aim of the present study was to investigate the effects of storage method (chilled, superchilled and frozen) and time on the quality of salmon as raw material and the subsequent behavior during production of lightly salted salmon. Enzymatic activity (α-glucosidase), protein denaturation and degradation (as changes in the protein solubility, molecular weight distribution by SDS–PAGE and free amino acids), texture attributes, water holding capacity (WHC), water activity ($a_w$), pH, and mass transfer phenomena (measured as weight, salt and water changes), were used as quality indicators.

## 2. Materials and methods

### 2.1. Raw salmon and sample preparation

Farmed Atlantic salmon (*Salmo salar*) ($n = 48$) was supplied by a commercial plant, Salmar Processing AS, located in mid-Norway. The salmon was slaughtered commercially and packed on ice in Styrofoam boxes. The boxes were sent to the research facilities in Trondheim. The fish were divided evenly into 6 batches, where the pooled mean weight and length of all fish were 3.5 ± 0.3 kg and 72.1 ± 2.8 cm, respectively. Three batches of salmon were kept on crushed ice at +4 °C for 2 days (raw material I0), 9 days (raw material I1) and 16 days (raw material I2), one batch was frozen at −40 °C for 30 days (raw material F) and two batches were superchilled at −1 °C without ice, for 9 days

(raw material S1) and 16 days (raw material S2). After the different storage treatments, each batch of salmon ($n = 8$) was filleted with skin on, tagged and dry salted without drainage ($n = 16$) at +4 °C. The average weight and length values for all batches of whole gutted salmon and their fillets are shown in Table 1.

### 2.2. Salting and fillet sampling

After the storage treatment, each batch ($n = 16$) was dry salted with ordinary commercial refined salt (Jozo salt, Akzo Nobel Salt, Göteborg, Sweden) for 24 h at 4 ± 1 °C at a ratio 1 kg fish/0.5 kg salt, in plastic containers without drainage. Lightly salted salmon as raw material for the smoking industry was considered to be salmon salted between 12 and 24 h. During salting, the fillet weight of the salmon ($n = 8$) was recorded after partly removing the salt crystals on the fillet surface, to calculate yields at 0, 4, 8, 12, and 24 h.

Samples for physicochemical analysis were taken from two fillets chosen at random. The piece near the head was used for texture analysis. The middle part of the fillet was used for determination of chemical parameters: contents of salt, water and fat ($x^{w}$, $x^{NaCl}$ and $x^{fat}$). In addition water activity ($a_w$), pH, water holding capacity (WHC), protein solubility (amount of water and salt soluble protein: $x^{WSP}$, $x^{SSP}$), amount of free amino acids (free aa), molecular weight distribution (SDS–PAGE), and α-glucosidase activity were determined (Fig. 1).

### 2.3. Analytical determinations

#### 2.3.1. Textural properties

Texture attributes were measured by double compression using a Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, UK) equipped with a load cell of 5 kg and a flat-ended cylindrical plunger (12.5 mm diameter), using a modification of the method described by Einen and Thomassen (1998). The probe was pressed downwards at a constant speed of 1 mm $s^{-1}$ into the fillets until it had reached 40% of the sample depth and the holding time between the compressions was 5 s. The force (N) was recorded continuously during compression in a texture profile curve (texture profile analysis (TPA 40)). Textural parameters were calculated as described by Bourne (1978) ($F_{hardness}$ maximum force of the first compression in the force–time curve, cohesiveness, elasticity, gumminess, chewiness). The measurements were carried out 6–8 times on each fillet ($n = 12$) and the area tested was between the gills and the dorsal fin, above the mid-line (Fig. 1).

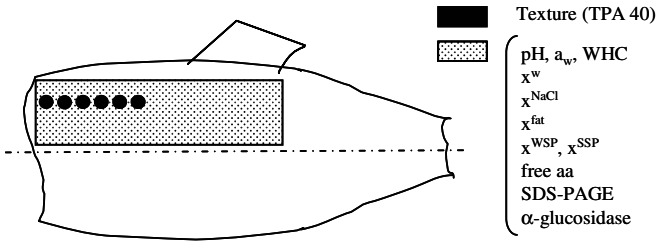


Fig. 1. Illustration of sampling for analyses of salmon fillets; for details see text.

#### 2.3.2. Protein solubility

After texture measurements, samples for determination of protein solubility were cut out and the proteins were extracted by a modification of the method of Anderson and Ravesi (1968), and Licciardello et al. (1982). The extractions were done in a cold room (+4 °C). Four grams of white muscle were homogenised for 20 s in 80 ml of phosphate buffer (0.05 M, 0.5% Triton X-405, pH 7.0) using an Ultra-Turrax. After centrifugation (9000 rpm) for 20 min at 4 °C, the supernatant was filtered and the volume was made up to 100 ml with phosphate buffer. Neglecting the initial salt content of the sample, this was regarded as the water soluble protein fraction (WSP). The precipitate was homogenised for 10 s in phosphate buffer with KCl (0.6 M KCl in 0.05 M phosphate buffer, 0.5% Triton X-405, pH 7.0), and centrifuged as above. The supernatant was made up to 100 ml with KCl–phosphate buffer. This was the salt soluble fraction (SSP). The extraction procedure was carried out twice on each fillet ($n = 4$). The amount of protein in the extracts was determined with the BioRad protein assay, using bovine serum albumin as a standard (Bradford, 1976). Absorbance was measured at $\lambda = 595$ nm in an Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Uppsala, Sweden). The analyses were run in triplicate.

Table 1
Weight and length fish and fillets used

| Storage method | Chilled (4 °C, crushed ice) | | | Frozen (−40 °C) | Superchilled (−1 °C, without ice) | |
|---|---|---|---|---|---|---|
| Time | Day 2 | Day 9 | Day 16 | Day 30 | Day 9 | Day 16 |
| Sample | I0 | I1 | I2 | F | S1 | S2 |
| Weight (kg) ($n = 8$) | 3.4 ± 0.4 | 3.6 ± 0.3 | 3.6 ± 0.2 | 3.4 ± 0.2 | 3.5 ± 0.3 | 3.7 ± 0.2 |
| Length (cm) ($n = 8$) | 71.4 ± 2.6 | 72.0 ± 3.5 | 72.3 ± 1.1 | 72.5 ± 2.2 | 71.0 ± 4.1 | 73.3 ± 2.7 |
| Fillet weight (g) ($n = 16$) | 1090 ± 144 | 1150 ± 187 | 1190 ± 97.5 | 1150 ± 97.6 | 1130 ± 74.2 | 1190 ± 57.7 |
| Fillet length (cm) ($n = 16$) | 42.7 ± 2.1 | 42.9 ± 2.7 | 42.2 ± 1.6 | 42.8 ± 1.6 | 40.8 ± 1.8 | 43.2 ± 1.7 |
| Thickness (cm) ($n = 16$) | 3.0 ± 0.3 | 2.8 ± 0.7 | 2.9 ± 0.5 | 3.1 ± 0.5 | 3.0 ± 0.4 | 3.1 ± 0.4 |

Values are given as mean ± sd.

#### 2.3.3. Free amino acids

The content of free amino acids was determined in the water soluble extract, after precipitation of the water soluble proteins in 2% sulphosalicylic acid, centrifugation and filtration (Osnes & Mohr, 1985) and diluting the supernatant with deionised water. Reversed phase HPLC by precolumn fluorescence derivatisation with *o*-phthaldialdehyde (SIL-9A Autoinjector, LC-9A liquid chromatograph, RF-530 Fluorescence HPLC Monitor (Shimadzu Corporation, Japan), was performed using a NovaPak C18 cartridge (Waters, Milford, MA) using the method of Lindroth & Mopper (1979), as modified by Flynn (1988). Amino acids concentrations were determined twice in each extract ($n = 4$). Glycine and arginine were determined together, as their peaks coeluted.

#### 2.3.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed on the water and salt soluble proteins, and carried out according to Laemmli (1970), using PhastGel Gradient 4–15 gels, SDS buffer strips, and high and low molecular weight calibration kit, on a Pharmacia LKB PhastSystem (Pharmacia LKB, Uppsala, Sweden). The gels were stained with Coomassie Brilliant Blue. The analyses were carried out according to the instructions of the manufacturer. The SDS sample buffer was composed of 10 mM Tris, 1 mM EDTA, 5% SDS and 10% β-mercaptoethanol ME, pH 8.0.

