

Acknowledgements

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Summary

Superchilling is a method that can be used to prolong the shelf life of foods by partial freezing. Knowledge of the effects of this method on both the shelf life and quality of foods is important in order to find optimal processing and storage conditions and is of great importance both for the industry and for the consumers. Different raw materials of muscle foods were studied with the purpose of creating a basis for further improvements of both the process and the storage conditions. Products from the commercially important species pork (both roasts and rib steaks), Atlantic salmon (both vacuum packed and wrapped fillets) and Atlantic cod (vacuum packed fillets) were chosen to represent different muscle foods.

Based on both sensory and microbial evaluations, the superchilled storage improved shelf life of pork roasts from 2 to 16 weeks, and shelf life might even be further prolonged if temperature is kept more constant. The H₂S-producing bacteria in superchilled cod fillets did not exceed the limit of consumption during the whole storage period of six weeks, while the microbial shelf life of the ice chilled fillets was estimated to be 8 days after processing.

Quality changes have been studied with focus on biochemical and physiochemical properties. One of the major goals in meat and fish processing is the ability to retain water and it was observed that drip loss was lower in superchilled samples compared to traditionally chilled samples in all species studied. However, the subsequent liquid loss was higher both in pork roasts and in cod fillets.

The extractability of protein was used as a tool to monitor protein denaturation. It was found that myofibrillar proteins denatured more easily during superchilled than during traditionally chilled storage both in salmon and in cod fillets. This was confirmed by electrophoresis in salmon. The amount of free amino acids were higher in cod than in salmon and increased more during superchilled storage than during ice chilled storage indicating exoproteolytic activity during storage. Activities of cathepsins B and B + L in salmon fillets were relatively stable during the storage period in all storage groups,

demonstrating that these enzymes are not deactivated at the selected storage temperatures and may therefore lead to softening during subsequent chilled storage.

In superchilled samples of all species studied, white spots emerged on the product surface. However, the appearance of spots did not correspond either to higher drip loss or to higher microbial growth on surface of these samples. On the contrary, the total plate counts of superchilled samples were lower than of the other storage groups. This observation also applied to iron agar counts on cod fillets. These findings are interpreted as a strong indication of that the spots most likely were not of microbial origin.

The optimal superchilling process and storage conditions remains to be found for the products studied. From the present results it is reasonable to suggest that optimal storage temperature for the vacuum packed salmon fillets is found between the superchilled temperatures in the present experiments. The quality both of the pork roasts and the cod fillets would probably benefit from a storage temperature slightly closer to 0 °C than those studied. It can also be assumed that the control of the superchilling process is more essential to cod than to salmon and pork. In addition, the properties of the raw material are crucial in order to obtain high quality of products after processing and storage.

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List of appendix papers

This thesis is based on a series of papers, which are referred to in the text by their Roman numerals.

- Paper I Duun, A. S., Hemmingsen, A. K. T., Haugland, A. and Rustad, T. (2008). "Quality changes during superchilled storage of pork roast." LWT - Food Science and Technology. *In press, corrected proof*.
- Paper II Duun, A. S. and Rustad, T. (2008). "Quality of superchilled vacuum packed salmon (*Salmo salar*) fillets stored at -1.4 and -3.6 °C." Food Chemistry, 106(1), 122-131.
- Paper III Duun, A. S. and Rustad, T. (2007). "Quality changes during superchilled storage of cod (*Gadus morhua*) fillets." Food Chemistry, 105(3), 1067-1075.
- Paper IV Duun, A. S. and Rustad T. (2008). "Quality of superchilled salmon (*Salmo salar*) fillets and rib steak (*M. psoas major*) of pork stored at two temperature levels." *Manuscript in preparation*.

1 Introduction

1.1 Muscle foods

1.1.1 GENERAL ASPECTS

Muscle from animals used for food is a major component of the human diet (Eskin, 1990; Mackie, 1993). Conventional sources of muscle food include cattle, pig, sheep, poultry and fish. Although all skeletal muscle is composed of the same main constituents, some of their properties are fundamentally different. Worldwide, fish muscle used as food is obtained from a variety of species. In Norway the main fish species used as food are cod, herring, saithe, mackerel, salmon and trout (Statistics Norway, 2008).

Skeletal muscle is part of the motional system of animals. However, based on diversity in muscle function, there are differences both among and within species. Some of the greatest differences are found between fish and meat. As stated by Foegeding, Lanier and Hultin (1996), these variations are due to three basic factors. Firstly, extensive strong connective tissues to maintain and support the muscles are not required in fish because the fish body is supported by water. Secondly, the proteins of fish muscles have different properties from those of warm-blooded species since most commercially important fish are poikilothermic animals and live in a cold environment. Finally, the structural arrangement of fish muscle is markedly different from that of terrestrial animals (Foegeding et al., 1996). This arrangement (Figure 1) is related to the characteristic movement of fish. An additional difference between meat and fish is the variation in properties of fish muscle with age and season (Archer, Evans, Jessen, Nielsen & James, 1998). Main constituents of skeletal muscle are water, protein, fat, carbohydrate and other soluble compounds. The chemical composition of muscle can vary greatly between species (Haard, 1992a; Toldra, 2003). Examples of the variation in composition of lean muscle between species are given in Table 1. Yet, there are many structural similarities between pork and fish muscle. The major structural proteins on the myofibrillar level (actin, myosin, troponin and tropomyosin) are arranged in a similar way (Venugopal & Shahidi, 1996).

Table 1: Proximate composition of edible muscle tissue from different species.

Species or specific product	Chemical composition (%)				Reference
	Water	Protein	Lipid	Ash	
Beef	70-77	20-22	4-8	1	Watt and Merrill 1963; Kolczak, Krzysztoforski et al. 2007
Beef rib steak	74	21.1	3.7		NFSA 2008
Pork	50-79	13.6-22.2	1.8-37	1.4	Watt and Merrill 1963; NFSA 2008
Pork rib steak	75	20.7	3.6		NFSA 2008
Pork roast	75	21.1	3.1		NFSA 2008
Chicken	67-75	18.6-23.8	1-14	1	Watt and Merrill 1963; NFSA 2008
Chicken breast	75	23.8	1.0		NFSA 2008
Lamb	59-75	16.8-21.3	2.1-24	1.6	Watt and Merrill 1963; NFSA 2008
Lamb roast	70	19.3	9.3		NFSA 2008
Fish	46-85	14-24	0.3-25		Watt and Merrill 1963; NFSA 2008
Atlantic cod	76-83	17.6-19.4	0.3	1.2	Watt and Merrill 1963; Venugopal and Shahidi 1996; NFSA 2008
Atlantic salmon	56.6-74.6	15.4-21.8	3.3-21.4	1.0-5.3	Watt and Merrill 1963; NFSA 2008; NIFES 2008

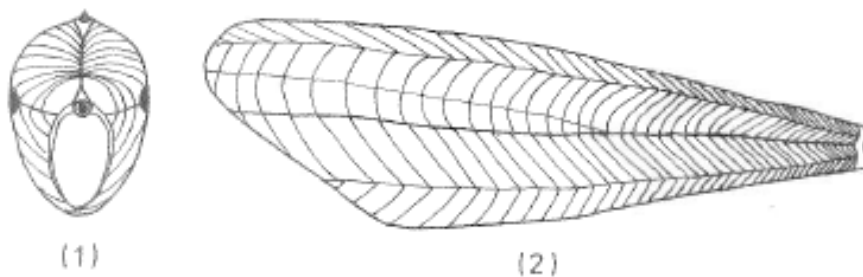


Figure 1: The metameric structure of fish muscle. The pattern of the lines on the cross (1) and longitudinal (2) sections represents the arrangement of the sheets of connective tissue in the muscles (Dunajski, 1980).

The texture of meat improves post mortem, as it becomes more tender. Fish is naturally tender and becomes even softer during storage. Contrary to mammalian muscle, where weakening of the muscle structure leads to a desirable flavor and texture in the product, fish tend to deteriorate rapidly post mortem (Archer et al., 1998; Cheret, Delbarre-Ladrat, de Lamballerie-Anton & Verrez-Bagnis, 2007; Shahidi & Botta, 1994). Therefore preservation of fresh quality is even more important in fish than in meat.

About one tenth of the mammalian muscle protein is collagen, which is the main component of the connective tissues. The connective tissue contributes significantly to texture in mammalian muscle (Foegeding et al., 1996). In contrast, the amount of collagen in fish muscle is about one tenth of that in mammalian muscle (Eskin, 1990). Moreover, the collagen of fish is less tough, less cross-linked, resulting in a lower melting temperature than that of meat (Sikorski, Scott & Buisson, 1984).

During the first few hours after death of an animal, the pH of the skeletal muscles decline from the physiological pH. This is due to the biochemical basis of muscle function. In live animals, contraction and relaxation of striated muscle occurs by the sliding action of the thin actin filaments and the thick myosin filaments with the length of the filaments remaining the same (Chen & Brenner, 1993; Eskin, 1990). When muscle stimulation occurs, the myosin head interacts with actin resulting in a contraction forming actomyosin and using one molecule of ATP. Subsequently the actin releases itself from the myosin and the muscle returns to its resting state. When the animal dies, the enzyme creatine phosphatase resynthesizes ATP from ADP and creatine phosphate (CP). Thereby the level of ATP is maintained until CP depletion (Eskin, 1990). The major source of ATP supply to the muscle fiber is thus lost and as a consequence actin can not be released from myosin and the muscle cell is in the state of rigor mortis. The development of rigor mortis in animals or fish is therefore the direct response to the decline of ATP. Glycogen can no longer be oxidized to carbon dioxide and water and is then anaerobically converted to lactic acid. The production of lactic acid is usually considered to cause the pH of the muscle to drop during the initial hours post mortem (Etherington, 1984; Scheffler et al., 2007). In warm-blooded animals the pH drops from the physiological pH of 7.2-7.4 to the ultimate post mortem pH of around 5.3-5.5 (Eskin, 1990; Etherington, 1984). The pH of fish muscle is lower than of meat, e.g. typically 6.7-6.9 in wild cod (Hultmann et al., 2002; Kristoffersen et al., 2007; Ofstad et al., 1996a; Rustad, 1992) and 6.1-6.4 in salmon (Ofstad et al., 1996a; Sigholt et al., 1997). In cod, it is shown that pH is 0.5 to 0.9 pH units lower in farmed cod than in wild cod (Rustad, 1992). The process of lactic acid causing the fall in pH has been termed lactic acidosis. However, whether the lactate is a consequence rather than the cause of cellular events leading to acidosis has recently become a subject of debate (Robergs, Ghiasvand & Parker, 2004).

