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Quality changes during superchilled storage of cod (*Gadus morhua*) fillets

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Abstract

Superchilling is a method with potential for extending the shelf life of food products by partial freezing. For centuries, Atlantic cod (*Gadus morhua*) has been the most important commercial species in the North Atlantic fisheries and is now regarded as a very promising species in cold water fish farming. In the present work, superchilled storage at −2.2 °C of fillet portions of farmed cod was investigated. Superchilled cod showed increased shelf life with respect to reduced growth of sulphide producing bacteria compared to ice chilled. Drip loss was lower in superchilled cod. However, liquid loss by low-speed centrifugation was higher in superchilled cod fillets compared to ice chilled. This can be explained by freeze denaturation of muscle proteins, which is supported by the lower extractability of salt soluble proteins. There is a need for process optimization to minimize protein denaturation.

Keywords: Superchilling; Cod; *Gadus morhua*; Bacterial count; SPB; Drip loss; Liquid loss; Protein extractability; pH; Free amino acid

1. Introduction

For centuries, Atlantic cod (*Gadus morhua*) has been the most important commercial species in the North Atlantic fisheries and is now regarded as a very promising species in cold water fish farming. Fish farming may provide an opportunity to obtain a seasonally independent supply of fresh fish to the market. Norway is one of the largest net exporters of seafood and in recent years the annual harvest has been more than 3 million metric tonnes of fish and seafood. In 2005, fish farming was responsible for almost half the value of Norwegian seafood exports. Cod has traditionally been sold as whole fish, while the tendency now is more toward fillets and prepacked dishes. The demand for fresh cod fillets is rapidly increasing at the expense of frozen fillets (Norwegian Seafood Export Council, 2005). Since the shelf life of fillets are lower than for whole fish, the need to develop methods for maintaining good post mortem quality of the fish on its way to the market increases. Extending the shelf life of fish may also be a way to increase profitability, since product prices in the fresh market are higher than in the frozen market. Potentials for extending shelf life can be found in using hurdle technologies such as different gas mixtures (Sivertsvik, 2007), additives (Boskou & Debevere, 2000) or new temperature regimes.

The most important factor for increasing shelf life is the temperature from catch to consumer. Superchilling (also called partial freezing or deep chilling) 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 initial freezing points of most foodstuffs are between −0.5 °C and −2.8 °C (Fennema, Powrie, & Marth, 1973). The surrounding temperature is set below the initial freezing point of the food, and depending on the method used some ice is formed in the outer few millimeters. In this way refrigeration capacity is stored inside the product, making it possible to maintain a low product temperature during storage or in a distribution chain where a considerable

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heat load is assumed. In this temperature range, a drop in temperature of about 1 °C will often result in doubling the amount of water frozen out (Power, Morton, & Sinclair, 1969). With the high latent heat of ice (3.65 kJ/kg), this will give a certain degree of aftercooling of the product if the cooling is interrupted. A low product temperature can thereby be ensured even with a short cooling time. Since fish is more highly perishable than meat, the temperature is even more important.

For many food products, superchilling results in better quality compared to conventional chilling. The shelf life of superchilled food can be extended by 1.5–4 times compared to chilled food and should be an attractive alternative to freezing and conventional chilling (Einarsson, 1988). Normal shelf life of good quality ice chilled cod is about 11–14 days (Dalgaard, Gram, & Huss, 1993; Einarsson, 1994). Power et al. (1969) concluded that reducing the storage temperature of round cod to just below the freezing point could approximately double the storage life, as measured by a taste panel. Based on the content of total volatile base nitrogen (TVB-N) and hypoxanthine, Nowlan and co-workers stated that the shelf life of ice chilled or superchilled cod at −1.6 or −4 °C – were 10, 14 and 18–20 days, respectively (Nowlan, Dyer, & Keith, 1975). Mullet (*Mugil* spp.) had a shelf life of 10 days stored at −2 °C compared to 7 days stored in ice (Lee & Toledo, 1984). Extension of shelf life was attributed to delayed microbial growth and reduced rates of biochemical processes.

More recently, Olafsdottir, Lauzon, Martinsdottir, Oehlenschlager, and Kristbergsson (2006) investigated superchilling of aerobically packed cod fillets by combined blast and contact (CBC) chilling. They found the shelf life of superchilled cod based on Torry score and TVB-N to be 15 days at −1.5 °C compared to 11 days for ice chilled cod.