#### 2.3.5. Enzymatic activity (α-glucosidase)

Centrifugal cell tissue fluid (CTF) and homogenate (HOM) were prepared according to Nilsson (1994). The α-glucosidase (E.C. 3.2.1.20) activity was measured according to Milanesi & Bird (1972) and Rehbein (1979). Na citrate buffer (0.3 ml, 0.05 M), pH 4.0, was mixed with 0.2 ml of 1.0 M NaCl, 0.2 ml of 4.2 mM *p*-nitrophenyl-α-glucopyranoside solution and 0.8 ml sample solution. The mixture was incubated at 37 °C for 60 min and the reaction was stopped by adding 1 ml of 0.2 M KOH; and the absorbance was measured immediately ($\lambda = 405$ nm, $\varepsilon_M = 19{,}500\ M^{-1}\ cm^{-1}$) using an Ultrospec 2000 (Pharmacia Biotech, Uppsala, Sweden). Mean values were calculated from four replicates.

#### 2.3.6. Salt, moisture and fat content

After determination of texture and sampling for protein solubility, the rest of the fillet sample, indicated in Fig. 1, was minced and used for chemical analyses. Salt content was measured using Volhards method (AOAC, 1990a). Moisture content was determined gravimetrically after drying 2 g of minced fish at 104 °C for 24 h (AOAC, 950.46, 1990). The total amount of fat was determined by the Bligh and Dyer method (1959), as modified by Hardy & Keay (1972). Mean values were calculated from three replicates per sample.

#### 2.3.7. Water activity, pH and water holding capacity

Water activity ($a_w$), was determined on minced samples ($n = 2$), using a CX-2 AQUALab (Decagon Devices Inc., Pullman, WA) and each sample was read 3 times. pH was determined in a suspension made by mixing equal volumes of fish mince and 0.15 M KCl, as described by Bendall (1973), using a PHM210 Standard pHmeter (METERLab, Copenhagen, Denmark) with a glass electrode (Mettler Toledo inlab 413). The determination was done in triplicate. Water holding capacity (WHC) was determined on minced muscle by low-speed centrifugation as described by Eide, Børrensen, & Strøm (1982) with a centrifugation force of 210*g*. The WHC is expressed as the percentage of water retained in the mince after centrifugation for 5 min. Mean values were calculated from four replicates per sample.

#### 2.3.8. Statistical analysis

Significant differences between the measured parameters were evaluated using one way and multifactor ANOVA at a significant level of 95%, using the software Statgraphics version 5.0 (Manugistics, Inc., Rockville MD). Principal component analysis (PCA) (Esbensen, 2000) using the software Unscrambler version 9.2 (CAMO A/S, Oslo, Norway) was applied, to study the texture attributes (hardness, cohesiveness, elasticity, chewiness and gumminess) in the different batches. PCA was performed on weighted samples of all objects, in order to compensate for the different scales of the variables, applying cross validation at full cross.

## 3. Results and discussion

### 3.1. Influence of time and storage method on the quality of salmon as raw material

#### 3.1.1. Characterisation of raw material

The characterisation of whole gutted fresh salmon placed in open boxes and stored under different conditions: ice-chilled for 2, 9 and 16 days (I0, I1 and I2), frozen for 30 days (F), and superchilled for 9 and 16 days (S1 and S2), is shown in Table 2.

Although the ANOVA results indicate that there are significant differences between the groups, with regard to the determined parameters, no clear tendency was found in relation to the effect of the storage conditions. Therefore, differences in composition may be explained as a result of fish-to-fish variations in the same batch, and may not be derived from the chilling conditions and storage time. The freshest raw material (I0) had the highest water content, coinciding with a lower fat content.

#### 3.1.2. Enzymatic activity

Table 3 shows the activity of the α-glucosidase in the homogenate (HOM) and in the cell tissue fluid (CTF) of fresh salmon after the different storage conditions:

Table 2
Fresh salmon characterisation (weight fraction of water $x_0^w$ and fat $x_0^f$; water activity ($a_w$), water holding capacity (WHC) and pH) stored under different conditions (ice chilled (I), frozen (F) and superchilled (S))

| Storage method | Chilled (4 °C, crushed ice) | | | Frozen (−40 °C) | Superchilled (−1 °C, without ice) | |
|---|---|---|---|---|---|---|
| Time | Day 2 | Day 9 | Day 16 | Day 30 | Day 9 | Day 16 |
| Sample | I0 | I1 | I2 | F | S1 | S2 |
| $x_0^w$(w/w) ($n = 6$) | $0.712 \pm 0.003^{a}$ | $0.687 \pm 0.006^{cd}$ | $0.694 \pm 0.002^{c}$ | $0.687 \pm 0.008^{cd}$ | $0.684 \pm 0.006^{d}$ | $0.702 \pm 0.001^{b}$ |
| $x_0^f$(w/w) ($n = 6$) | $0.081 \pm 0.014^{a}$ | $0.089 \pm 0.005^{ab}$ | $0.105 \pm 0.009^{bc}$ | $0.120 \pm 0.017^{c}$ | $0.105 \pm 0.015^{bc}$ | $0.102 \pm 0.007^{bc}$ |
| $a_w$($n = 6$) | $0.981 \pm 0.002^{a}$ | $0.972 \pm 0.003^{b}$ | $0.973 \pm 0.002^{b}$ | $0.946 \pm 0.002^{d}$ | $0.970 \pm 0.002^{bc}$ | $0.968 \pm 0.003^{c}$ |
| WHC (%) ($n = 8$) | $94.8 \pm 0.9^{d}$ | $93.5 \pm 0.9^{bc}$ | $94.5 \pm 1.3^{cd}$ | $96.1 \pm 0.9^{e}$ | $91.6 \pm 1.2^{a}$ | $92.5 \pm 0.8^{ab}$ |
| pH ($n = 6$) | $6.36 \pm 0.02^{a}$ | $6.19 \pm 0.05^{d}$ | $6.24 \pm 0.03^{cd}$ | $6.28 \pm 0.02^{b}$ | $6.26 \pm 0.02^{bc}$ | $6.18 \pm 0.04^{d}$ |

Significant differences between the storage conditions in the measured parameters are indicated with letter superscripts ($p < 0.05$). Mean values in the same raw with no letters in common are significantly different.

Table 3
α-Glucosidase activity (μmol/g* min) in the homogenate (HOM) and the cell tissue fluid (CTF) of fresh salmon, as a function of storage conditions (ice-chilled (I0, I1, I2), frozen (F), and superchilled (S1, S2)), ($n = 4$)

| Storage method | Chilled (4 °C, crushed ice) | | | Frozen (−40 °C) | Superchilled (−1 °C, without ice) | |
|---|---|---|---|---|---|---|
| Time | Day 2 | Day 9 | Day 16 | Day 30 | Day 9 | Day 16 |
| Sample | I0 | I1 | I2 | F | S1 | S2 |
| HOM | $0.961 \pm 0.080$ | $0.140 \pm 0.036$ | $0.062 \pm 0.012$ | $0.076 \pm 0.052$ | $0.286 \pm 0.101$ | $0.156 \pm 0.083$ |
| CTF | $0.004 \pm 0.002$ | $0.008 \pm 0.012$ | $0.008 \pm 0.004$ | $0.126 \pm 0.036$ | $0.005 \pm 0.007$ | $0.009 \pm 0.009$ |

ice- chilled for 2, 9 and 16 days (I0, I1, I2), frozen for 30 days (F), and superchilled for 9 and 16 days (S1, S2).

α-Glucosidase is one of the lysosomal enzymes that has been used for detection of freeze–thaw treatment of fish (Rehbein, 1979). An increase in activity in the CTF indicates membrane damage, resulting in the enzyme leaching out of the lysosome (Nilsson, 1994). The higher activity in the CTF in the frozen samples, compared to the superchilled samples, shows that the damage to the membrane structure is larger in the frozen samples, compared to the superchilled and the ice-chilled ones. This is in accordance with the results from Lee & Toledo (1984), who found minor structural changes in mullet, due to ice crystals at −2 °C, compared to those occurring during freezing at −20 °C. In yellowtail fish, however, a breakdown of glycogen granules and some damage to the mitochondrial inner membrane was found in tissue stored at −3 °C, indicating that some deleterious changes occur at these temperatures. Nevertheless, in samples stored at −20 °C, broken fibrils were frequently found, with most damage occurring at the Z-disk (Dion, 1995).