1.1.2 QUALITY ASPECTS

Shelf life can be defined as the period of time during which a perishable product under a given appropriate set of conditions can be stored or displayed until it is considered as unsuitable for sale or consumption (Barbosa, Bremner & Vaz-Pires, 2002). Shelf life is highly variable among different types of food products and several quality and safety parameters determine its values. These parameters can be divided into the categories of microbiological, chemical and sensory properties.

Fresh meat and fish held at refrigeration temperature have a limited shelf life, primarily because of microbial and enzymatic spoilage (Ashie, Smith & Simpson, 1996; Foegeding et al., 1996). It is well documented that Gram-negative psychrophilic or psychrotrophic organisms are important in spoilage of fish. A large number of bacteria (10^7 - 10^8 CFU/g) are normally found on spoiling fish, but only part of this flora may be classified as active spoilers (Gram, Trolle & Huss, 1987). Two of the most prominent characteristics of fish spoilage bacteria are regarded as the ability to reduce trimethylamine oxide (TMAO) into dimethylamine (DMA) and formaldehyde or to produce hydrogen sulphide (H_2S) (Gram et al., 1987). In cod, *Shewanella putrefaciens* (Gram et al., 1987; Jorgensen & Huss, 1989) and *Photobacterium phosphoreum* (Dalgaard, 1995) have been identified as the main spoilage bacteria. In cold smoked salmon, lactic acid bacteria are found to produce off-odors causing sensory rejection. However, in lightly preserved fish spoilage is in general likely to be caused by *Enterobacteriaceae*, *Brochothrix thermosphacta*, yeasts and *Photobacterium phosphoreum* (Gram & Huss, 1996).

On the other hand, the aerobic spoilage flora of fresh meat stored at chill temperatures is usually dominated by the rapidly growing *Pseudomonas spp.* However, other slower growing organisms such as lactic acid bacteria, psychrotrophic *Enterobacteriaceae* and *Brochothrix thermosphacta* and spore-forming bacteria, such as *Clostridium spp.*, in addition to yeasts and moulds may be present (Gill, 1996; Huis in't Veld, 1996).

Although microbial activity is the most essential parameter determining shelf life, enzymatic activity is also important. The loss of freshness is caused by both chemically and enzymatically controlled autolytic processes (Lindsay, 1991). Products of autolysis are then used as substrates for bacteria leading to the unpleasant flavors and eventually spoilage (Gram

et al., 1987). Even in lean fish and meat lipid oxidation (both chemical and enzymatic) may lead to rancidity. Interaction of lipid oxidation products with protein chains may result in cross-linking of protein and toughening as well as discoloration of muscle tissues (Mackie, 1993; Shahidi et al., 1994). Post mortem biochemical changes in proteins and non-protein nitrogenous compounds are deteriorating factors in seafoods (Sotelo, Pineiro & Perezmartin, 1995b; Van Waarde, 1988; Venugopal et al., 1996), and rapid loss of fresh quality may also be due to a variety of changes caused by hydrolysis, polymerization, deamination, decarboxylation or oxidative processes (Shahidi et al., 1994). Moreover, the activity of proteolytic enzymes in fish muscle is higher than in meat (Cheret et al., 2007). In meat, however, protein degrading enzymes contributes to maturation of the product. Proteinases (endoproteases) of muscle can be divided between the nonlysosomal enzymes which act preferentially near neutral pH, and the cathepsins of the muscle lysosomes, which are the most active under mildly acidic conditions (Etherington, 1984).

In addition to retardation of microbiological and enzymatical spoilage, the minimizing of exudate losses is of commercial concern. Drip loss (also called free moisture, purge or exudate) is a collective term which denotes the liquid lost during processing, storage and/or thawing (Cheng & Sun, 2008; Huff-Lonergan & Lonergan, 2007; Jeremiah, Gibson & Argnosa, 1995). Such losses influence the profitability of fresh products by affecting processing yields and palatability. Moreover, processed products often have poorer consumer acceptance because of the unappealing appearance of the product when excessive liquid is present in the package (Huff-Lonergan et al., 2007). Drip loss contains both water and substances leaking from cells as these are ruptured during processes such as cooling, storage and thawing (Kristoffersen, Vang, Larsen & Olsen, 2007; Offer & Knight, 1988b; Savage, Warriss & Jolley, 1990), and has a higher concentration of sarcoplasmic proteins than the muscle itself (Offer & Cousins, 1992). The ability of meat to retain both inherent water and added water is a main goal in meat processing and is defined as water-holding capacity (WHC) (Cheng et al., 2008; Huff-Lonergan & Lonergan, 2005; Offer & Knight, 1988a) or liquid-holding capacity (Nielsen, Hyldig, Nielsen & Nielsen, 2005; Ofstad, Kidman, Myklebust & Hermansson, 1993).

Development of pork quality attributes is mainly determined by the rate and degree of postmortem pH decline (Figure 2). The combination of high temperature at low pH or abnormally low ultimate pH causes denaturation of sarcoplasmic and myofibrillar proteins,

resulting in paler color and reduced water-holding capacity (Scheffler & Gerrard, 2007). Pork meat is prone to two major post-slaughter defect carcass conditions described as dark, firm, dry (DFD) and pale, soft, exudative (PSE) meat. These are the extreme cases on each side of normal good quality meat regarding color, texture and water-holding capacity. DFD pork has a dark, unattractive appearance and a firm, dry, and sticky texture due to enhanced water-holding capacity. In contrast, PSE pork is characterized by pale color, soft texture, and low water-holding capacity, and has limited functionality in further processing. PSE pork represents considerable economic losses for the industry due to its limited functionality and undesirable appearance. Despite exhaustive research efforts, there is still a relatively high occurrence of PSE pork (Scheffler et al., 2007).

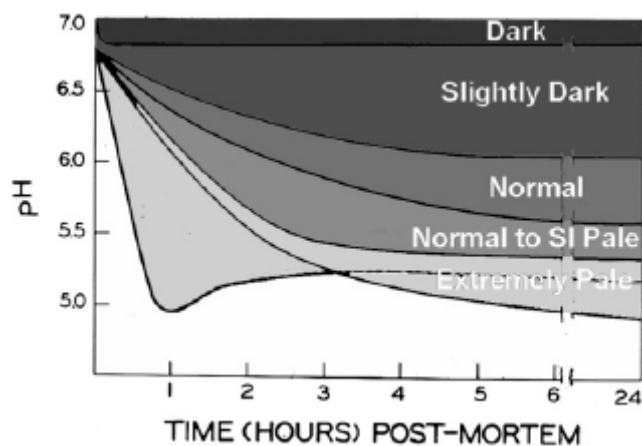


Figure 2: Various pH declines occurring postmortem and associated pork quality characteristics (Briskey, 1964) as modified by (Scheffler et al., 2007).

As in meat, high WHC of fish muscle is of great importance both to the industry and to the consumers. WHC is closely related to textural properties, and low WHC has often been described as an effect of post mortem structural changes in the muscle. Such alterations could be shrinkage of the myofilament lattice, myosin denaturation and increased extracellular space (Olsson, Ofstad, Lødemel & Olsen, 2003). WHC as expressed by liquid loss (LL) is generally twice as high in cod than in salmon, and muscle proteins in cod are less stable than those in salmon (Ofstad et al., 1993). Other factors affecting post mortem quality of fish muscle include feeding regime, pre-slaughter handling and the degree of pre-slaughter stress, temperature and processing conditions (Kiessling, Espe, Ruohonen & Mørkøre, 2004; Kristoffersen et al., 2006; Sigholt, Erikson, Rustad, Johansen, Nordtvedt & Seland, 1997; Sigurgisladdottir, Sigurdardottir, Ingvarsdottir, Torrissen & Hafsteinsson, 2001).

Although the most important extrinsic parameter in food preservation is low temperature, the chilling post mortem can be too rapid since the temperature and the pH of the muscle at the onset of rigor mortis are decisive factors of the degree of cold shortening (Savell, Mueller & Baird, 2005). More rigid cell membranes at lower temperatures cause Ca^{2+} to accumulate in the cell, resulting in ATP exhaustion and strong rigor contractions referred to as cold shortening. The resulting muscle texture is tough. This effect is of concern for many species, such as beef, mutton and poultry. To prevent cold shortening, the onset of rigor can be accelerated by electrical stimulation in such a way that ATP is consumed and pH declines while the muscle is still warm. However, too slow chilling is unfortunate since it facilitates microbial growth.

1.2 Need for longer shelf life

The global production from capture fisheries and aquaculture supplied about 106 million tons of food fish in 2004, providing an apparent per capita supply of 16.6 kg (live weight equivalent) (FAO, 2006). In 2004, the global meat production was 260 million tons (FAO, 2007), which corresponds to a supply of 40.7 kg per capita. In Norway, the average total annual consumptions of meat and seafood in 2004-2006 were 47.1 and 16.1 kg per capita, respectively (Statistics Norway, 2007). Table 2 shows that among unprocessed meat, consumption of pork was the highest, representing 7.8 kg per capita per year. Unprocessed fish represents about the same amount of consumption (6.4 kg per capita per year) as for poultry (6.3 kg per capita per year). Regarding both quality and demand, fresh or chilled foods represent higher profit potentials than frozen food. A high share of fresh or chilled food of consumption is thus desirable both for consumers and for industry.

Norway seafood export represents values of more than 3600 million USD, which after China (5200) and Thailand (3900) is the third highest value on world basis (NSEC, 2005). Norway is the world's leading producer of Atlantic salmon and nearly half of the country's seafood export value (47% in 2005) is related to the export of Atlantic salmon. Fish farming provides an opportunity to obtain a seasonally independent supply of fresh fish to the market, and the demand for fresh salmon is rapidly increasing at the expense of frozen (NSEC, 2005).

Table 2: Quantity consumption of meat and fish in Norway, by commodity group in 2004-2006 (SSB, 2007). Numbers represents kilograms per person per year.