Superchilling of fatty fish has shown promising results. Based on both sensory and microbial analyses, superchilled salmon stored at −2 °C had a 21 days sensory shelf life, whereas fillets stored at chilled conditions were spoiled after 7 days (Sivertsvik, Rosnes, & Kleiberg, 2003).

When some of the water freezes out, the concentration of solutes in unfrozen solutions increases. This may lead to denaturation of the muscle proteins as well as structural damage of membranes, which can result in increased drip loss, loss of water holding capacity and textural changes. Increased enzymatic activity has also been suggested during storage at subzero temperatures (Foegeding, Lanier, & Hultin, 1996). The shelf life of superchilled food is far shorter than for frozen food, but could be more attractive since the amount of water frozen out is lower in superchilling compared to freezing. Less water frozen out will lead to less change in microstructure, which in turn may result in a lower degree of freeze denaturation and less drip loss (Einarsson, 1988). Structural changes due to ice crystals at subzero temperature storage have appeared to be minor compared to those which occurred during freezing at −20 °C, as revealed by microscopic examination (Lee & Toledo, 1984). The ice fraction curve is very steep in the superchilling temperature area. Fluctuating temperature may give melting and refreezing of water resulting in larger ice crystals, which can be disruptive to the microstructure (Mackie, 1993).

Superchilling has emerged as a potential method for extending shelf life, but still there is a need to know more about how the degree of superchilling (the amount of water frozen out) affects biochemical changes, such as protein denaturation, enzymatic activity and liquid retention. Most of the studies on superchilling have focused on microbiology, sensory analysis and spoilage indicators such as TVB-N (Olafsdottir et al., 2006; Sivertsvik, Jeksrud, & Rosnes, 2002; Sivertsvik et al., 2003; Zeng, Thorarinsdottir, & Olafsdottir, 2005), and have only to a minor extent studied the effect of superchilling on biochemical processes and how they influence quality parameters, such as loss of juiciness and negative textural changes.

The aim of this study was to investigate the effects of a superchilling process on selected quality parameters of farmed cod in order to extend shelf life without freezing the fish.

2. Materials and methods

2.1. Raw materials and processing

Twenty Atlantic cod (*G. morhua*) from a fish farm in mid-Norway were slaughtered and transported on ice to the laboratory in October 2005. After three days of ice chilled storage, the cod were headed, filleted and deboned. The fish had a weight of 2348 ± 509 g, the length was 53.6 ± 3.5 cm and the hepatosomatic index (HSI), calculated as the ratio of liver weight to ungutted fish weight, was 13.9 ± 1.7%. From the middle of each skin-on fillet with thickness of 15–25 mm, two portions of 158 ± 31 g were vacuum packed and labeled. Samples from the right side of the cod fillets were subsequently cooled down for 14 min in a freezing tunnel at a temperature of −24 °C and air velocity of 4 m/s. The duration of the cooling was based on previous experiments and simulations (Hardarson, 1996). After cooling the surface ice layer was 3–5 mm and the core temperature approximately −1 °C. The samples were immediately transferred to a cold storage room at −2.2 ± 0.2 °C for temperature equalization and storage for up to 34 days. The day of processing was defined as day 0. Samples from the left side of the cod fillets were used as references and kept on ice or frozen at −21 or −40 °C, respectively. Prior to analysis (1–2 h depending on fillet thickness) subzero stored samples were transferred to room temperature for thawing. During the superchilled storage air temperature was logged by logs of the type StowAway-IS Temp with an internal sensor.

2.2. Sampling

Analysis were made on superchilled samples on day 7, 14, 21, 28, 35 and 42 and on ice stored samples on day 1,

8, 15, 22 and 37. In addition, frozen samples stored at −21 or −40 °C were analyzed on day 36 and 43, respectively. On each of the sampling days a group of six samples were analyzed. At each sampling time, samples for the first storage group were randomly drawn. For the second group, samples from corresponding left-right fillets of same individual were selected when possible.

2.3. Microbiological analyses

The vacuum packed bag was opened and samples were collected as described by NMKL method No. 91 (2002). A 1–2 mm thick piece of surface was aseptically cut using a 10 cm^2 template and transferred to a Stomacher filter bag. Saline peptone diluent (0.85% w/v NaCl, 0.10% w/v peptone, 9 ml) was added and the sample was homogenized for 2×30 at 230 rpm. The homogenate was defined as dilution 10^{-1}. Dilution series of the homogenate were made in test tubes with saline. The same samples were used both for total plate count and iron agar count.