The total activity was determined in the homogenate (HOM) and the enzyme activity was higher in HOM than in the CTF. The α-glucosidase in HOM was reduced with longer storage time, and the reduction during superchilled storage was between the reductions observed during ice-chilled and frozen storage. The residual activity was found to be 32.5% after 9 days superchilled (S1) and 20% after 16 days superchilled (S2). Nine days ice chilled (I1) had a residual activity of 17.8%, followed by 16 days ice chilled (I2) with 10%, comparable with frozen storage (F) with 11.4%. Alemán et al. (1982), Chang et al. (1998), Lee and Park (1985), Losada et al. (2004), Losada et al. (2005), Piñeiro et al. (2005) and Uchiyama and Kato (1974) reported lower $k$-values in fish superchilled at −3 °C and with slurry ice, compared to ice storage, and they also concluded that in superchilled storage the rate of enzymatic reactions was reduced, compared to ice-chilled storage.

### 3.1.3. Protein solubility

Changes in the proteins during storage and processing will be reflected in changes in solubility properties. The myofibrillar proteins in fish are easily denatured during processing, resulting in loss of solubility. The properties of the salt soluble proteins are therefore important for the water-holding capacity and thereby the texture of the fish muscle (Hultmann & Rustad, 2002).

In general, the total amount of extractable proteins was reduced during storage, independent of the storage method (Fig. 2). This is in accordance with the results of Gómez-Guillén, Montero, Hurtado, and Borderías (2000) and Hultmann, Røra, Steinsland, Skåra, and Rustad (2004). The amount of salt soluble protein (SSP) decreased with storage time, independent of the storage method. Erikson, Hultmann, and Steen (2006), however, found an increase in the amount of SSP between 1 and 2 weeks storage on ice, but the salmon in their experiment was smaller than in this study. It is interesting to note that the amount of SSP in salmon superchilled for 9 days (S1) was comparable to the amount of SSP in samples 2 days ice-chilled (I0), and was higher than in the samples ice stored for 9 days (I1). This could be an indication that superchilling at −1 °C does not result in a high degree of protein denaturation (Kato et al., 1974). Nevertheless a decrease in water holding capacity was observed; this could be due to some structural changes in the muscle during superchilled storage.

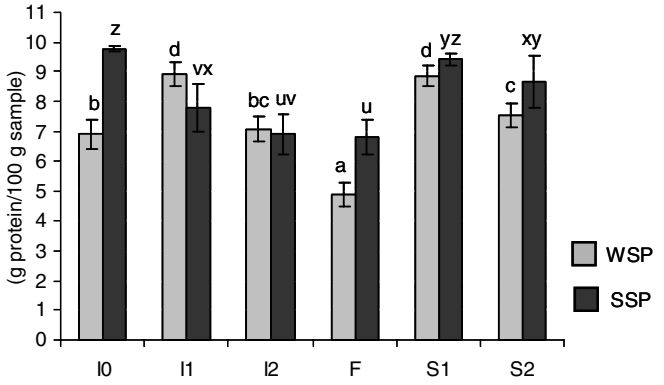


Fig. 2. Changes in water and salt soluble protein (WSP, SSP) of fresh salmon as a function of the storage conditions. I0, I1, I2 (ice chilled for 2, 9 and 16 days), F (frozen for 30 days), S1 and S2 (superchilled for 9 and 16 days). Significant differences in WSP and SSP between the storage conditions are indicated with letters on top of each column. Samples with no letters in common are significantly different ($p < 0.05$), ($n = 4$).

The amount of water soluble protein (WSP) increased in samples ice-chilled and superchilled for 9 days (I1, S1), followed by a decrease after 16 days. This is in accordance with previous studies of Hultmann and Rustad (2002), who found that WSP decreased during iced storage for 2 weeks, whereas the amount of salt soluble proteins remained constant. Frozen storage (F) significantly reduced the amount of water and salt soluble proteins, compared to I0 ($p < 0.05$). This is in agreement with Mackie (1993), who reported a decrease in salt soluble proteins, reflected in the insolubilisation of myofibrillar proteins, and loss of functional properties during frozen storage, while the solubility of the sarcoplasmic proteins was little affected.

### 3.1.4. Free amino acids

An increase in the amount of free amino acids is a result of enzymatic degradation of proteins. Table 4 shows the total amount of free amino acids (μmol/g wet weight) and the fraction (%) in fresh salmon fillets stored under different conditions (ice-chilled (I0, I1, I2), frozen (F), and superchilled (S1, S2)).

The total content of free amino acids in the stored salmon was slightly higher (I1, F, S2), than in the freshest salmon (I0), but no significant differences ($p < 0.05$) between the storage conditions were found. The increase in free amino acids can be related to the increase in amount of water soluble proteins observed during the first week of storage. The total amount of free amino acids in salmon superchilled for 9 days (S1) was comparable to the 2 days ice-chilled (I0) and the batch superchilled for 16 days (S2) was comparable to the 9 days ice-chilled (I1). Even if S1 was stored for a longer period than the freshest batch, the enzymatic protein degradation was minor.

The decrease in the total amount of free amino acids after 16 days in the ice- chilled batch (I2) could be due to a higher bacterial load. In this experiment the microbial growth was however not determined. Jackson, Acuff, and Dickson (1997) reported that bacteria such as Pseudomonas species could be able to compete successfully on aerobically stored and refrigerated muscle foods. The high water content of fresh muscle tissue together with refrigerated temperatures, and with the readily available glycogen, peptides and amino acids, as a source of energy, may have contributed to its suitability as a substrate to support this microbial growth. A higher bacterial load in iced salmon compared to superchilled salmon has been observed (Rustad, unpublished results) and is in agreement with significantly lower counts obtained with slurry ice and ozonised slurry ice treatment of farmed turbot and sardine (Piñeiro et al., 2005; Campos et al., 2005; Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2006).

Regarding the relative amounts of the different amino acids, small changes in the different amino acids were observed. Independent of the time and storage method, glycine/arginine (coeluting), and alanine constituted about 45–51% of the total amount of free amino acids (wt.%), followed by threonine, glutamic acid, histidine, and valine. This is in agreement with Hultmann and Rustad (2004), who also found a decrease in the relative amount of glycine/arginine during iced storage of salmon. Lund and Nielsen (2001) investigated changes in free amino acids (using ethanol extraction) during cold storage of fresh salmon for up to 23 days. The dominating amino acid in all samples was histidine and it constituted about 48% (wt.%) of total free amino acid content in salmon. Alanine, glutamic acid and glycine were also important, constituting about 22% of total amino acid content. Hultmann et al. (2004) found lower amounts of histidine, and this is in accordance with the results in this study.

### 3.1.5. Electrophoretic profiles of myofibrillar and sarcoplasmic protein

Changes in the composition of the salt and water soluble proteins were investigated by eletrophoretic profiles (Fig. 3). SDS–PAGE has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergson, 2001). In the salt soluble extracts (Fig. 3a), myosin and actin were the dominating proteins. No large changes in intensity in the actin (42 kDa) and myosin heavy chain (200 kDa) bands were observed for the different storage procedures, except for salmon ice stored for 2 days (I0); this is probably because the fish was not completely out of rigor after 2 days (Wang, Tang, Correia, & Gill, 1998). In the salmon ice-chilled for 9 and 16 days (I1, I2) as well as in the frozen (F) and 9 days superchilled (S1), two bands with molecular weights in the range of 76 and 170 kDa appeared. In the salmon superchilled for 16 days (S2), there was a decrease in band intensity. An increase in new bands in the range of 45 kDa was observed with longer storage. This could be due to proteolytic degradation during storage. These results agree with previous studies of Lund and Nielsen (2001), who reported

Table 4
Free amino acids in fresh salmon fillets stored under different conditions (ice-chilled (I0, I1, I2), frozen (F), and superchilled (S1, S2))

| Storage method | Chilled (4 °C, crushed ice) | | | Frozen (−40 °C) | Superchilled (−1 °C, without ice) | |
|---|---|---|---|---|---|---|
| Time | Day 2 | Day 9 | Day 16 | Day 30 | Day 9 | Day 16 |
| Sample | I0 | I1 | I2 | F | S1 | S2 |
| Aspartic acid | 2.1 | 3.7 | 4.0 | 4.7 | 2.9 | 4.2 |
| Glutamic acid | 7.5 | 7.0 | 7.1 | 6.8 | 6.4 | 4.8 |
| Asparagine | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Histidine | 4.4 | 8.2 | 5.2 | 6.4 | 5.2 | 7.9 |
| Serine | 3.2 | 4.3 | 4.0 | 7.4 | 3.2 | 3.4 |
| Glutamine | 2.7 | 3.3 | 6.6 | 2.3 | 1.0 | 4.0 |
| Glycine/arginine | 18.8 | 13.2 | 14.5 | 12.9 | 12.3 | 12.7 |
| Threonine | 9.2 | 9.2 | 6.7 | 6.1 | 8.3 | 6.0 |
| Alanine | 32.5 | 32.5 | 32.8 | 29.6 | 34.8 | 34.0 |
| Tyrosine | 2.8 | 3.0 | 2.2 | 2.9 | 3.6 | 3.3 |
| Methionine | 1.1 | 1.0 | 1.2 | 1.3 | 1.1 | 1.1 |
| Valine | 4.6 | 4.5 | 4.9 | 5.2 | 5.4 | 5.4 |
| Phenyalanine | 1.3 | 1.7 | 2.4 | 3.1 | 2.2 | 2.8 |
| Isoleucine | 2.3 | 1.7 | 1.7 | 1.9 | 2.0 | 1.6 |
| Leucine | 3.9 | 3.4 | 4.0 | 4.8 | 4.6 | 4.4 |
| Lysine | 2.3 | 2.2 | 1.9 | 4.0 | 3.8 | 2.8 |
| Total amino acid amount | $6.8 \pm 1.5^{a}$ | $11.7 \pm 0.5^{ab}$ | $7.8 \pm 1.1^{ab}$ | $9.9 \pm 0.8^{ab}$ | $7.0 \pm 0.7^{ab}$ | $12.1 \pm 1.3^{b}$ |

Free amino acids fraction (%) of total amount (from μmol/g wet weight). Total amounts of amino acids (μmol/g wet weight), on the bottom line, are given as average ± sd of the mean ($n = 4$).