Commodity group	Quantity
Dried, salted or smoked meat and edible meat offal	13.1
Other preserved or processed meat and meat preparation	11.7
Fresh, chilled or frozen meat of swine	7.8
Fresh, chilled or frozen meat of poultry	6.3
Fresh, chilled or frozen meat of bovine animals	3.6
Fresh, chilled or frozen meat of sheep and goat	2.9
Other fresh, chilled or frozen edible meat	1.8
Meat total	47.2
Fresh, chilled or frozen fish	6.4
Fresh, chilled or frozen seafood	2.1
Dried, smoked or salted fish and seafood	1.4
Other preserved or processed fish and seafood and fish and seafood preparations	6.2
Fish total	16.1

Once established, there are several advantages of fish farming compared to traditional capture fisheries. Farmed fish is available regardless of season, which implies a steady supply, quality and price of the fish to the market. To retain these advantages, it is important to be aware of any risks of quality deterioration in all elements of the distribution chain. Norwegian salmon is already being exported to long distance markets. Shelf life extension of the salmon products would further increase the distribution and profit potentials.

Cod farming is currently an industry on the rise. In addition, cod has traditionally been sold as whole fish, while the tendency now is toward fillets and prepacked dishes. The demand for fresh cod fillets is rapidly increasing at the cost of frozen fillets (NSEC, 2005). Since the shelf life of fillets is shorter 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.

Pork is the world's most widely eaten meat, and production of pork in Norway is high. However, the production is not always in balance with the demand. Thus, the industry would benefit substantially from improvement of preservation methods as increased shelf life permits a higher degree of flexibility in supplying the market. Methods that minimize meat quality loss would enhance the consumer contentment and thus the industrial profitability.

With the large increase in demand for fresh food, focus is on shelf life and food safety. The commercial trend is a shift from frozen storage with a shelf life of months or years to fresh products with a shelf life of days or weeks, and has substantially changed the demands for chilling and the cold chain. A long shelf life is required in order to achieve sufficient time for distribution and sale. Improving shelf life is particularly important for highly perishable food such as muscle foods. Hence, a huge effort is put into improving shelf life by a variety of techniques, such as different gas mixtures (Ashie et al., 1996; Farber, 1991; Sivertsvik, Jeksrud & Rosnes, 2002), new additives (Boskou & Debevere, 2000; No, Meyers, Prinyawiwatkul & Xu, 2007) or new temperature regimes. However, the most important factor for improving shelf life is the product temperature from catch or slaughter to consumer (James, Vincent, de Andrade Lima & James, 2006; James, 1996; Ronsivalli & Baker, 1981). This increases the importance of a well functioning cold chain. In this perspective, superchilling can be a valuable tool.

1.3 Cold chain

Process and storage conditions exert a strong effect on the quality and safety of food products. The cold chain of food can be defined as the control and maintenance of satisfactory low temperature through the supply chain from production to preparation. The cold chain commences with the initial chilling of the freshly caught and slaughtered fish or slaughtered carcass and continues through processing, packaging, distribution, retail display, transport, and storage and preferably lasts until preparation or cooking (Gould, 1996; James, 1996; Ronsivalli et al., 1981). An unbroken cold chain is where the food is processed, stored and transported at a cold storage temperature within a minimum of fluctuation (Hemmingsen, 2002).

The cold chain can be divided into two main parts; i.e. chilling process and storage conditions. Through the initial post-production procedures, the aim is to reduce the average temperature of the food. In order to achieve a sufficient decrease in product temperature, heat has to be removed primarily by the use of equipment and facilities designed for this purpose. From this point on, maintaining the low temperature of the food or food product is the prime aim. In order to provide safe food products of high quality, attention must be paid to every aspect of the cold chain. The critical stages of the cold chain are during distribution, in the retail part and in domestic refrigerators (Hemmingsen, 2002; Likar & Jevsnik, 2006). One or more defects in this chain of processes may result in excessive weight loss, higher energy use,

reduced shelf life or other deteriorations of product quality (James, 1996). The appropriate temperature regime of the cold chain is dependent of the product being discussed and the purpose and duration of the storage.

The most commonly used technique for preservation of meat is refrigeration. At low temperatures growth of microorganisms is retarded and many chemical and enzymatic reactions are slower (Foegeding et al., 1996). The preferred system for chilling and storage of fish at temperatures down to 0 °C has traditionally been by means of adding ice (flake ice) made from fresh water (Ronsivalli et al., 1981). The ice melts as it chills the fish and the combination of ice and water is a more effective heat remover than ice alone. Ice has to be refilled as it melts. Ice and water can also be added together to achieve better contact between the fish and the cooling media. In addition, systems involving refrigerated seawater (RSW) or chilled seawater (CSW) can be used for chilling fish. In contrast to RSW, CSW usually involves mechanical refrigeration, and thus temperatures lower than 0°C can be obtained (Ronsivalli et al., 1981).

While refrigeration is used for short periods of time, freezing is a process for preserving the quality of food for long periods. Although, some microorganisms survive storage at very low temperatures, there is generally no opportunity for growth of microorganisms if recommended storage temperatures are maintained. The retention or loss of quality during freeze preservation is, however, dependent on the intrinsic characteristics of the fish or the meat and on how the freezing process is conducted (Foegeding et al., 1996).

Frozen food is to be stored at maximum –18 °C (NMFCA, 1996) (less than 0 °F) and storage of chilled food is supposed to be carried out in the region between -1 and 4 °C. The latter also concerns packaged fresh fish and fish products, while fresh fish should be stored in ice (NMFCA, 1996). Fresh food in this context means unprocessed food without additives. Food that is or has been frozen is not considered as fresh food, and should therefore not be sold as fresh food. Superchilling (further defined in section 1.5) implies temperatures in the borderline between chilling and freezing.

Distribution is one of the most critical points of the cold chain and the refrigeration systems used in most transport containers are not designed to extract heat from the load but to maintain the temperature of the cargo. Hence, it is important that the food is at the right

temperature prior to loading. In the large containers used for long distance transportation, food temperatures can be kept within ± 0.5 °C of the set point (James, James & Evans, 2006).

Another critical point in maintaining an unbroken cold chain is at delivery, storage and display conditions at the retail dealers. Inadequate practice and handling often result in incorrect product temperatures at this stage (Hemmingsen, 2002; James, 1996).

Despite numerous recommendations on handling and storage temperatures, consumer use and the performance of refrigerators has remained remarkably unchanged throughout the world over the last 30 years (James, Evans & James, 2008).

To ensure correct temperature handling of food, one can consider use of time-temperature monitors accompanying the products or included in the product packaging. Several types of time-temperature monitors are currently available or being developed (Kerry, O'Grady & Hogan, 2006). Until now a serious drawback of this technology is that the costs of such equipment are rather high, but has recently become affordable (e.g. 0.02-0.20 USD per unit).

1.4 Water in muscle foods

Water is the principal component of skeletal muscle and the water content is typically in the range of 70-80% (Table 1). The amount of water affects edible quality as well as processing of the food. In fish muscle there is usually an inverse relationship between the water and fat content, which together constitutes about 80% of the muscle. The majority of the water in the muscle is located within the structure of the muscle and the muscle cells. Water is found within and between the myofibrils and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Huff-Lonergan et al., 2005; Offer et al., 1992).

The water can be divided into three main categories, based on the degree of freedom. A small part of muscle water is free, unbound water, while the rest is mainly held by myofibrillar proteins and the structures. The strength of interaction between water and proteins varies with distance and with the charge of the proteins. Bound water is that which strongly interacts with hydrophilic sites of proteins to form monolayers, and water that forms several additional layers around hydrophilic groups. This water is very resistant to freezing and constitutes less than 10% of the total muscle water (Offer et al., 1988b; Wolfe, Bryant & Koster, 2002). The

third, and major, fraction of water is referred to as entrapped, immobilized or bulk water. This water is not directly bound to the proteins but may be held within the structure by steric effects (volume of space) and/or attraction to the bound water. The immobilized water is the water that is most affected by alterations in the muscle structure and some of it can eventually be lost as drip. The immobilized water is easily converted to ice upon freezing (Huff-Lonergan et al., 2005; Offer et al., 1988b).

One of the major characteristics of foodstuffs in general is that, as they are cooled below their initial freezing point, ice formation takes place. Fundamental factors of ice formation are relatively complex. The freezing process, which includes undercooling, nucleation (homogeneous and heterogeneous), ice crystal propagation, and maturation, is strongly influenced by thermodynamics (heat transfer properties), kinetics (mass transfer properties), and product (e.g. composition, shape and size) variables (Sahagian & Goff, 1996). Figure 3 shows some typical food freezing curves.

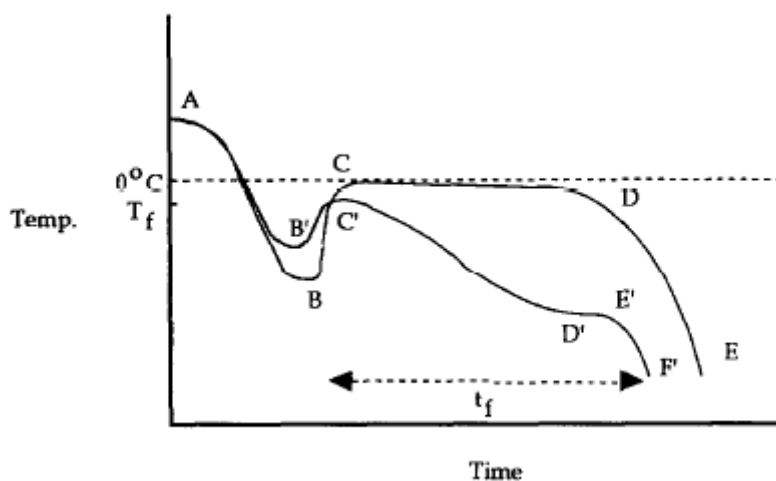


Figure 3: A temperature-time curve of water (ABCDE) and an aqueous solution, e.g. 10% sugar (AB'C'D'E'F') during freezing. T_f represents the temperature of the initial freezing point of the liquid, while t_f represents the freezing time as determined by such an experiment (Goff, 1992).

The bottleneck of the freezing process is the nucleation stage, as a nucleus is required for a crystal to grow. When the temperature of the food is reduced below 0 °C, undercooling occurs. Rapid heat removal leads to a higher degree of undercooling and a larger number of

nuclei are formed (Devine, Bell, Lovatt & Chrystall, 1996; Reid, 1983). The growth of extracellular crystals also takes place at the expense of intracellular water, leading to a partial dehydration of muscle fibers and subsequent distortion (Devine et al., 1996). Crystal growth is possible once nucleation has taken place. As long as a stable crystal is present, further growth is possible. The size of the crystal formed is dependent on the initial freezing, and in contrast to rapid freezing, which tend to form many small crystals, slow freezing often results in fewer and larger crystals leading to greater tissue distortion (Devine et al., 1996; Reid, 1983). Larger crystals due to recrystallization are also an effect of storage, especially with fluctuating storage temperatures. Smaller crystals tend to decrease in size and larger crystals grow in size (Reid, 1983; Santos-Yap, 1996).