Total plate count analysis was performed according to NMKL method No. 86 (1999). One milliliter of three dilutions was transferred in triplicate on Petri dishes containing 15 ml commercial plate count agar (Plate Count Agar, Difco). Colonies were counted after 72 ± 3 h incubation at 25 °C. Mean values of colony forming units (CFU) were calculated as the average of two dilutions.

Iron agar counts were determined by the method of Gram, Trolle, and Huss (1987). One milliliter of three dilutions and 15 ml of commercial iron agar (Lyngby) (Oxoid) with 4% w/v separately added sterile filtered L-cysteine (Sigma) were transferred in triplicate to sterile Petri dishes. Five milliliters iron agar was added on top of the plates of the first agar gel. Black and white colonies were counted after 72 ± 3 h incubation at 20 °C. Mean values of CFU were calculated as average of two dilutions.

2.4. Biochemical analyses

For quantification of the drip loss, the sample was removed and the liquid left in the bag was dried off using preweighed filter paper (Schleicher & Schuell). Mean values were calculated from six replicates.

White muscle for the rest of the analyses was cut from an identical location of each sample. The fish meat was minced in a small food mill and kept on ice.

The pH was determined in approximately 2 g of minced sample mixed with an equal amount of 0.15 M KCl. The analysis was run in duplicate. Mean values were calculated from six 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 230*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 ~2 g of sample for 5 min. The analyses were run in duplicate.

Water content in the mince was determined by drying minced sample of ~2 g at 105 °C for 24 h. The analyses were run in duplicate. Mean values were calculated from six replicates.

Protein extraction was performed in two steps resulting in a water soluble and a salt soluble fraction by a modification (Hultmann & Rustad, 2002) 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, pH 7.0) at 4 °C using an Ultra Thurrax and centrifuged (20 min, 10,400*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 rehomogenized in 80 ml of buffer 2 (50 mM KH_2PO_4, 0.6 M KCl, pH 7.0) and recentrifuged. 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. Protein content in the extracts was determined in triplicate in suitable dilutions of both fractions by the method of Bradford (1976) with bovine serum albumin (BSA) as a standard. Mean values were calculated from six replicates.

Amount of free amino acids was determined in the water soluble protein extracts. Proteins were precipitated by mixing 4 ml of extract with 1 ml sulphosalisylic acid (10%) and leaving overnight at 4 °C (Osnes & Mohr, 1985). The samples were centrifuged (10 min, 600*g*, 4 °C), the supernatant were diluted 1:10 with doubly distilled water, sterile filtered and frozen (−20 °C) until analyzed. Reverse phase HPLC by precolumn fluorescence derivatization with *o*-phtaldialdehyde (SIL-9A Auto Injector, LC-9A Liquid Chromatograph, RF-530 Fluorescence HPLC Monitor, all parts from Shimadzu Corporation, Japan) was performed using a NovaPak C18 cartridge (Waters, Milford, MA, USA), by the method of Lindroth and Mopper (1979) as modified by Flynn (1988). Glycine/ arginine and methionine/ tryptophan were determined together, as their peaks merged. The analysis was performed once on each sample. Total amount of free amino acids were calculated as percent of wet weight. Mean values for each time and temperature group were calculated from six replicates.

2.5. Statistics

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

3. Results and discussion

3.1. Total plate count and iron agar count

The total plate count numbers on ice chilled references increased from 5.4 log units CFU/ cm^2 of surface on day

1 to 7.5 and 7.7 log units after 22 and 37 days of storage, respectively (Fig. 1a). Values above 5.7 log units are considered as high and 6.7 log units represents a limit of what is recommended for consumption, although total plate count number alone cannot be used as an absolute limit (Norwegian Food Safety Authority, 2002). Superchilled samples generally had lower plate count numbers (5.2–5.8 log units of CFU/cm^2) during the 6-week storage. Similar total plate counts are found after 1 week of storage (6.7 log units CFU/g) of ice chilled farmed cod, but much higher after 2 weeks (9.3 log units CFU/g) (Olsson, Seppola, & Olsen, 2007).

After 2 weeks, the total number of colonies on iron agar is generally much higher in ice chilled samples compared to superchilled. A maximum value of 7.6 log units CFU/g was reached after 5 weeks of ice chilled storage (Fig. 1b). Chang, Chang, Shiau, and Pan (1998) found that, with a safety limit of 6.5 log units CFU/g aerobic plate counts, the shelf life of sea bass at 5, 0 and −3 °C was 3 days, about 2 weeks and >4 weeks, respectively.