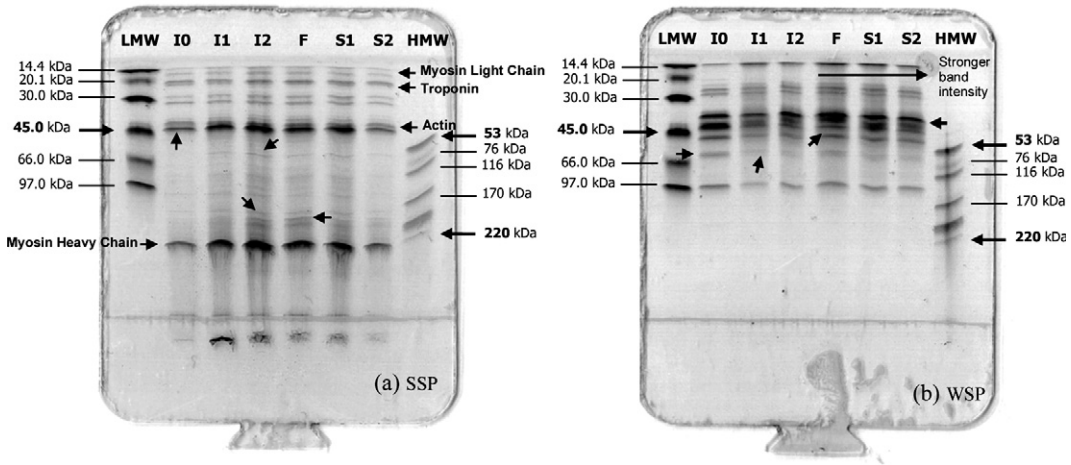


Fig. 3. SDS–PAGE profile of the salt and water soluble protein extract (SSP (a), WSP (b)) of fresh salmon. Ice chilled (I0, I1, I2), frozen (F), and superchilled (S1, S2) are the different times and storage methods. Arrows indicate other bands whose intensity increase during time and storage method. Low molecular weight standard (LMW): α-lactalbumin (14.4 kDa); trypsin inhibitor carbonic (20.1 kDa); anhydrase (30.0 kDa); ovalbumin (45 kDa); albumin (66.0 kDa); phosphorylase (97.0 kDa). High molecular weight standard (HMW): glutamic dehydrogenase (53.0 kDa); transferrin (76 kDa); β-galactosidase (116 kDa); α2-macroglobulin (170 kDa); myosin heavy chain (220 kDa).

the appearance of several bands in the 43-150 kDa range after different storage periods. Hultmann et al. (2004) also found that iced storage of salmon did not seem to cause a great increase in large fragments, due to breakdown or aggregation of proteins. New proteins might be present at too low concentrations to be detected by SDS–PAGE, and small changes in the molecular weights of proteins will not be detected.

The bands in the water soluble fraction of salmon (Fig. 3b), were in the range of 14.4 up to 97.0 kDa. Electrophoresis showed slight traces of bands in the range of 45–97 kDa and in the range of 20.1–30 kDa, and a stronger band intensity was observed with further storage.

### 3.2. Influence of time and storage method on the quality of lightly salted salmon

#### 3.2.1. Protein solubility

The amount of water and salt soluble protein (WSP, SSP) after 12 and 24 h of salting is shown in Fig. 4. The state of the proteins depends on the salt content. Muscle proteins generally first show an increase in solubility (salting-in) with increasing salt concentration. This is followed by a decreased solubility (salting-out) on further addition of salt, mainly due to the protein denaturation (Martínez-Álvarez & Gómez-Guillén, 2005; Sigurgisladottir, Sigurdardottir, Torrissen, Vallet, & Hafsteinsson, 2000; Thora-

rinsdottir et al., 2001). Salting minimised the differences in protein solubility after storage, but for all batches, a slight increase in WSP and SSP compared to the unsalted samples was found. Similar amounts of WSP and SSP were extracted after 12 and 24 h of salting in the ice-chilled batches (I0, I1 and I2) and in the superchilled batches (S1 and S2), indicating a low degree of protein denaturation. The extractability of myofibrillar proteins (SSP), was significant lower ($p < 0.05$) in the frozen samples (F), compared to the rest of the batches after salting, due to denaturation of the protein and loss of functional properties during frozen storage prior to processing (Mackie, 1993). However, an increased extractability in WSP and SSP after salting, compared to the unsalted frozen batch (F), was found.

### 3.2.2. Free amino acids

After 24 h of salting, there was a significant ($p < 0.05$) increase in the total amount of free amino acids, compared to fresh salmon, except for the frozen batch (F) (Table 5). The salted samples showed the same pattern as salmon before processing (Table 4). The total amount of amino acids in salmon superchilled for 9 days (S1) was comparable to the batch ice-chilled for 2 days (I0) and salmon superchilled for 16 days (S2) was comparable to 9 days ice-chilled (I1).

Small changes in the relative amounts of the different free amino acids were observed during salting. Glycine/arginine (coeluting), and alanine constituted about 37–47% of the total amount, followed by threonine, glutamic acid, histidine, and valine. The storage method and salting did not seem to influence the relative amount of the different free amino acids.

### 3.2.3. Electrophoretic profiles of myofibrillar proteins

The composition of the salt soluble proteins in salmon salted for 24 h is shown in Fig. 5. No clear differences in the protein distribution between unsalted (Fig. 3) and lightly salted samples were observed. No marked differences between the salted salmon from batches stored under different conditions were observed. The dominating bands were actin and myosin heavy chain. Electrophoresis showed slight traces of bands in I2-1, F-1, S1-1 and S2-1, in the range of 45 kDa, and some others in I2-1 and F-1, in the range of 220 kDa.

During dry salting, the salt uptake by the salmon muscle results in competition with muscle proteins for water molecules; after the "salting-in" phenomena, there is a denaturation and aggregation of these proteins by the "salting-out" process, resulting in reduced hydration capacity (Martínez-Álvarez & Gómez-Guillén, 2005, 2006). Even in the production of lightly salted salmon, the salt concentration in the outer layers of the muscle may be high enough to cause salting out. The myofibrillar proteins in fish therefore, are easily denatured during processing, resulting in loss of solubility (Hultmann et al., 2004). When comparing other previous studies on the effect of salting and different pHs on the functional properties of cod muscle proteins, either after subsequent lightly salting or heavy salting of cod, Martínez-Álvarez and Gómez-Guillén (2005, 2006) found that cod proteins were easily degraded. Myosin heavy chain was mostly absent and this could be due to possible protein aggregation in the early stages of salting. However, salmon proteins showed more protein stability degree than cod proteins (Ofstad, Kidman, Myklebust, & Hermansson, 1993) as Fig. 5 shows.