1.5 Superchilling: principle and system

The storage of food in the temperature area from 0 °C to a few degrees below is in most cases, but not always, referred to as superchilling (Anelich, Hoffman & Swanepoel, 2001; Carlson, 1969; Fik, Surowka & Leszczynska-Fik, 1988; Gallart-Jornet, Rustad, Barat, Fito & Escriche, 2007; Haugland, Aune & Hemmingsen, 2005; Love, 1969; Magnussen, Nordtvedt & Torstveit, 1998; Nowlan, Dyer & Keith, 1974; Nowlan, Dyer & Keith, 1975; Olafsdottir, Lauzon, Martinsdottir, Oehlenschlager & Kristbergsson, 2006; Power, Morton & Sinclair, 1969; Rosnes, Kleiberg, Sivertsvik, Lunestad & Lorentzen, 2006; Rosnes, Sivertsvik, Skipnes, Nordtvedt, Corneliussen & Jakobsen, 1998; Sivertsvik et al., 2002; Sivertsvik, Rosnes & Kleiberg, 2003; Wang, Sveinsdottir, Magnusson & Martinsdottir, 2008; Waterman & Taylor, 1967; Zeng, Thorarinsdottir & Olafsdottir, 2005; Zhao, Ezeike, Doyle, Hung & Howell, 2003) or partial freezing (Aleman, Kakuda & Uchiyama, 1982; Bilinski et al., 1981; Borchert & Briskey, 1964; Borchert & Briskey, 1965; Chang, Chang, Shiau & Pan, 1998; Gibbard, Lee, Gibbard & Bilinski, 1981; Haard, 1992b; Kato, Umemoto & Uchiyama, 1974; Kramer, French, Kennish, Schwartz & Mcneil, 1984; Lanari & Zaritzky, 1991; Mackenzie, 1981; Magnussen et al., 1998; Okuzumi, Shimizu & Matumoto, 1980; Razavishirazi, Murata, Sakaguchi & Kuwana, 1990; Roach & Tomlinson, 1969; Roach, Tomlinson, Geiger & E., 1966; Sadok, Abdelmoulah & El Abed, 2004; Tomlinson, Geiger, Kay, Uthe & Roach, 1965; Uchiyama, Ehira, Uchiyama & Masuzawa, 1978; Uchiyama, Kakuda & Uchida, 1984; Uchiyama & Kato, 1974) or both superchilling and partial freezing (Aune & Nordtvedt, 1999; Carlson, 1969; Gallart-Jornet et al., 2007; Hemmingsen, 2002; Haard, 1992b; Lanari et al., 1991; Magnussen, Johansen, Nordtvedt, Hardarson, Haugland & Hemmingsen, 2007; Magnussen et al., 1998; Power et al., 1969; Simpson & Haard, 1987; Sivertsvik et al., 2003).

In a few cases the terms subfreezing (Urbaniak, 1975) or near ultra-chilling (Sun, Singh & O'Mahony, 2005) are also utilized. However, despite the different names, the processes are often similar.

Superchilling is often used to describe a process where food products are stored between the initial freezing point of the products and 1-2 °C below this in order to prolong shelf life (Bøgh-Sørensen, 1976; Carlson, 1969; Fik et al., 1988; Love, 1969; Nowlan et al., 1974; Power et al., 1969; Sivertsvik et al., 2003). The initial freezing point is the temperature where ice starts to form, and is in most foods found in the range of -0.5 °C to -2.8 °C (Fennema, Powrie & Marth, 1973). In superchilling, the surrounding temperature is set below this, and, depending on the method used, some ice is formed in the outer few millimeters. Superchilling can either be used prior to traditionally chilled distribution or be maintained throughout storage and distribution to store refrigeration capacity in the products. Superchilling can also be applied in relation to fisheries in distant waters where the use of ice and/or seawater is not sufficient for maintaining the catch in a good quality.

As with the general cold chain, the superchilling process consists of two stages; chilling and storage. In the chilling procedure, heat is removed relatively quickly from the food. Rapid temperature change is essential in chilling as well as in freezing technology. The driving force of the heat removal is dependent on the temperature difference between the cooling media and the product (Hemmingsen, 2002). Fast heat removal is possible with a large temperature difference and/or good heat transfer, i.e. blast chilling is more effective than still air and immersion chilling is more effective than air chilling (Fellows, 1988). In superchilling, ice in the external few millimeters absorbs heat from the interior of the product. As a consequence, the residential time in the chilling equipment needed for lowering the temperature can be reduced compared to ordinary chilling. After chilling, the food is immediately placed at the desired temperature for storage. During the first part of the storage the temperature in the product is equalized as a result of the outer part of the product removing heat from the inner part making the temperature move towards equalization throughout the product (Magnussen et al., 2007). The surrounding temperature should remain as constant as possible for the rest of the storage time to avoid melting and refreezing of ice crystals. The shelf life of superchilled food is far shorter than of frozen food and in superchilling a higher amount of the bulk water remains unfrozen. In the unfrozen water, however, the concentration of solutes will increase and affect the proteins and enzymes in the muscle. In this temperature area, even

small changes in temperature leads to great changes in the amount of ice frozen out (Mackie, 1993; Power et al., 1969). To maintain a stable amount of ice in the product, it is therefore especially important to obtain good control of the process. It is most probably a relation between the properties of the raw material and the optimal process, but the research on this area is scarce.

Superchilling can be achieved by several approaches. One of the methods is air chilling, where food is blast chilled in air (Power et al., 1969). A similar method is called contact blast chilling (CBC) (Olafsdottir et al., 2006; Zeng et al., 2005), where the food product is placed on Teflon coated aluminum conveyer belt and chilled in air. An alternative to the former two systems is liquid chilling, which means soaking packed or unpacked foodstuffs in a cooled aqueous solution, such as chilled refrigerated seawater (Magnussen, Hardarson & Nordtvedt, 2001; Toledo-Flores & Zall, 1989) or other subzero temperature baths (Lee & Toledo, 1984). The foodstuffs may then be stored in air or remain in chilled liquid at the desired temperature.

Transport is likely to be more effective when there is no external ice accompanying the product. With superchilling technology, refrigeration capacity is stored within the products and no extra ice is needed. If ice was added to superchilled fish, the fish would in fact be a source of keeping the ice cold rather than the opposite. The use of ice in traditional chilling of fish represents weight, volume and latent spill water in fish transport. The ice usually constitutes about 20-30% of the weight of each box of fish (Magnussen et al., 2001), and sufficiently ice has to be added to each box of fish to maintain the temperature during transport (NMFCA, 1996). Theoretically, the number of trucks exporting salmon from Norway to other European countries thus can potentially be reduced by 20-30% (Magnussen et al., 2001). More effective transport and significantly reduced amounts of spill water represent economical and environmental gains.

The requirements for transporting superchilled products is nevertheless dependent on whether the product is intended to be thawed and stored as chilled or further stored as superchilled. In case of further superchilled storage in the retail part of the cold chain, the storage compartments of the transport vehicles have to be designed to maintain superchilling temperatures. In addition, in the case of co-distribution with other food products potential conflicts between their required temperatures have to be clarified.

1.6 Superchilling: applications

It is claimed (Carlson, 1969; Waterman et al., 1967) that a technique referred to as superchilling was applied onboard Portuguese fishing vessels in the 1930's. These vessels were used for fishing in distant waters, and the fish were superchilled onboard to maintain the quality as good as possible for the time it took to return for delivery of the catch. The superchilling principle was also utilized in the same manner in Alaska (Roach et al., 1966; Tomlinson et al., 1965), especially in the early eighties (Bilinski et al., 1981; Gibbard et al., 1981). However, a system for refrigerating fish in bulk on fishing vessels is first described in a French patent of 1920 (Le Danois, 1920).

In the field of superchilling, most research has been done in the product groups fish (Aleman et al., 1982; Anelich et al., 2001; Carlson, 1969; Chang et al., 1998; Gibbard et al., 1981; Jul, 1986; Kato et al., 1974; Magnussen et al., 1998; Nowlan et al., 1974; Nowlan et al., 1975; Okuzumi et al., 1980; Olafsdottir et al., 2006; Power et al., 1969; Roach et al., 1969; Roach et al., 1966; Rosnes et al., 2006; Sadok et al., 2004; Sivertsvik et al., 2003; Tomlinson et al., 1965; Uchiyama et al., 1978; Uchiyama et al., 1984; Uchiyama et al., 1974; Wang et al., 2008; Waterman et al., 1967; Zeng et al., 2005), poultry (Cortesi & Pagliazzo, 1981; Matyniak & Ziolencki, 1983; Zhao et al., 2003) and to some extent also red meat (Jeremiah & Gibson, 1997; Jeremiah & Gibson, 2001; Lanari et al., 1991). In later years, the method has often been applied in combination with storage in various modified gas atmospheres (discussed separately in section 1.8). This has shown interesting results with respect to keeping the products at more stable temperatures during short breaks in the cold chain.

Chang and coworkers (Chang et al., 1998) examined the influence of superchilling or refrigerated storage on the quality of cultured sea bass and compared the changes in freshness. The shelf life of sea bass stored at +10 °C and +5 °C was 2 and 3 days, respectively, but at 0 °C it was extended to about 2 weeks. Superchilling storage at -3 °C increased the shelf life to more than 4 weeks. It was stated that the long shelf life was due to the longer lag phase of bacterial growth and retardation of nucleotide breakdown rate. Conclusively they stated that superchilling appeared to be an effective freshness preservation method for fish, and that it deserves further investigation.

In superchilling of fatty fish, salmon and trout are among the species that have been studied most intensively. Superchilling of rainbow trout for short time storage might be attractive for industrial practice (Fik et al., 1988).