Shewanella putrefaciens is the predominant spoiler of seafood from cold and temperate water (Gram et al., 1987) and the dominating microbial spoilage organism in cod stored at 0 °C (Jorgensen & Huss, 1989). During growth on a medium containing ferricitrate and cysteine, *S. putrefaciens*-like organisms forms H_2S resulting in a black precipitate of FeS. The growth of H_2S producing microorganisms can thus be enumerated by counting black colonies (Gram et al., 1987). In the ice chilled samples, the black counts on iron agar increase during storage and the value is always higher than for the superchilled samples (Fig. 1c). After 7 days of superchilled storage, the fraction of H_2S producing colonies on cod fillet surface growing in iron agar is 17% (Fig. 1d). This fraction then decreases drastically to below 1%. On ice chilled cod fillet surface this fraction remains below 3% during the complete storage period. Both frozen reference groups shows low fractions (<1%) of black colonies. In contrast, others found that in cod fillets, the amount of H_2S-producing bacteria was 7.6 log units CFU/g after 15 days of −1.5 °C storage (Olafsdottir et al., 2006) and that vacuum packed ice chilled cod fillets had 7.7 log units CFU/g H_2S-producing bacteria after 14 days (Dalgaard et al., 1993).

Jorgensen and Huss (1989) found that *S. putrefaciens* was responsible for spoilage of vacuum packed cod fillets and suggested that the low number of bacteria found at rejection, 5–6 log units of CFU/g, was caused by variation