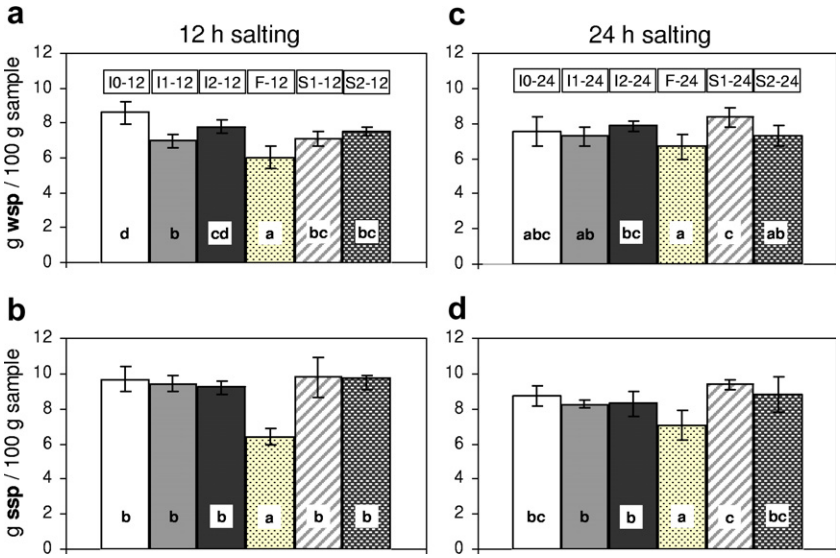


Fig. 4. Changes in water and salt soluble protein (WSP, SSP) after 12 (a, b) and 24 h (c, d) of dry salting. I0, I1, I2 (ice chilled for 2, 9 and 16 days), F (frozen for 30 days), S1 and S2 (superchilled for 9 and 16 days) are the different batches used under different time and storage methods before salting. Significant differences in water soluble and salt soluble protein among the samples after 12 and 24 h of salting are indicated with letters in the columns. Samples with no letters in common are significantly different ($p < 0.05$), ($n = 4$).

Table 5
Free amino acids in salmon salted for 24 h

| Storage method | Chilled (4 °C, crushed ice) | | | Frozen (−40 °C) | Superchilled (−1 °C, without ice) | |
|---|---|---|---|---|---|---|
| Time Salted batch (24 h) | Day 2 | Day 9 | Day 16 | Day 30 | Day 9 | Day 16 |
| Sample | I0 | I1 | I2 | F | S1 | S2 |
| Aspartic acid | 1.3 | 1.6 | 2.9 | 4.7 | 2.6 | 3.6 |
| Glutamic acid | 8.0 | 9.8 | 6.3 | 6.0 | 7.4 | 8.6 |
| Asparagine | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Histidine | 8.6 | 5.2 | 5.0 | 6.3 | 8.1 | 4.9 |
| Serine | 5.4 | 11.5 | 16.0 | 9.1 | 4.6 | 5.3 |
| Glutamine | 0.7 | 0.9 | 0.5 | 0.8 | 0.3 | 0.5 |
| Glycine/arginine | 13.9 | 14.5 | 11.8 | 9.8 | 12.9 | 13.9 |
| Threonine | 9.8 | 7.8 | 7.7 | 7.0 | 7.3 | 7.9 |
| Alanine | 33.4 | 30.4 | 25.2 | 30.9 | 32.9 | 27.8 |
| Tyrosine | 2.3 | 2.2 | 2.4 | 3.1 | 3.3 | 2.8 |
| Methionine | 0.9 | 0.9 | 1.2 | 0.8 | 0.9 | 1.4 |
| Valine | 4.4 | 4.2 | 4.4 | 5.6 | 5.0 | 4.7 |
| Phenyalanine | 1.1 | 1.3 | 2.2 | 3.4 | 2.1 | 2.4 |
| Isoleucine | 2.0 | 1.9 | 1.7 | 2.0 | 2.0 | 1.8 |
| Leucine | 3.5 | 3.1 | 3.6 | 5.1 | 4.0 | 3.9 |
| Lysine | 3.2 | 3.5 | 4.0 | 4.3 | 2.7 | 5.6 |
| Total amino acid amount | $10.3 \pm 1.3^{ab}$ | $14.7 \pm 1.6^{cd}$ | $17.4 \pm 0.9^{d}$ | $7.9 \pm 1.3^{a}$ | $12.6 \pm 0.9^{bc}$ | $15.3 \pm 1.1^{cd}$ |

I0, I1, I2 (ice chilled), F (frozen) and S1, S2 (superchilled) are the different batches used under different time and storage methods before salting. Free amino acids fraction (%) of total amount (from μmol/g wet weight). Total amounts of amino acids (μmol/g wet weight), on the bottom line, are given as average ± sd of the mean ($n = 4$). Significant differences along the storage time and conditions are indicated with superscript letters. Samples with no letters in common are significantly different ($p < 0.05$).

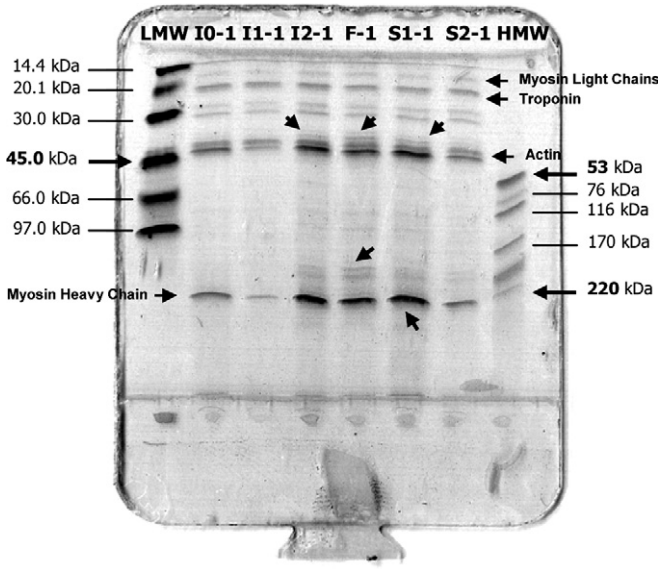


Fig. 5. SDS–PAGE profile of the salt soluble protein extract in salted salmon for 24 h. Ice chilled (I0, I1, I2), frozen (F), and superchilled (S1, S2) are the different times and storage methods underwent prior to salting. The number 1 in each of the batches means 1 day of salting. Arrows indicate other bands whose intensity increase during storage. Low molecular weight standard (LMW): α-lactalbumin (14.4 kDa); trypsin inhibitor carbonic (20.1 kDa); anhydrase (30.0 kDa); ovalbumin (45 kDa); albumin (66.0 kDa); phosphorylase (97.0 kDa). High molecular weight standard (HMW): glutamic dehydrogenase (53.0 kDa); transferrin (76 kDa); β-galatosidase (116 kDa); $\alpha_2$-macroglobulin (170 kDa); myosin heavy chain (220 kDa).

### 3.2.4. Mass transfer

The main components transferred between salmon and the brine are water and NaCl. In addition, some proteins and some fat are lost into the formed pickle. Mass changes were determined during the salting experiments using the methodology described by Fito and Chiralt (1996) and Barat et al. (2006).

The total, water and NaCl salmon weight changes ($\Delta M_t^{o}$, $\Delta M_t^{w}$ and $\Delta M_t^{NaCl}$, respectively), determined by means of Eqs. (1)–(3) ($M_t^{o}$ and $M_0^{o}$ being the salmon weight at the sampling time $t$ and 0, $x_t^{w}$ and $x_0^{w}$ being the salmon water weight fractions, and $x_t^{NaCl}$ and $x_0^{NaCl}$ being the salmon NaCl weight fraction at time $t$ and 0, respectively), during the salting process, as a function of the raw material used were

$$\Delta M_t^{o} = \left(\frac{M_t^{o} - M_0^{o}}{M_0^{o}}\right) \quad (1)$$

$$\Delta M_t^{NaCl} = \left(\frac{M_t^{o} \cdot x_t^{NaCl} - \mathrm{M}_0^{o} \cdot x_0^{NaCl}}{\mathrm{M}_0^{o}}\right) \quad (2)$$

$$\Delta M_t^{w} = \left(\frac{M_t^{o} \cdot x_t^{w} - M_0^{o} \cdot x_0^{w}}{M_0^{o}}\right) \quad (3)$$

The total, NaCl and water weight changes after 12 ($\Delta M_{12}^{o}$, $\Delta M_{12}^{NaCl}$ and $\Delta M_{12}^{w}$) and 24 h salting $\Delta M_{24}^{o}$, ($\Delta M_{24}^{NaCl}$ and $\Delta M_{24}^{w}$) are shown in Fig. 6. After 12 h of salting, the total weight changes are quite small for all of the batches. However a significant positive weight increase ($p < 0.05$) after 12 h salting was observed in batches stored 9 days on ice (I1) and superchilled (S1), while batches I0, I2 (2 and 16 days ice-chilled), F (frozen) and S2 (16 days superchilled) lost weight. After 24 h of salting, the yield was negative for all of the batches; I2 and S2 (16 days ice chilled and superchilled) had the lowest yield. This fact could be explained by the higher degree of structural damage caused