Superchilling has been employed on poultry with success. It is currently a well established method within the US poultry industry. Poultry is seldom referred to as superchilled in the literature, since in the USA poultry meat kept above 26 °F (-3.3 °C) legally can be marketed as fresh (US Food Safety and Inspection Service). Goose meat superchilled and stored at -2 °C for 30 days retained a quality similar to that of goose meat chilled and stored at +2 °C for about 3 days (Urbaniak, 1975), and superchilling did not have an adverse effect on the ultrastructure of chicken breast muscles. In fact, the structure of the muscles was particularly well preserved (Smolińska & Abdul-Halim, 1992). The turning point for the sensory attributes of chicken carcasses, structural and microbiological changes, came on the tenth day of aging. Smolinska et al. (1992) stated that the results show that superchilling extended shelf life of carcasses up to 4 days longer than conventional chilling.

Investigation of long distance transport of superchilled Atlantic salmon has been made (Aune et al., 1999; Rosnes et al., 1998). Whole superchilled salmon were transported by refrigerated truck from Norway to France in recyclable cardboard cartons without the addition of ice. It was found that the salmon were at a satisfactory level of quality on arrival (Aune et al., 1999).

1.7 Comparable subzero refrigeration systems

Along with superchilling, there are also similar systems that utilize the same main principle. Food products are kept at temperatures that lie on the borderline between chilling and freezing in order to prolong the shelf life. These methods have primarily been applied on fish.

Deep chilling (Cortesi et al., 1981; Einarsson, 1988; Jul, 1986; Matyniak et al., 1983; Smolińska et al., 1992) is a process related to superchilling. However, the temperature area is restricted to the narrow interval between 0 °C and the initial freezing point of the food.

In a system referred to as slurry ice (also known as fluid ice, slush ice, liquid ice, flow ice and binary ice), addition of salts and other soluble compounds to an ice-water mixture enables subzero temperatures (Losada, Pineiro, Barros-Velazquez & Aubourg, 2004b). Pineiro and coworkers (Pineiro, Barros-Velazquez & Aubourg, 2004) reviewed the effects of newer slurry

ice systems versus flake-ice chilling methods on the quality of aquatic food products. An ice-water mixture provides a faster chilling rate than flake ice. It is also claimed that the physical damage on the fish flesh in a slurry ice system is reduced compared to flake ice and RSW due to the small size and spherical shape of the particles. Typical ice-water ratio of the slurry is 40:60 and temperatures are around -1.5 °C (Aubourg, Losada, Prado, Miranda & Barros-Velazquez, 2007; Losada et al., 2004b; Pineiro, Bautista, Rodriguez, Losada, Barros-Velazquez & Aubourg, 2005; Rodriguez, Barros-Velazquez, Pineiro, Gallardo & Aubourg, 2006). The slurry ice method is currently being investigated by Spanish (Aubourg et al., 2007; Barros-Velazquez, Gallardo, Calo & Aubourg, 2008; Losada, Barros-Velazquez, Gallardo & Aubourg, 2004a; Losada et al., 2004b; Losada, Pineiro, Barros-Velazquez & Aubourg, 2005; Pineiro et al., 2004; Pineiro et al., 2005; Rodriguez et al., 2006; Rodriguez, Losada, Aubourg & Barros-Velazquez, 2004) and Turkish (Cakli, Kilinc, Cadun & Tolasa, 2006; Cakli, Kilinc, Dincer & Tolasa, 2006; Kilinc, Cakli, Cadun, Dincer & Tolasa, 2007) research groups.

One example of a non-salt compound that can be added to the slurry mixture for better microbiological control is ozone (Aubourg, Losada, Gallardo, Miranda & Barros-Velazquez, 2006; Campos, Losada, Rodriguez, Aubourg & Barros-Velazquez, 2006; Campos, Rodriguez, Losada, Aubourg & Barros-Velazquez, 2005; Losada et al., 2004a).

In order to further extend the shelf life using RSW or CSW, the systems can be modified. This can be done by adding CO₂ to the seawater, and thus lowering the pH of the cooling agent (Bullard & Collins, 1978; Collins, Reppond & Bullard, 1980; Reppond, Bullard & Collins, 1979; Reppond & Collins, 1983). Dry ice has been applied both alone (Jeyasekaran, Ganesan, Anandaraj, Shakila & Sukumar, 2006) and mixed with water (Jeyasekaran, Anandaraj, Ganesan, Shakila & Sukumar, 2008) in order to prolong microbial shelf life of seafood.

Another preservation method involving rapid freezing and subsequent chilled storage, is freeze chilling (Fagan, Gormley & Mhuircheartaigh, 2003; Patsias, Badeka, Savvaidis & Kontominas, 2008). The principle of this method is, however, quite different from that of superchilling technology. In this system, products are deep frozen and stored at deep freeze temperatures. When required, it is thawed and stored at refrigerate temperatures.

1.8 Combination with other techniques

In order to prolong product shelf life further, subzero storage temperatures can be used in combination with several preservation factors or hurdle techniques such as vacuum packaging, modified atmosphere packaging (MAP), microbial inhibitors (Sadok et al., 2004) or combinations of these processes.

In recent years, superchilling has to a larger extent than previously been combined with the use of MAP. This has especially been a preferred method in storage of meat. MAP products are surrounded by a gas mixture to prevent or delay spoilage (Farber, 1991; Sivertsvik et al., 2002). As this gas layer also serves as an additional barrier to the surroundings, the rate both of chilling and of heating is relatively slow (Hemmingsen, 2002). The product temperature may thus be more stable even with short breaks in the cold chain. However, after longer breaks in the cold chain leading to a rise in the product temperature, it would also take longer for the product to return back to the desired temperature.

MAP in combination with superchilling has shown promising results. These two techniques have been combined for prolonging shelf life of muscle food from a variety of species, such as pork (Liu, Yang & Li, 2006; Livingston, Brewer, Killifer, Bidner & McKeith, 2004; McMullen & Stiles, 1994), beef (Gill, 1996; Hemmingsen, 2002), cod (Einarsson, 1994; Wang et al., 2008), salmon (Rosnes et al., 1998; Sivertsvik et al., 2003), mackerel (Hong, Leblanc, Hawrysh & Hardin, 1996), hake (Ruiz-Capillas & Moral, 2001), snapper (Scott, Fletcher & Hogg, 1986) and wolf-fish (Rosnes et al., 2006). MAP storage of whole superchilled salmon showed that enough refrigeration capacity was stored to keep a core temperature below 0.0 °C during long distance transport (Rosnes et al., 2006). The combination of superchilling and MAP on cod loin has a potential of keeping the fresh and sweet taste longer than by each technique alone. However, some meaty texture of the superchilled MAP loins was observed after one week (Wang et al., 2008).

Hemmingsen (2002) compared biochemical and microbial quality changes of superchilled and ordinary chilled MAP sirloin steaks during storage for 9 and 16 days at constant and varying temperature. Storing at varying storage temperatures gave low water-holding capacity and amount of acid soluble peptides, but resulted in higher extractability of salt soluble proteins. Varying storage temperature also gave high plate counts, whereas superchilling gave

somewhat lower plate counts. Hemmingsen (2002) state that the superchilling process is very challenging and should be optimized further. However, Hemmingsen (2002) conclude that storage at varying temperature is very unfortunate for the MAP products and should be avoided.

1.9 Summary of introduction

Improving shelf life is particularly important for highly perishable food such as muscle foods. The shelf life of meat and fish held at refrigeration temperature is limited, primarily due to microbial and enzymatic spoilage. However, the minimizing of exudate losses is of commercial concern, since such losses influence the profitability of fresh products by affecting processing yields and palatability. The water-holding capacity (WHC) is closely related to textural properties, and low WHC has often been described as an effect of post mortem structural changes in the muscle. The immobilized water is the most affected by alterations in the muscle structure and some of it can eventually be lost as exudate.

The most important extrinsic parameter in food preservation is low temperature, since it retards microbial growth. Superchilling implies temperatures in the borderline of chilling and freezing and can be a valuable tool in strengthening the cold chain.

It has previously been stated that superchilling can prolong microbial shelf life of muscle foods, and the studies performed have mainly focused of microbiology, sensory parameters and to some extent on exudate losses. There is a need to investigate other quality parameters of the muscle to gain knowledge about the relationship between the process and the quality. The results can serve as a basis for optimizing the superchilling process.

2 Aims and scope

Superchilling is a method that can be used to prolong the shelf life of foods by partial freezing. Knowledge of the effects of this method on both the shelf life and quality of foods is important in order to find optimal processing and storage conditions and is of great importance both for the industry and for the consumers.

The objective was to study biochemical processes taking place during superchilling of muscle foods and how the physiochemical changes relate to these processes and thereby the quality of the raw material. In order to enhance the knowledge of the biochemical and physiochemical changes in superchilled products that comes from different origins, raw materials from Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and pork (*Sus scrofa domesticus* of LYLD breed) were chosen to represent fish and meat products.

Effects of different temperature levels were studied in order to find the optimal superchilling parameters. In addition, the consequences of an interrupted cold chain during superchilled storage were also studied.

Consequently, the main issues addressed in this thesis are:

- How is the shelf life affected by superchilling compared to traditional iced, chilled or frozen storage?
- How is the amount of drip loss and water-holding capacity influenced by superchilling?
- How are biochemical properties, such as of proteins and enzymes, affected by superchilling?

3 Methodology

3.1 Raw materials

The main purpose of this thesis is to investigate biochemical and physiochemical effects of superchilling on different muscle foods, more exactly both meat and fatty and lean fish. Pork, salmon and cod were chosen to represent meat and fish products. Pork is the predominant species within the Norwegian meat consumption. Salmon and cod are both commercially important species that are sold fresh. Still, salmon and cod are different raw materials for processing mainly because they are fatty and lean fish species, respectively.

Salmon (*Salmo salar*) is well established in the Norwegian fish farming and export industry. A better technology that prolongs the shelf life compared to the traditional ice chilling or freezing would be valuable. Two superchilling experiments are described in this work. The salmon products in these experiments were vacuum packed portions of skin-on fillets (Paper II) and wrapped whole skin-on fillets (Paper IV). In the first experiment, two portions of 469 ± 46 g were cut from the middle of each of the two fillets and vacuum packed. Samples were superchilled at two different temperatures, -1.4 °C or -3.6 °C, and compared to traditionally ice chilled or frozen (-40 °C) references. In the other experiment, whole fillets of 1320 to 2170 g were wrapped in thin sheets of plastic and stored in expanded polystyrene (EPS) boxes. Samples were superchilled at a lighter or deeper temperature level, denoted as sup 1 and sup 2, and stored at -1.4 and -1.7 °C, respectively. References were traditionally ice chilled or frozen (-40 °C) samples.