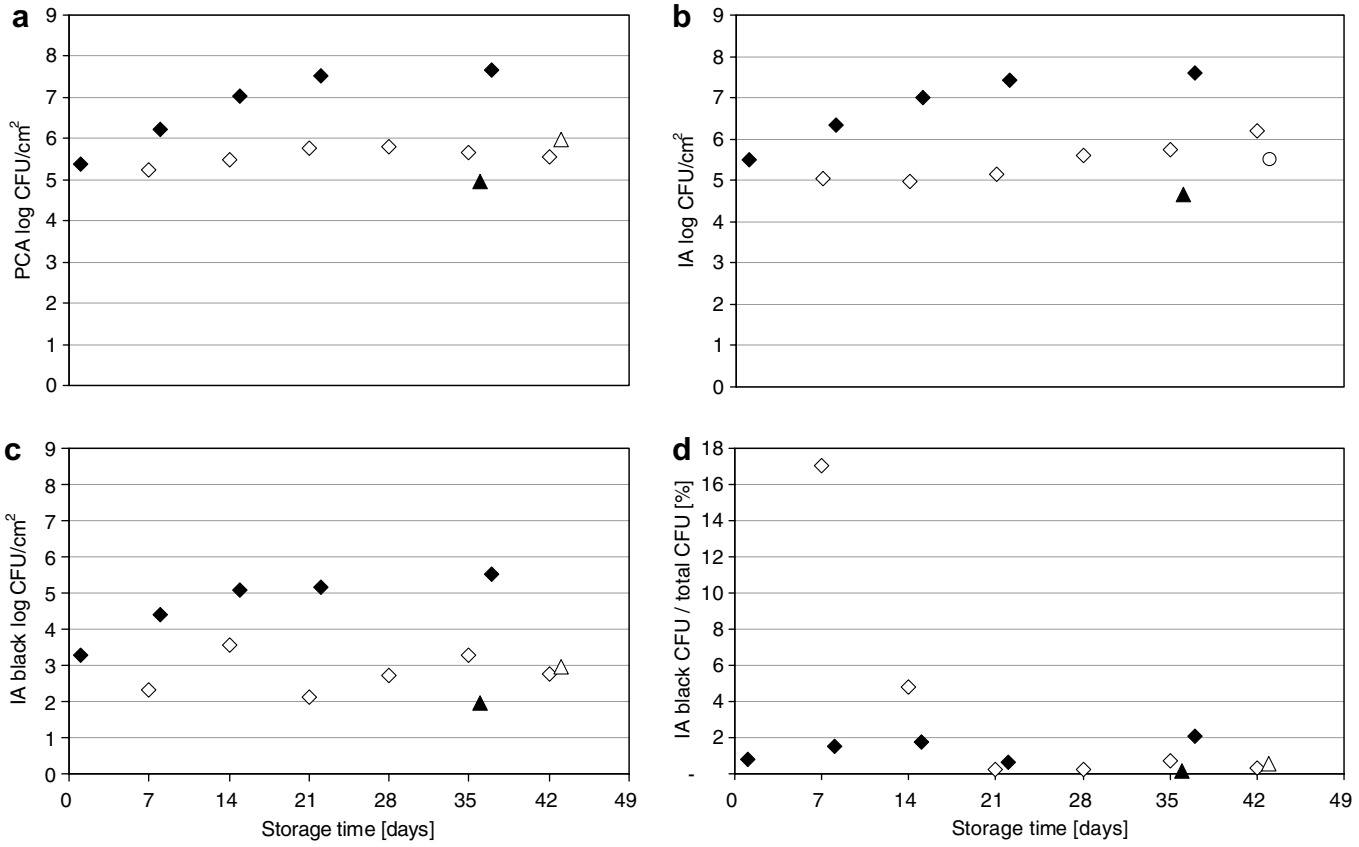

Fig. 1. Development in the total plate counts (PCA) and iron agar (IA) counts on the surface of vacuum packed portions of cod fillet at different storage conditions. (a) Total plate counts after incubation for 72 ± 3 h at 25 °C, and (b)–(d) total counts, black counts and the percentage of black counts on iron agar after incubation for 72 ± 3 h at 20 °C, respectively. Open diamonds indicates superchilled storage at −2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at −21 °C and open triangles indicates frozen storage at −40 °C. All values are means of six samples.

in spoilage potential among different strains of H_2S-producing organisms. The shelf life is dependent on raw material factors such as degree of starvation prior to slaughtering and hygiene of fish handling. Based on our results, the microbial shelf life of vacuum packed iced cod fillets is estimated to be 8 days after processing. The bacterial counts of superchilled fillets did not exceed the limit of consumption for the whole storage period. This disagrees with findings of shelf life of 15 days for superchilled cod based on TVB-N and sensory rejection (Olafsdottir et al., 2006). No bad odor that could cause sensory rejection of our superchilled samples was detected at any point during storage when opening the vacuum bags.

3.2. Drip loss

Drip loss results in reduced value and the drip liquid is also a good growth medium for bacteria. It is therefore of great interest to reduce the drip. The drip loss is higher in ice chilled compared to superchilled cod fillets (Fig. 2). Superchilled cod fillets never lost more than 1.5% of their weight as drip. The drip loss of ice chilled fillets increased with storage time and amounted to more than 5% after 15 days of storage. This is in agreement with Dalgaard et al. (1993), who found the drip loss of ice chilled vacuum packed cod fillets to be of 4.7% at the time of sensory rejection (14 days). The drip loss in fillets frozen at −40 °C was comparable to the superchilled fillets, while those stored at −21 °C lost about 2.7% of their original weight.

Drip loss was less in superchilled than in the ice chilled or the −21 °C frozen samples, but higher than in the −40 °C frozen references. The higher drip loss in the ice chilled samples might be due to increased proteolysis, resulting in a looser structure and increased amount of water in the extracellular room (Olsson, Ofstad, Lodemel, & Olsen, 2003; Olsson et al., 2007). Bacterial enzymes may also contribute to the degradation and thereby affect the ability to retain water (Olsson et al., 2003). Bacterial counts were higher on the surface of ice chilled than on superchilled fillets and could thus account for some of the differences in drip loss values. During superchilling a fraction of the water is frozen and the result seems to be that the water is better retained by the muscle structure as long as no outer force is applied. The reason for the difference between samples frozen at −21 °C compared to −40 °C might be that the degree of freeze denaturation is higher in the former samples resulting in less ability of the tissue to retain the water. In contrast to the present results, Simpson and Haard (1987) found that free drip increased during storage of Atlantic cod at 0 or −3 °C, and the increase was largest for partial frozen samples. Their samples were superchilled at −3 °C which may result in a higher degree of freeze denaturation than at −2 °C.