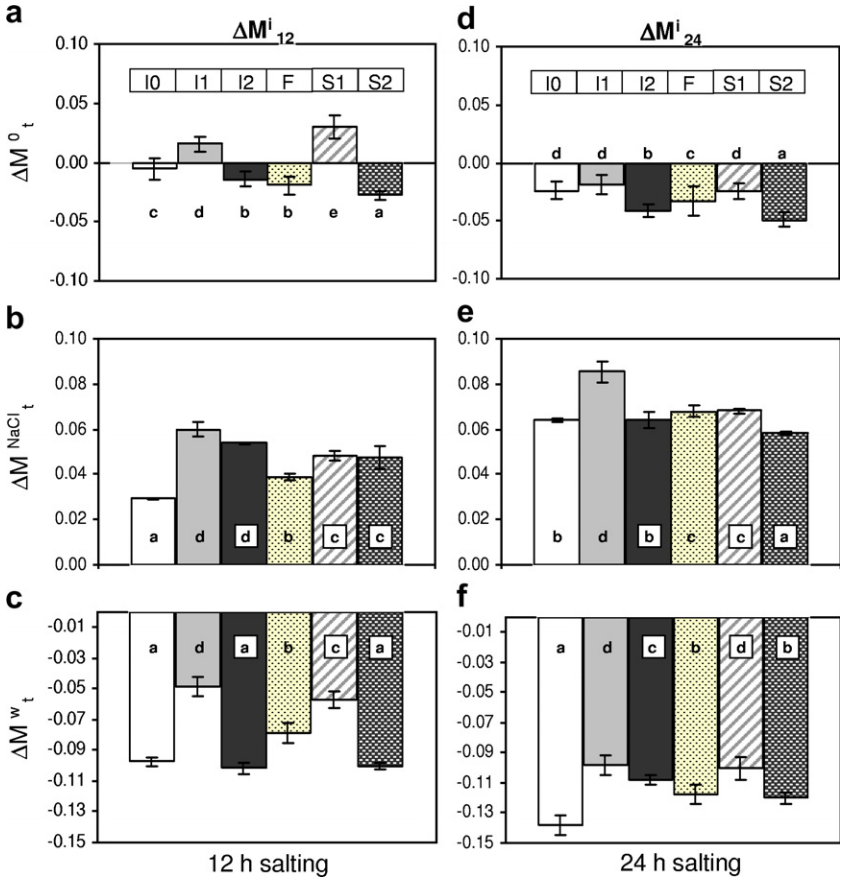


Fig. 6. Total mass, salt and water weight change after 12 (a–c) and 24 h (d–f) of dry salting. I0, I1, I2 (ice chilled for 2, 9 and 16 days), F (frozen for 30 days), S1 and S2 (superchilled for 9 and 16 days) are the different batches used under different time and storage methods before salting. Significant differences in total mass, salt and water weight changes after 12 and 24 h of salting are indicated with letters on each column. Samples with no letters in common are significantly different ($p < 0.05$), ($n = 8$).

due to different reasons. In the case of I2 samples, the higher damage to the structure could be explained by the intense proteolytic activity during the ice storage; while for S2 samples, small fluctuations in the storage temperature range (−1 to −2 °C), would lead to intense recrystalisation phenomena, contributing to an increase the structural damage (Mackie, 1993). Over this temperature range, the amount of ice in fish muscle varies from 10% to 56% of the total water in the tissue (Huss, 1998).

Regarding the salt uptake weight changes (Fig. 6b), the lowest value was for I0 (the freshest raw material stored 2 days on ice), indicating a more intact tissue structure. It seems that when the structure of the muscle is not damaged, it is limiting the salt uptake (Barat et al., 2006; Wang et al., 1998). However I0 achieved the highest water loss (Fig. 6c), probably due to the lower NaCl uptake previously commented. After 9 days of ice-chilling (I1), the salmon structure is losing its integrity, as a consequence of degradation of cellular structures by enzymes, which means that salt ions have much more freedom to penetrate (Wang et al., 1998). Nevertheless the protein matrix is still capable of retaining water after 12 h of salting, and therefore less dehydration was observed. The same phenomenon occurred in S1 (9 days superchilled), probably mainly caused by mechanical damage rather than biochemical reactions. Therefore, 9 days old salmon obtained a higher yield in the salting step, independent of the storage method but for the aforementioned reasons. Concerning the batches I2 and S2 (16 days ice-chilled and superchilled) the structure may have become more degraded, and therefore, the protein might have lost the ability to retain water, resulting in more dehydration and contributing to an increase in salt uptake (Wang et al., 1998).

The frozen batch (F), behaved similarly to the fresh batch (I0). Although the F batch has been frozen (according to Mackie (1993), 92% of the water is frozen when reaching –30 °C), it seems that there is not a marked variation in the tissue structure after salting, either due to the use of very low freezing temperatures (−40 °C), or because the fatty tissue nature of the salmon may tolerate vigorous temperature changes, or a combination of both. Although the structure of frozen salmon would be different from the structure of 16 days superchilled salmon (Kato et al., 1974; Mackie, 1993), salting minimised the differences.

When salting time increases from 12 to 24 h, there is little difference in the behaviour of the samples. The behaviour of the superchilled samples seems to be between the freshest batch and the oldest refrigerated batch. I1 seems

to have the optimum structure followed by S1, enabling a high uptake of salt with a good capability to retain water, and so is the least dehydrated. This is in agreement with other studies (Barat et al., 2006; Lauritzsen et al., 2004). The negative yield obtained after 24 h of salting (a 3.6–7.4% reduction in fillet weight) was in accordance with other results by (Cardinal et al., 2001; Mørkøre, Vallet, Cardinal, Gómez-Guillén, & Montero, 2001).

### 3.2.5. Texture attributes

During ice storage the fish muscle softens or tenderises, due to a weakening of the Z-discs of the myofibrils, a degradation of connective tissue, or a weakening of myosin–actin junctions (Hultmann & Rustad, 2002; Kato et al., 1974; Wang et al., 1998). The texture of fish muscle is influenced by several factors such as rate of pH decline and extent of proteolysis causing breakdown of myofibrils (Hultmann & Rustad, 2002).

In this study, the fillet hardness (force recorded at 40% compression) was significantly reduced ($p < 0.05$) during storage (Fig. 7). The highest value was obtained for the 2 days ice stored batch (I0), while the lowest recorded hardness was for S2 (16 days superchilled) batch. This decrease could be due to the slow freezing of fish flesh during a long storage period. Some of the water freezes out and the salt concentration in the tissue increases, leading to denaturation of the muscle proteins, as well as structural damage of membranes (Huss, 1998). In addition, temperature fluctuations during storage promote an increase in the size of the ice crystals, causing more mechanical damage of the structure (Mackie, 1993), resulting in increased drip loss, loss of water holding capacity, textural changes and poorer appearance (Huss, 1998). On the other hand, the hardness

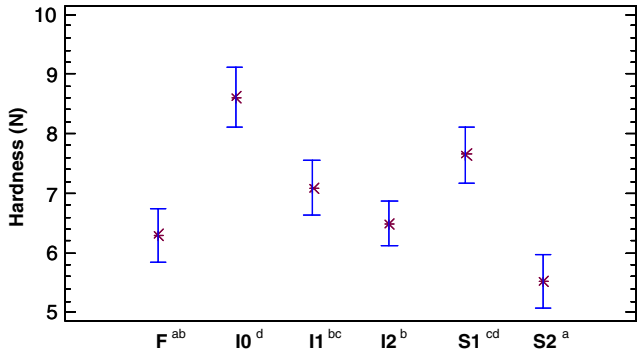


Fig. 7. Mean values of hardness ($N$) for fresh salmon ($n = 12$–14). F (frozen for 30 days), I0, I1, I2 (ice chilled for 2, 9 and 16 days), S1 and S2 (superchilled for 9 and 16 days) are the different batches used under different time and storage methods before salting. Significant differences in hardness are indicated with letters superscripts on each of the batches name. Samples with no letters in common are significantly different ($p < 0.05$), ($n = 12$–14).

of the S1 (9 days superchilled) batch, was between that of the batches stored 2 and 9 days ice chilled (I0 and I1). It seems superchilling for shorter periods will have less effect on texture.