Cod (*Gadus morhua*) is the most important commercial species in the North Atlantic fisheries and is now regarded as a very promising species in cold water fish farming. The demand for fresh cod fillets is rapidly increasing at the expense of frozen fillets (NSEC, 2005). Since the shelf life of fillets is 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. In this work, two portions of 158 ± 31 g were cut from the middle each of the two skin-on fillets and vacuum packed. Superchilled samples were compared to traditionally ice chilled samples and to samples frozen at -21 °C or -40 °C.

The choice of pork was done in collaboration with one of the major food companies in Norway, which was looking for an alternative technology that could be used for prolonging shelf life of pork and thereby reduce the need of freezing. In this work two experiments are described. The pork products in these experiments (Figure 4) were vacuum packed boneless roasts from leg (Paper I) and vacuum packed rib steaks (Paper IV). The samples were delivered at the lab after cutting and packing by the manufacturer. The pork was of the Norwegian breed Noroc (LYLD), progeny of the crossing of a LY sow and a LD boar from the races Norwegian Landrace (L), Finnish Yorkshire (Y) and Norwegian Duroc (D). The commercial brand of this breed is “Edelgris”. The average product weight of the roasts and the rib steaks was 1076 ± 259 g and 334 to 700 g, respectively. The superchilled roasts were compared to traditionally chilled or frozen samples and to superchilled samples with an interrupted cold chain. Some superchilled samples were after one month also transferred to chilled storage to observe some effects on subsequent chilled storage. Rib steaks of pork were superchilled at a lighter or deeper temperature level, denoted as sup 1 and sup 2, and stored at -1.4 and -1.7°C , respectively. References were traditionally chilled samples.



Figure 4: Pork products represented in this work are roast (left) and rib steak (right) (Gilde).

3.2 Chilling system

The food products were chilled in air until the core temperature of the products reached $\sim -1^{\circ}\text{C}$. The surrounding temperature during the chilling procedure in the different experiments

varied between -24 °C and -30 °C and the air velocity was between 2 and 4 m/s. Chilling in air was chosen because the equipment needed for the superchilling procedure is close to the existing industrial chilling equipment. It was also a currently available system, adjusted through calculations and simulations and tested at the thermal laboratory (Sintef Energy Research). With the exception of whole salmon fillets (Paper IV), the samples were vacuum packed. The vacuum packaging was chosen to prevent the samples from drying out. After the chilling procedure, the products were immediately transferred to storage at the chosen temperature.

3.3 Analytical parameters

Most of the studies on superchilling have focused on microbiology, sensory analysis and spoilage indicators such as TVB-N (Olafsdottir et al., 2006; Sivertsvik et al., 2002; Sivertsvik et al., 2003; Zeng et al., 2005), and have only to a minor extent investigated the effect of superchilling on biochemical processes and how they influence quality parameters, such as loss of juiciness and negative textural changes. In this work, focus has been on liquid-holding capacity, drip loss and protein stability. In addition, some investigations on the microbiological, textural and enzymatical aspects have been conducted.

Drip loss and water-holding capacity can be measured in several different ways (Cheng et al., 2008; Otto, Roehe, Looft, Thoelking & Kalm, 2004). In this work, the drip liquid has been collected and quantified immediately after storage or after thawing. The water content of the drip loss has also been examined. Water-holding capacity was analyzed by low-speed centrifugation by a modification (Hultmann & Rustad, 2002) of the method of Eide, Børresen and Strom (1982), and expressed liquid loss (LL), the percentage of weight of the mince lost during centrifugation.

Microbiology was analyzed by total counts, psychrotrophic counts and/or sulphide-producing bacteria (SPB). As further described in the papers, NMKL (Nordic Committee on Food Analysis) methods or commercially available kits (ColiFAST) were used.

Measurements of pH were performed by mixing minced muscle with KCl (150 mM) in 1:1 ratio (Bendall, 1973).

Changes in solubility properties has been shown to be related to process induced changes in muscle foods (Mackie, 1993). Extractions for determination of protein solubility was performed in two steps resulting in a water-soluble and a salt soluble fraction by a modification (Hultmann et al., 2002) of the methods of Anderson and Ravesi (1968) and Licciardello, Ravesi, Lundstrom, Wilhelm, Correia and Allsup (1982).

The amount of free amino acids can be used as an indication of proteolytic activity in the muscle. Analysis was conducted in order to monitor preservation of enzymatic activity during superchilled storage. Amount of free amino acids was determined in the water-soluble protein extracts by the procedure of Osnes and Mohr (1985) and analyzed with reverse phase high performance liquid chromatography (RP-HPLC) by the method of Lindroth and Mopper (1979) as modified by Flynn (1988).

The extractions of proteolytic enzymes in salmon muscle (Paper II) were preformed at +4 °C as described by Hultmann, Rørå, Steinsland, Skåra and Rustad (2004a).

Textural properties of salmon fillets were investigated by the method of Einen and Thomassen (1998) on a TA.XT2 Texture Analyzer (Stable Micro Systems, UK) equipped with a flat-ended cylindrical plunger (12 mm diameter) (Hultmann et al., 2002).

4 Results and discussion

4.1 Drip loss and liquid loss

4.1.1 DRIP LOSS

Fish and meat are sold on weight basis. When the industry is considering use of superchilling technology, it is thus economically important to know whether it would result in increased drip loss or not. The current work shows that superchilling does not necessarily lead to increased drip loss compared to ordinary chilled or iced storage. Indeed, the tendency was more drip loss in the chilled or iced references than in the superchilled fish or meat products.

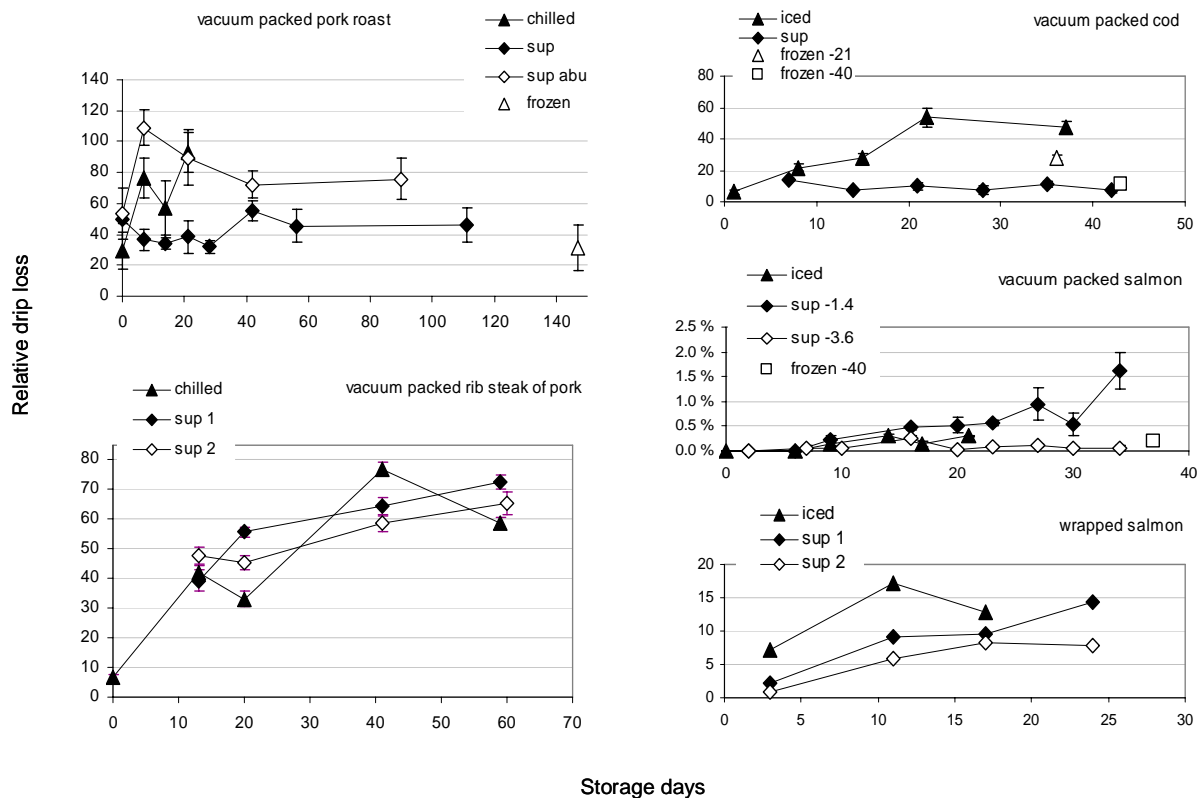


Figure 5: Comparison of drip loss relatively to product weight of various pork, cod and salmon products subjected to different chilling and storage conditions. Mean values of drip loss are given in percent of product weight. Y-error bars represent the standard error of the mean. Values of for wrapped salmon are pooled measurements of six fillets.

According to the supplier, freezing and thawing pork under industrial conditions may lead to a loss of as much as around 10% of the product weight. The superchilled pork roasts (Paper I) only lost between 3.2 and 5.5% of the product weight during the complete course of the superchilling process, superchilled storage and thawing, while traditionally chilled references lost 3-9.5% during storage (upper left diagram in Figure 5). One batch of superchilled pork roasts were subjected to temperature abuse during storage. These roasts lost 5.4-11% of the product weight, which was the highest relative drip loss amongst all experimental groups, regardless of species.

In the experiment where superchilling was applied on rib steak of pork (Paper IV), the quantities of drip loss generally increased during storage (lower left panel in Figure 5). The rib steaks of the lighter superchilled group (sup 1) lost 3.9-7.3%, while the group that was deeper superchilled (sup 2) lost 4.5-6.5%. Traditionally chilled rib steaks lost 3.3-7.7% of the product weight. The increase in drip loss probably contributed to the decrease in muscle water content during storage.

Vacuum packed superchilled salmon stored at -3.6 °C and ice chilled or frozen references all lost less than 0.3% of the weight during storage, while samples stored at -1.4 °C lost maximum 1.6% (middle right panel in Figure 5). Values of 1-2% drip loss cannot be regarded as high (Dalgaard, Gram & Huss, 1993; Einen, Guerin, Fjæra & Skjervold, 2002) and therefore drip loss cannot be considered as a problem in vacuum packed superchilled salmon (Paper I).