3.3. Liquid loss

The water holding capacity (WHC), and thus the liquid loss, of muscle is regarded as an essential quality parameter and a high WHC is of great importance both to the industry and the consumer. It influences the appearance of the muscle before cooking, its behavior during cooking and its juiciness when consumed (Olsson, 2003). The liquid loss was significantly higher in ice chilled compared to superchilled cod fillets (Fig. 3). Initially, the liquid loss of cod fillets was 13%. The value declined to 9% after 3 weeks of ice chilled storage, while in superchilled fillets the liquid loss was 30% after 2 weeks and declined to about 21% after 6 weeks. This must be viewed in context with the higher drip loss of the ice chilled fillets. The drip loss represents the most loosely bound water, and samples with a high drip

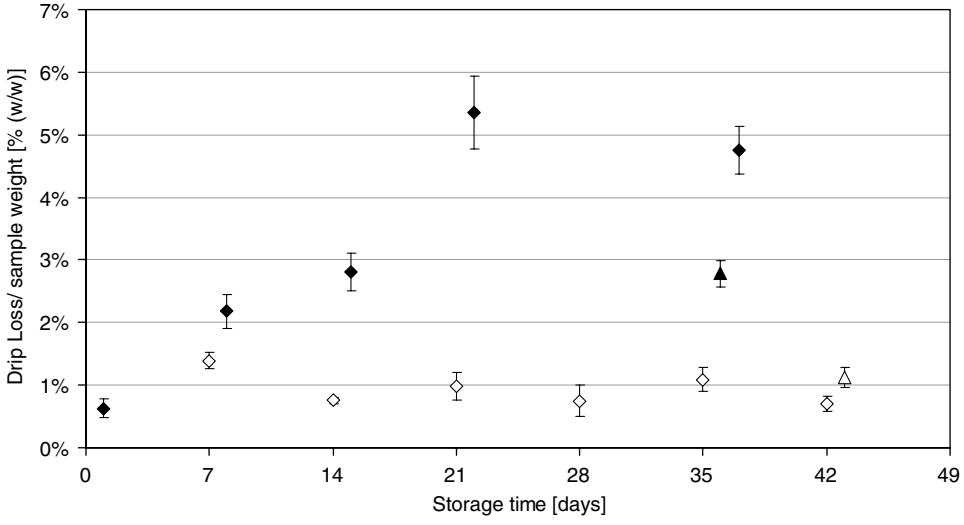

Fig. 2. Development in drip loss from vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at −2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at −21 °C and open triangles indicates frozen storage at −40 °C. Values are given as means of six samples with standard error of the mean as *y*-error bars.

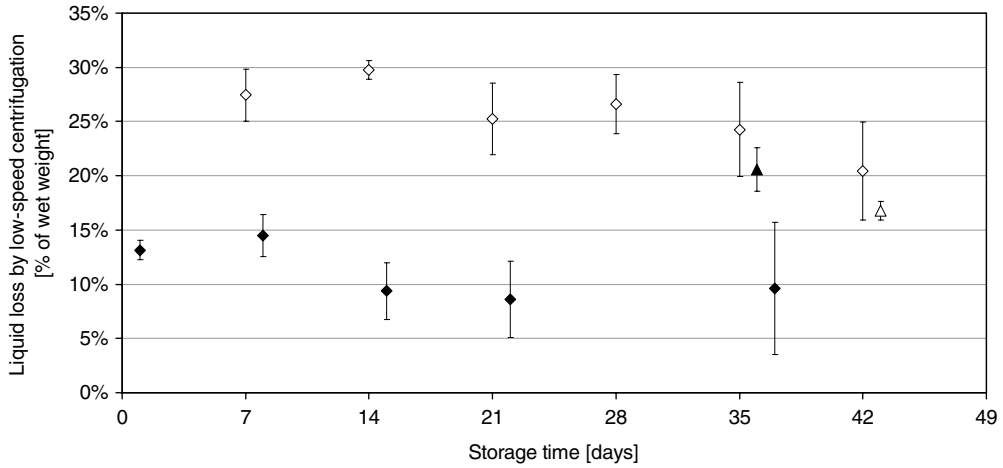

Fig. 3. Development in water holding capacity expressed as liquid loss (LL) of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at −2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at −21 °C and open triangles indicates frozen storage at −40 °C. Values are given as means of six samples with standard error of the mean as *y*-error bars.

loss will therefore be able to hold on to a higher share the remaining water during the centrifugal procedure.

3.4. pH

On day 1, the average pH value in the fish was 6.13. During the first week, pH increased for both storage groups, with ice chilled cod fillets having a slightly higher (i.e. ~0.05) pH value than superchilled (Fig. 4). Thereafter the values decreased somewhat until week 5, after this a slight increase was observed in both groups. Cod fillets frozen at −21 or −40 °C had pH values of 6.25 and 6.16, respectively. The water holding capacity is related to the pH of the muscle (Rustad, 1992), but the minor differences in pH cannot fully explain the large differences in drip loss or LL between the superchilled and chilled fillets.