Regarding chewiness, all the batches showed the same significant trend as in hardness ($p < 0.05$). The highest value recorded was for the freshest raw material (I0) followed by superchilled (I1), while the lowest was recorded for the frozen (F) and superchilled for 16 days (S2). Cohesiveness and gumminess had the same significant group trend, the highest cohesiveness was recorded for batches I0 and I1 and the lowest values for F and S2. Samples ice-chilled for 9 and 16 days (I1 and I2) were in between the others. On the contrary, I0 followed by I1 were found

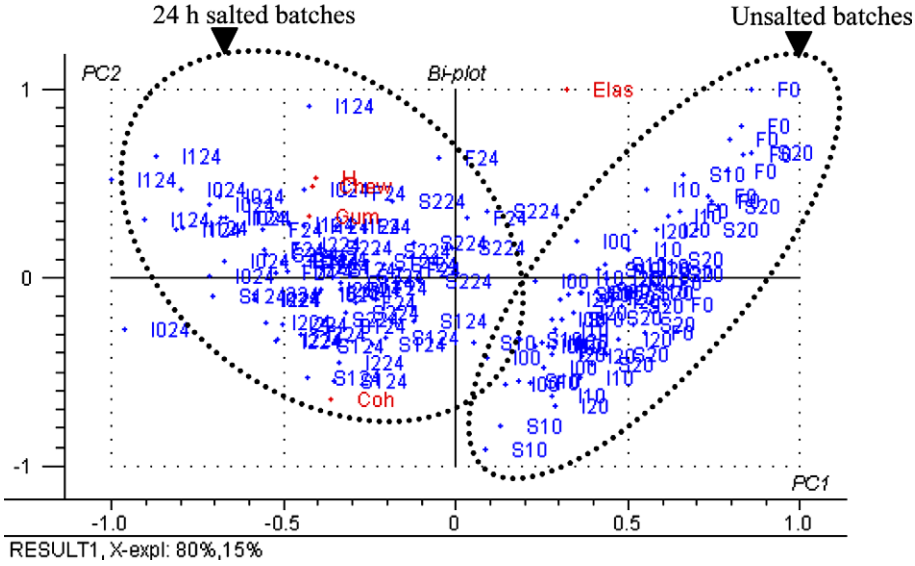


Fig. 8. PCA of the texture attributes on the different stored batches. Bi-Plot for the first two principal components PC1 (80%) and PC2 (15%) of the PCA model. The texture attributes are identified by (H: hardness; Coh: cohesiveness; Elas: elasticity; Gum: gumminess and Chew: chewiness). I0, I1, I2 (ice chilled for 2, 9 and 16 days), F (frozen for 30 days), S1, S2 (superchilled for 9 and 16 days) are the different batches used under different time and storage methods before salting.

to be significantly ($p < 0.05$) the least elastic and F the most elastic.

In order to obtain an overview of the main effects of the salting process on the textural attributes (H: hardness; Coh: cohesiveness; Elas: elasticity; Gum: gumminess and Chew: chewiness), for salmon stored under different conditions (I0, I1, I2, F, S1 and S2), a multivariate analysis was performed to identify similarities and differences between the treatments. The results are presented in a PCA bi-plot of scores and loadings (Fig. 8). It was found that two PCs explained 95% of the variance in the data set. The salting time played an important role on texture parameters, independently of the storage method. The behaviour of unsalted salmon was different from the lightly salted salmon. Twenty-four hours of salting was found to cause an increase in fillet hardness of 200% in all of the different batches studied. The PCA plot showed that the salted samples were associated with an increased hardness, gumminess, chewiness and cohesiveness, compared to the unsalted samples. Unsalted samples were more associated with elasticity.

## 4. Conclusions

Salmon stored on ice, super chilled or frozen, seems to behave differently with regard to biochemical and physical changes. Superchilling seems promising for storing raw material before salting, since biochemical quality degradation that easily happens under chilled storage is slowed down, the degree of protein denaturation is low and the degree of structural damage is less than in frozen storage.

The raw material superchilled for 9 days was comparable to material ice-chilled for 2 days and better than 9 days ice-chilled, especially with regard to protein denaturation and degradation (measured as changes in protein solubility and free amino acids), as well as structural changes. Longer superchilled storage had a marked negative effect on textural properties. Therefore, to prolong the superchilled storage time and to avoid mechanical damage, it is necessary to control both the superchilling process and the storage conditions, minimising temperature fluctuations.

Regarding the salting step, the effect of the different storage methods was minimised. Nevertheless the highest yield of lightly salted salmon was obtained for salmon superchilled for 9 days followed by 9 days ice-chilled. Further sensory and microbiological studies of the final smoked product under the different preserving methods used should be addressed.

## Acknowledgements

This research has been supported by a Marie Curie Fellowship, PHD20 of the European Community Programme *Valuefish Training Site* IHP at the Institute of Biotechnology, Norwegian University of Science and Technology under contract number HPMT-CT-2001-00301 and by a FPI-UPV grant at the Universidad Politécnica de Valencia (Spain). The efforts of Christian Bagioni at Department of Biotechnology concerning laboratory work are much appreciated.

## References

Alemán, M., Kakuda, K., & Uchiyama, H. (1982). Partial freezing as a means of keeping freshness of fish. *Bulletin of the Tokai Region Fish Research Laboratories, 106*, 11–25.

Anderson, M. L., & Ravesi, E. M. (1968). Relation between protein extractability and free fatty acid production in code muscle aged in ice. *Journal of the Fisheries Research Board of Canada, 25*(10), 2159–2169.

AOAC (1990a). Moisture in meat. Method 950.46. In P. Cunniff (Ed.), *Official methods of analysis of AOAC international*. Gaithersburg, MD, USA.

AOAC (1990b). Salt (chlorine as sodium chloride) in seafood. Method 937.09. In P. Cunniff (Ed.), *Official methods of analysis of AOAC international*. Gaithersburg, MD, USA.

Aune, E. J., & Nordtvedt, T. S. (1999). An environmentally transport of salmon from Norway to France by means of super-chilling and a new packaging method. In *20th International congress of refrigeration* (pp. 1–6). Sydney.

Barat, J. M., Gallart-Jornet, L., Andres, A., Akse, L., Carlehög, M., & Skjerdal, O. T. (2006). Influence of cod freshness on the salting, drying and desalting stages. *Journal of Food Engineering, 73*, 9–19.

Bendall, J. R. (1973). Structure, part 2 (2nd ed.. *The structure and function of muscle* (Vol. 2, pp. 243–309). New York: Academic Press.

Bligh, E. G., & Dyer, W. J. (1959). A Rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62–66, 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.

Campos, C. A., Rodríguez, O., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2005). Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sadina pilchardus*). *International Journal of Food Microbiology, 103*, 121–130.

Campos, C. A., Rodríguez, O., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2006). Evaluation of an ozone-slurry ice combined refrigeration system for the storage of farmed turbot (*Psetta maxima*). *Food Chemistry, 97*, 223–230.

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Mørkøre, T., Thomassen, M., et al. (2001). Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research International, 34*(6), 537–550.

Chang, K. L. B., Chang, J., Shiau, C. Y., & Pan, B. S. (1998). Biochemical, microbiological, and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. *Journal of Agricultural and Food Chemistry, 46*, 682–686.

Dion, P. (1995). *Pursuit of prime quality seafood – introduction to superchilling*. Plymouth, MA 02360: Paul Dion Associates, Inc. Available at http://members.aol.com/pdionpda/. Accessed February 2006.

Eide, O., Børrensen, T., & Strøm, 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), 347–354.

Einarsson, H. (1998). Deep chilling (superchilling, partial freezing) a literature survey. *SIKs service-serie* (Vol. 784, pp. 30). Göteborg, Sweden: Chalmars University of Technology.

Einen, O., & Thomassen, M. (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*, 37–53.

Elvevoll, E. O., Sørensen, N. K., Østerud, B., Ofstad, R., & Martínez, I. (1996). Processing of marine foods. *Meat Science, 43*, S265–S275.

Erikson, U., Hultmann, L., & Steen, J. E. (2006). Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia I. Establishing a method for large-scale processing of farmed fish. *Aquaculture, 252*, 183–198.

Esbensen, K. H. (2000). *Multivariate data analysis – in practice. An introduction to multivariate data analysis and experimental design* (4th ed.). Oslo: CAMO ASA.

Fito, P., & Chiralt, A. (1996). Osmotic dehydration. An approach to the modeling of solid food–liquid operations. *Food engineering 2000* (Vol. 13, pp. 231–252). New York: Chapman & Hall.

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.

Gómez-Guillén, M. C., Montero, P., Hurtado, O., & Borderías, A. J. (2000). Biological characteristics affect the quality of farmed Atlantic salmon and smoked muscle. *Journal of Food Science, 65*(1), 53–60.

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

Hardy, R., & Keay, J. N. (1972). Seasonal variations in the chemical composition of *Cornish mackerel* (*Scomber scombrus* L.), with detailed reference to the lipids. *Journal of Food Technology, 7*, 125–137.