As with the vacuum packed salmon, the level of drip loss in wrapped superchilled salmon (Paper IV) was not high. The total weight loss during storage and thawing for both the lighter (0.2-1.4%) and the deeper superchilled fillets (0.1-0.8%) was less than for the iced references (0.7-1.3%) (Lower right panel in Figure 5).

Superchilled vacuum packed cod fillets never lost more than 1.5% of their weight as drip (Paper III). The drip loss was highest in ice chilled fillets, lower in fillets frozen at -21 °C (2.7%) and lowest in superchilled fillets and fillets stored at -40 °C (Upper right panel in Figure 5). The drip loss of ice chilled fillets increased with storage time and amounted to more than 5% after 15 days of storage.

When comparing the two experiments involving salmon (Paper II and IV), the lighter superchilled samples lost somewhat more weight as drip than the deeper superchilled samples. This was more evident in the vacuum packed salmon (Paper II). For the ice chilled references it was also observed that wrapped salmon loses more weight as drip than vacuum packed salmon.

Despite the fact that fish proteins are less stable than those of meat are, meat generally lost more of its weight as drip during storage and thawing in the present experiments than fish did (Figure 5). This was the case both when comparing superchilled meat and fish and when comparing traditionally chilled meat and fish. This difference may partially be explained by the fact that the pH of meat is lower than that of fish. When pH of the muscle low, the ionic repulsion of the muscle proteins is lower (i.e. pH is closer to the pI of myosin), the space between the fibrils becomes smaller and can thus hold less water. Within the fish species studied, cod lost more drip than salmon. This may be explained by the more unstable muscle proteins (Ofstad, Egeland, Kidman, Myklebust, Olsen & Hermansson, 1996a) and the higher water content in cod compared to salmon. Differences in muscle structure between the species (Ofstad, Kidman & Hermansson, 1996b) may also contribute to the higher drip.

From the present results it can be concluded that superchilling did not lead to increased drip loss in the raw materials studied. The muscle structure is apparently equally or better preserved during storage at superchilled temperature than during chilled storage. This can be explained by reduced enzymatic activity and generally lower reaction rates of processes at subzero temperatures.

The formation of drip loss is critically related to post mortem physiological and structural factors (Cheng et al., 2008). Some of the principal factors are considered to be changes that take place at the molecular level, such as post mortem fiber shrinkage due to pH fall and actomyosin cross bridges, myofibrillar shrinkage and contraction, and myosin denaturation (Offer et al., 1992; Schäfer, Rosenvold, Purslow, Andersen & Henckel, 2002). Fluid, especially that accumulating between fiber bundles, is the likely source of drip (Offer et al., 1992). Changes in the permeability of the cell and basement membrane to water are also likely to be of importance for drip formation (Cheng et al., 2008; Schäfer et al., 2002). Structural changes at the fiber and fiber bundle level can lead to an increase of the extracellular space where expelled fluid accumulates (Cheng et al., 2008).

4.1.2 LIQUID LOSS

The ability of muscles to retain liquid or water is referred to as the liquid- or water-holding capacity and it is often determined as liquid loss (LL) by a centrifugation procedure (Eide et al., 1982; Ofstad et al., 1996a). Drip loss and WHC is influenced by muscle structures, activity of enzymes and by properties of the muscle proteins which in turn is affected by the pH of the muscle. WHC or LL influences the profitability of fresh pork products by affecting processing yields and palatability (Melody, Lonergan, Rowe, Huiatt, Mayes & Huff-Lonergan, 2004). The ability of the muscle to retain water reflects the moisture content and thereby influences the eating quality of the food product. While the drip loss directly quantifies the loss of saleable weight and/or deterioration of appearance and further facilitates surface microbial growth, a high liquid loss indicates toughening and reduced eating quality of the fish or meat. Muscle food with poor liquid-holding properties is likely to lose more moisture during further processing and cooking.

The chilled references (2-7%) and the temperature abused superchilled pork roasts (3-15%) showed better liquid retention than the superchilled roasts (3-19%) (Paper I). Liquid loss of rib steak of pork was similar in all storage groups (Paper IV). Between 13 and 60 days of storage the values varied from 3 to 7% (Upper left panel in Figure 6).

In superchilled vacuum packed salmon fillets the liquid loss was generally much lower (4-9% at -1.4 °C and 6-7% at -3.6 °C) than in cod fillets (21-30% at -2.2 °C). The liquid loss was significantly higher in superchilled (21%-30%) compared to ice chilled (9%-15%) cod fillets (Paper III) (Upper right panel in Figure 6).

In vacuum packed salmon fillets superchilled at -1.4 °C the loss decreased from initial 7% down to 4% during the first two weeks of storage (Paper II). During the remaining storage period, the liquid loss in samples stored at -3.6 °C (5-7%) was slightly higher compared to those stored at -1.4 °C (4-8%) (Middle right panel in Figure 6).

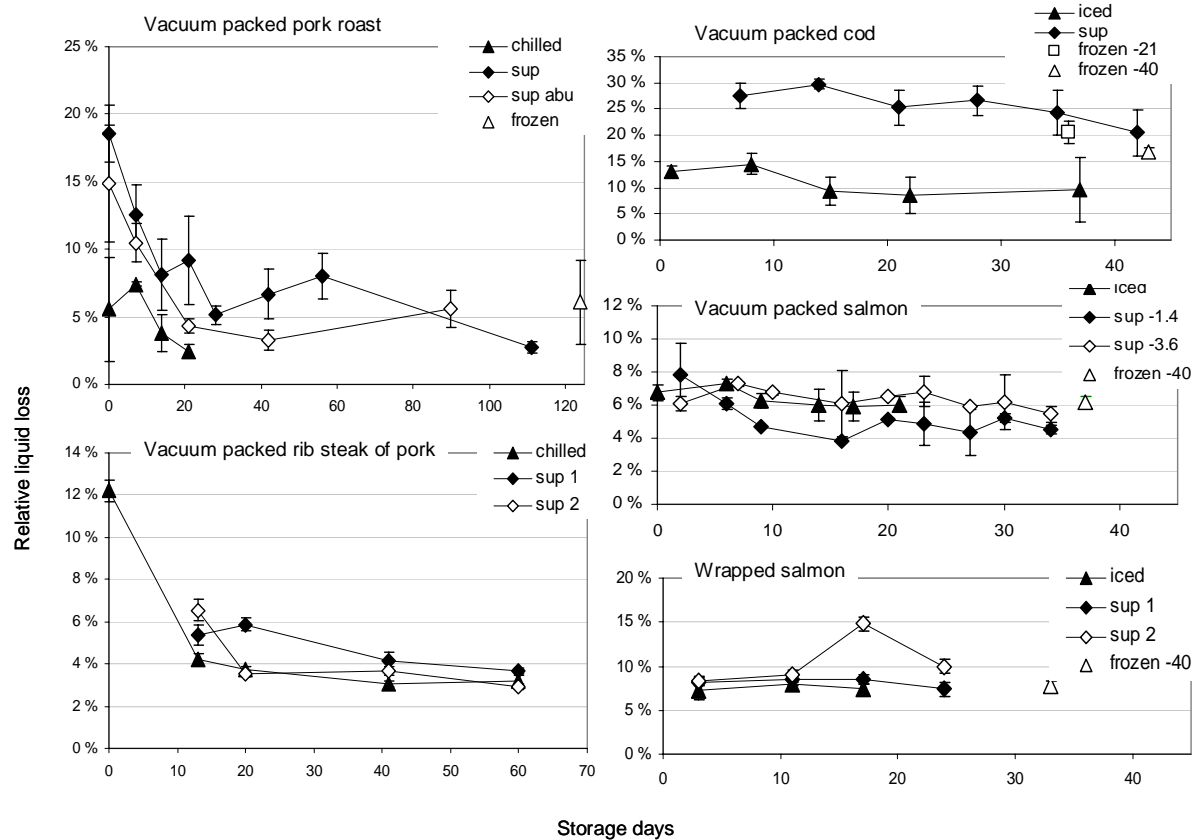


Figure 6: Comparison of liquid loss by low speed centrifugation relatively to product weight of various pork, cod and salmon products subjected to different chilling and storage conditions. Mean values of liquid loss are given in percent of product weight. Y-error bars represent the standard error of the mean.

The liquid loss of wrapped salmon fillet (Paper IV) was similar in all storage groups (7-10%), except from a higher value (15%) in the deeper superchilled fillets at day 17/18 (Lower right panel in Figure 6). The water content in the fillets varied between 57.6 and 62.4%. Except from during the first week, the water content was generally highest in the iced samples and lowest in the deeper superchilled samples.

The most loosely bound water or liquid is lost as drip loss. When the water-holding capacity analysis is conducted, some of the water is thus already lost. Therefore the level of the liquid loss has to be seen in context with the amount of drip loss. Samples with high drip loss are more likely to be able to hold better on to the remaining water during the centrifugation procedure of the water-holding analysis.

The superchilled cod fillets showed lower drip loss values than ice chilled fillets. However, the liquid loss of the superchilled fillets was higher than of the ice chilled fillets. Although to a much lesser extent, the superchilled pork roast also showed less drip loss and more liquid loss than chilled samples. There was an opposite tendency in vacuum packed salmon. The superchilled samples stored at -1.4 °C lost somewhat more weight as drip, but less weight as liquid loss compared to the other storage groups.

The higher liquid loss in cod compared to salmon is supported by Ofstad et al. (1993), who found the liquid loss in coarsely chopped cod muscle to be more than twice as high as for salmon muscle. Moreover, Atlantic salmon possess much better liquid-holding properties than Atlantic cod with similar low ultimate muscle pH (Ofstad et al. 1996a). In addition, as cod is a gadoid species, its muscle proteins are more susceptible to freeze denaturation than those of salmon (Sotelo et al., 1995b). The effect of pH on drip loss and liquid loss is further discussed in the following section.

4.2 pH

The pH value of the muscle has a large impact on the ability of the muscle to retain water (Cheng et al., 2008; Offer et al., 1989).

Changes in pH during storage can also be used as an indication of bacterial growth. It has been shown that inhibition of bacterial growth can delay the rise in pH, and thereby also retard the expected increase in water-holding capacity (Olsson et al., 2003). It has also been shown that low pH is associated with reduced water-holding capacity and increased myofibrillar shrinkage during heating of cod (Ofstad et al., 1996b).