3.5. Extractable water- and salt soluble proteins

Extracted water soluble proteins were between 3.4% and 3.7% of wet weight for ice chilled reference samples, and between 3.7% and 4.3% in the frozen fillets (Table 1). The amount of water soluble proteins in the superchilled samples was slightly lower and varied from 2.4% to 3.2%.

The amount of extracted salt soluble proteins was significantly lower in the superchilled samples (1.4–2.7%) than in the ice chilled samples (3.6–5.5%). The values for the superchilled samples are only slightly higher than for the frozen

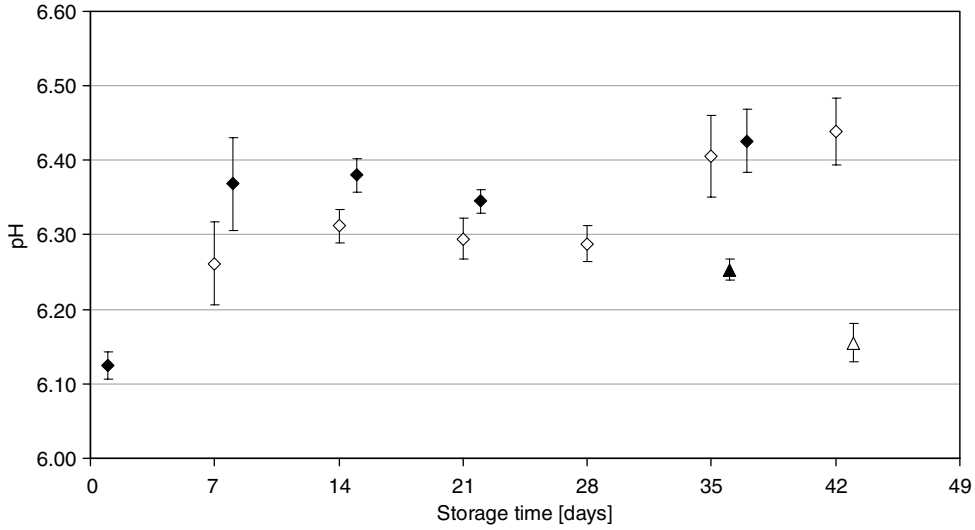

Fig. 4. Development in pH of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at −2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at −21 °C and open triangles indicates frozen storage at −40 °C. Values are given as means of six samples with standard error of the mean as *y*-error bars.

Table 1
Development in water- (WSP) and salt soluble proteins (SSP) extracted subsequently at pH 7.0 and +4 °C without and with 0.6 M KCl from vacuum packed portions of cod fillet during storage time at different storage conditions

Method	Storage time (days)	WSP (% w/w wet weight)	SSP (% w/w wet weight)
Ice chilled	1	3.5 ± 0.1	3.9 ± 0.2
	8	3.4 ± 0.1	3.8 ± 0.6
	15	3.5 ± 0.1	3.6 ± 0.6
	22	3.4 ± 0.2	4.4 ± 0.9
	37	3.7 ± 0.1	5.5 ± 0.8
Superchilled	7	2.4 ± 0.3	1.9 ± 0.2
	14	3.2 ± 0.2	1.4 ± 0.2
	21	3.1 ± 0.1	1.7 ± 0.3
	28	2.9 ± 0.2	1.7 ± 0.4
	35	2.6 ± 0.3	1.4 ± 0.4
	42	3.0 ± 0.4	2.7 ± 0.4
Frozen −21 °C	36	3.7 ± 0.1	1.1 ± 0.2
Frozen −40 °C	43	4.3 ± 0.2	2.2 ± 0.1

Values are given as means of six samples with standard error; $N = 6$.

samples (1.1% and 2.2%). In the ice chilled fillets, the extractability, especially for the salt soluble fraction, seems to increase after 3 weeks. This is not the case for the superchilled samples, where the values remain low for the whole storage period. The low extractability of muscle proteins from cod tissue stored at −2.2 °C indicates freeze denaturation of the proteins. In muscle, freeze denaturation result in lower extractability, especially for the myofibrillar proteins while the sarcoplasmic proteins are less affected (Mackie, 1993). Since the myofibrillar network retains most of the water in the muscle, the properties of the salt soluble proteins are important for the WHC. The high liquid loss in the superchilled fillets can therefore be explained by protein denaturation in the superchilled cod fillets. In order to maintain good quality of the cod fillets during superchilling, the process has to be optimized with regard to freezing rate and degree of superchilling to minimize protein denaturation and LL. At the same time, the temperature has to be sufficiently low to ensure an increase in microbial shelf life.