Hempel, E. (1998). Another decade of growth for salmon. *Seafood International*, 27–31.

Hempel, E. (1999). Bright future lies ahead for salmon. Norway: industry continues to grow. *Seafood International*, 22–23.

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

Hultmann, L., & Rustad, T. (2002). Textural changes during iced storage of Salmon (*Salmon 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*, 31–41.

Huss, H. (1998). *Fresh fish-quality and quality changes*. In H. Huss & Technological Laboratory Ministry of Agriculture and Fisheries (Eds.). *FAO Collection: Fisheries No. 29*. FAO.

Jackson, T. C., Acuff, G. R., & Dickson, J. S. (1997). Meat, poultry and seafood. In M. P. Doyle, L. R. Beuchat, & T. J. Montville (Eds.), *Food microbiology, fundamentals and frontiers* (pp. 83–100). Washington, DC: ASM Press.

Kato, N., Umemoto, S., & Uchiyama, H. (1974). Partial freezing as a means of preserving freshness of fish-II. Changes in the properties of protein during the storage of partially frozen sea bass muscle. *Bulletin of the Japanese Society of Scientific Fisheries, 40*(12), 1263–1267.

Laemmli, U. K. (1970). Cleavage and structural proteins during assembly of the head of bacteriophage $T_4$. *Nature, 227*, 680–685.

Lauritzsen, K., Akse, L., Johansen, A., Joensen, S., Sørensen, N. K., & Olsen, R. L. (2004). Physical and quality attributes of salted cod (*Gadus morhua* L.) as affected by the state of rigor and freezing prior to salting. *Food Research International, 37*, 677–688.

Lee, Y. W., & Park, Y. H. (1985). Effect of partial freezing as a means of keeping freshness. I. Changes in freshness and gel forming ability of mullet muscle during storage by partial freezing. *Bulletin of the Korean Fisheries Society, 18*(6), 529–537.

Lee, C. M., & Toledo, R. T. (1984). Comparison of shelf life and quality of mullet stored at zero and subzero temperature. *Journal of Food Science, 49*, 317–344.

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 *o*-phthaldialdehyde. *Analytical Chemistry, 51*(11), 1667–1674.

Losada, V., Rodríguez, A., Ortiz, J., & Aubourg, S. P. (submitted for publication). Quality enhancement of canned sardine (*Sardina pilchardus*) by a preliminary slurry ice chilling treatment. *European Journal of Lipid Science and Technology*.

Losada, V., Piñeiro, C., Barros-Velázquez, J., & Aubourg, S. (2004). Effect of slurry ice on chemical changes related to quality loss during European hake (*Merluccius merluccius*) chilled storage. *European Food Research Technology, 219*, 27–31.

Losada, V., Piñeiro, C., Barros-Velázquez, J., & Aubourg, S. P. (2005). Inhibition of chemical changes related to freshness loss during storage of horse mackerel (*Trachurus trachurus*) in slurry ice. *Food Chemistry, 93*, 619–625.

Lund, K. E., & Nielsen, H. H. (2001). Proteolysis in salmon (*Salmo salar*) during cold storage; effects of storage time and smoking process. *Journal of Food Biochemistry, 25*(5), 379–395.

Mackie, I. M. (1993). The effects of freezing on flesh proteins. *Food Reviews International, 9*(4), 575–610.

Magnussen, O. M., Nordtvedt, T. S., Hardarson, V. (2001). Developments in rapid chilling of fish. In *Proceedings from IIF-IIR-Commission C2-(Section 1: Primary Chilling of Meat, Fish, Fruit, Vegetables, Cereals and Food Products. Rapid cooling of food* (pp. 99–108). Bristol, United Kingdom.

Martínez-Álvarez, O., & Gómez-Guillén, M. C. (2005). The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*). *Food Chemistry, 92*, 71–77.

Martínez-Álvarez, O., & Gómez-Guillén, M. C. (2006). Effect of brine salting at different pHs on the functional properties of cod muscle proteins alter subsequent dry salting. *Food Chemistry, 94*, 123–129.

Milanesi, A. A., & Bird, J. W. C. (1972). Lysosomal enzymes in aquatic species. II. Distribution and particle properties of thermally acclimated muscle lysosomes of rainbow trout (*Salmo gairdneri**). *Comparative Biochemistry and Physiology, 41B*, 573–591.

Mørkøre, T., Vallet, J. L., Cardinal, M., Gómez-Guillén, M. C., Montero, R., et al. (2001). Fat content and fillet shape of Atlantic salmon: relevance for processing yield and quality of raw and smoked products. *Journal of Food Science, 66*, 1348–1354.

NF (2006). Toppår for lakseeksporten (in Norwegian). Kyst.no, Norsk Fiskeoppdrett (Ed. Gustav-Erik Blaalid). http://www.kyst.no/index.php?page_id=26&article_id=73732. Accessed February 2006.

Nielsen, J., Hyldig, G., & Larsen, E. (2002). Eating quality of fish. A review. *Journal of Aquatic Food Product Technology, 11*(3/4), 125–141.

Nilsson, K. (1994). Quality of frozen rainbow trout. *Effects of different freezing and thawing treatments*. Doctoral thesis, Chalmers tekniska høgskola, Göteborg.

Nordtvedt, T.S., Børresen, T., & Magnussen O.M., (1998). Partial freezing of salmon fillets. In: *Proceedings from IIF-IRR-Commission D1, D2/D3. Refrigerated transport, storage & retail display* (pp. 106–113). Cambridge, United Kingdom.

Norwegian Seafood Export Council (2005) (in Norwegian). http://www.akvafakta.no or http://www.godfisk.no. Accessed November 2005.

Nowlan, S., Dyer, W. J., & Keith, R. A. (1974). Temperature and deteriorative Changes in Postrigor cod muscle stored up to 14 days in the superchill range, −1 to −4 °C. *Journal of the Fisheries Research Board of Canada, 32*(9), 1595–1605.

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, 12*(2), 163–174.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., et al. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology, 8*, 258–263.

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

Piñeiro, C., Bautista, R., Rodriíguez, O., Losada, V., Barros-Velázquez, J., Santiago, P., et al. (2005). Quality retention during the chilled distribution of farmed turbot (*Psetta maxima*): effect of a primary slurry ice treatment. *International Journal of Food Science and Technology, 40*, 817–824.

Rehbein, H. (1979). Development of an enzymatic method to differentiate between sea-frozen and thawed fish fillets. I. Comparison of the applicability of some enzymes of fish muscle. *Zeitschrift fur Lebensmittel–Untersuchung und-Forschung A–Food Research and Technology, 169*, 263–265.

Roberts (1998). Atlantic salmon – the European scene. *Aquaculture Magazine*, 26–36.

Rørå, A. M., Kvåle, A., Mørkøre, T., Rørvik, K. A., Steien, S. H., & Thomassen, M. S. (1999). Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*) in relation to raw material characteristics. *Food Research International, 31*, 601–609.

Sigurgisladottir, S., Sigurdardottir, M. S., Torrissen, O., Vallet, J. L., & Hafsteinsson, H. (2000). Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. *Food Research International, 33*(10), 847–855.

Sigurgisladóttir, S., Torrissen, O., Lie, Ø., Thomassen, M., & Hafsteinsson, H. (1997). Salmon quality: methods to determine the quality parameters. *Reviews in Fisheries Science, 5*(3), 223–252.

Sivertsvik, M., Jeksrud, W. K., & Rosnes, 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*, 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.

Sveinsdottir, K., Martinsdottir, E., Hyldig, G., Jorgensen, B., & Kristbergsson, K. (2002). Application of quality index method (QIM) scheme in shelf life study of farmed Atlantic salmon (*Salmo salar*). *Journal of Food Science, 67*, 1570–1579.

Thorarinsdottir, K. A., Arason, S., Geirsdottir, M., Bogason, S. G., & Kristbergson, K. (2001). Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. *Food Chemistry, 77*, 377–385.

Uchiyama, H., & Kato, N. (1974). Partial freezing as a means of preserving fish freshness-I. Changes in free amino acids, trimethylamine nitrogen, ATP and its related compounds and nucleic acids during storage. *Bulletin of the Japanese Society of Scientific Fisheries, 40*, 1145–1154.

Wang, D., Tang, J., Correia, L. R., & Gill, T. A. (1998). Postmortem changes of cultivated Atlantic salmon and their effects on salt uptake. *Journal of Food Science, 63*(4), 634–637.

Zeng, Q. (2003). Quality Indicators of Northern Shrimp (*Pandalus borealis*) stored under different cooling conditions. In *Final Project 2003. Fisheries Training Programme*. The United Nations University.