3.6. Free amino acids

Changes in the amount of free amino acid can be used to observe the effect of proteolytic activity, since enzymatic assays are difficult to carry out at subzero temperatures. Both ice chilled and superchilled cod samples had higher levels of free amino acid compared to frozen samples after about 5 weeks (Fig. 5). The total level of free amino acids is initially 3–4 mg/g of wet weight. After 5–6 weeks of superchilling, it had increased to around 10 mg/g of wet weight, while for ice chilled samples the value was about 8 after 36 days. This agrees with the findings of Sotelo, Franco, Aubourg, and Gallardo (1995), who state that the content of free amino acids in hake increases with storage time at −5 °C, but not at −20 °C.

3.7. Spots

White spots were detected on surface of the fillets after about 1 week of superchilled storage. These spots were not present until the temperature was equalized in the fillets and were not of microbial origin. The latter is supported by the plate count numbers being no higher on superchilled samples and the fact that the spots were not of colony-like appearance as seen in light microscope as well as with the

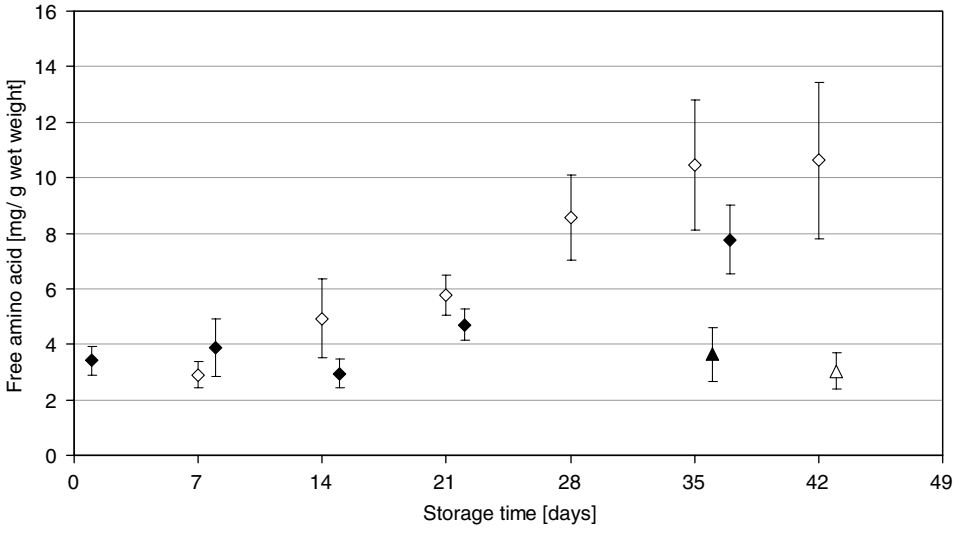

Fig. 5. Development in free amino acid content of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at −2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at −21 °C and open triangles indicates frozen storage at −40 °C. Values are given as means of six samples with standard error of the mean as *y*-error bars.

naked eye. Amino acid leakage is unlikely, since preliminary tests (results not shown) confirm no difference in amino acid amount or composition on surface and non-surface muscle tissue. We would rather suggest that this effect could be due to diffusional effects through drip channels from inside of muscle to muscle surface. It has been shown that such channels are formed in beef when the cell membrane lifts off from the cell body, while the attachment of the cell membrane to the cellular matrix remains unaffected (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). Transmembrane protein integrin degradation is also found to coincide with opening of drip channels (Lawson, 2004). Effort should be put into reducing this spot effect, because of the negative effect on the appearance of the fillets.

4. Conclusion

Superchilling extended the microbial shelf life of vacuum packed cod fillets by several weeks compared to traditional ice chilling. The total number of bacteria and the fraction of H_2S producers were lower in the superchilled cod fillets compared to the ice chilled.

Drip loss was lower in the superchilled samples while the LL was higher compared to the ice chilled samples. The high LL was due to freeze denaturation, which also was observed by the fact that the amount of extracted salt soluble proteins was significantly lower in the superchilled samples (1.4–2.7%) than in the ice chilled samples (3.6–5.5%).

In order to maintain good quality of the cod fillets during superchilling, the process has to be optimized with regard to freezing rate and degree of superchilling to minimize protein denaturation and LL. At the same time, the temperature has to be sufficiently low to ensure an increase in microbial shelf life. In addition, effort should be put into reducing formation of surface spots.

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