In the pork roasts (Paper I), the pH values of the temperature abused and the chilled roasts increased more than those of the superchilled roasts. After about four weeks, the values of the superchilled samples decreased from 5.9 to 5.6 (Bottom panel in Figure 7). The variation in the pH values of the superchilled samples was smaller than the variation in the other sample groups. The pH values of the rib steaks of pork (Paper IV) samples varied between 5.59 and 5.80 (Middle left panel in Figure 7).

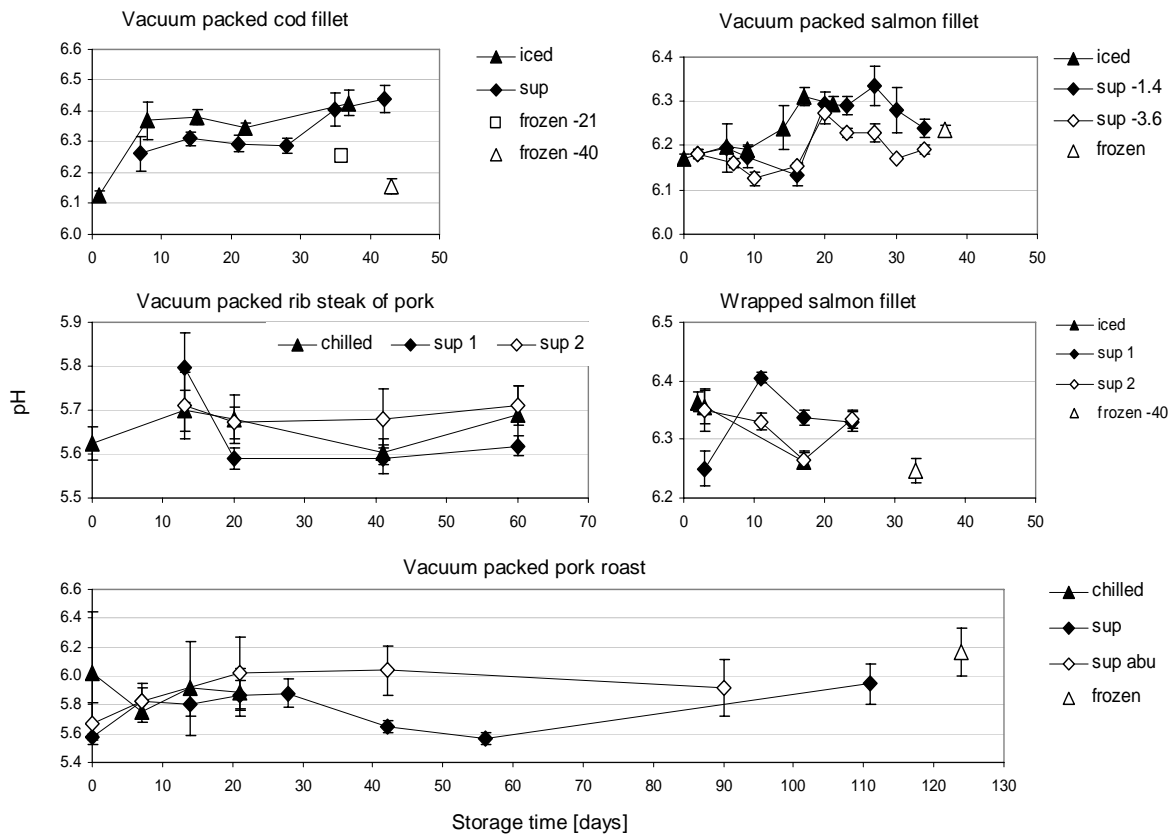


Figure 7: Changes in pH of various pork, cod and salmon products subjected to different chilling and storage conditions. Results are given as mean pH values. Y-error bars represent standard error of the mean.

The average pH value of the vacuum packed cod fillets (Paper III) were 6.13 on day 1 (day 3 post mortem) (Upper left panel in Figure 7). 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. 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 can not fully explain the large differences in drip loss or LL between the superchilled and chilled fillets. The pH values are low, but typical for farmed cod (Rustad, 1992).

The rise in pH values of both groups of superchilled vacuum packed salmon fillets (Paper II) were 4-6 days delayed compared to that of ice chilled fillets (Upper right panel in Figure 7). The pH values of wrapped salmon fillets (Paper IV) varied from 6.25 to 6.41 (Middle right

panel in Figure 7). The development in pH values of the lighter superchilled wrapped salmon fillets is different from those of the deeper superchilled and of the ice chilled samples.

The muscle pH of vacuum packed cod increased earlier than in salmon, and a further delay was observed in superchilled compared to ice chilled salmon. The delay in pH increase between the species can be part of the explanation of the higher liquid loss in cod compared to salmon. However, the delay in pH increase between storage groups did not cause similar differences in liquid loss of salmon fillets as in cod fillets.

4.3 Protein stability

Both muscle pH and the properties of the proteins are important for quality characteristics such as texture and the ability to retain water. Both for meat and for fish, intact muscle proteins are related to less soft textural quality, lower drip loss and better liquid retention. Fish has a better eating quality when the texture is firm and thus softening in fish is related to poor quality. In meat, a certain softening (maturation) is wanted because it tenderizes the meat. Muscle softening post rigor is a result of the enzymatic activity of proteases (Etherington, 1984). The function of proteases in live animals is to regulate the protein turnover. When the animal dies, the regulation of this biological function is lost and the proteases hydrolyze the muscle proteins, causing tenderization of the meat (Foegeding et al., 1996). Protein degradation caused by proteolytic activity may enhance extractability. However, a high degree of muscle protein denaturation caused by freezing is related to lower protein stability, and thus lower extractability of proteins. Protein denaturation is also associated with crosslinking which results in protein aggregation and reduced solubility. In frozen storage of gadoid fish species, such as cod, aggregation is often caused by reduction of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (Sotelo et al., 1995b).

It has been shown that storing fish at freezing temperatures close to 0 °C will result in a higher degree of freeze denaturation of protein (Mackie, 1993). It was therefore important to investigate protein solubility during superchilled storage and compare it with traditionally chilled and frozen references. In the present studies, protein extractability is used as a tool to monitor protein denaturation. In addition, process and storage effects on textural properties and activities of cathepsins can be valuable for understanding of the connection between

protein stability and superchilling. Free amino acids were quantified in salmon and cod in order to further support the findings.

The muscle proteins can be divided into three classes based on solubility properties; sarcoplasmic proteins (mainly enzymes) which are water-soluble, myofibrillar proteins (contractile network) which are salt soluble and insoluble proteins (mainly connective tissue) (Foegeding et al., 1996; Haard, 1992a). Analyses of the stability of sarcoplasmic and myofibrillar proteins of salmon (Paper II) and cod (Paper III) in the present work were performed by extractions in two consecutive steps. The first step revealed the extractability of the sarcoplasmic (water-soluble) proteins, while the second step revealed the extractability of the myofibrillar (salt soluble) proteins. Connective tissue proteins are not measured by this procedure.

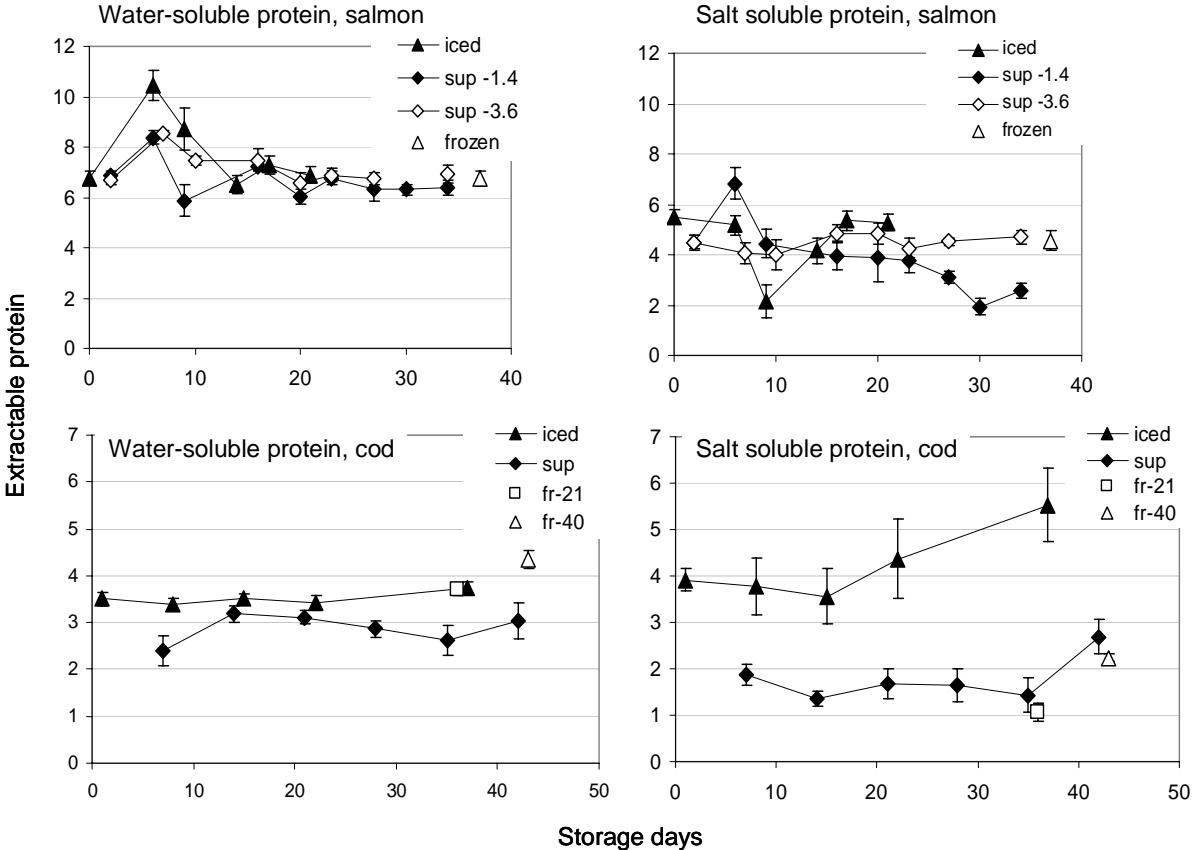


Figure 8: Changes in extractability of water- and salt soluble proteins of vacuum packed cod and salmon fillets subjected to different chilling and storage conditions. Mean values of protein extracted are given in percent of product weight. Y-error bars represent the standard error of the mean.

In cod (Paper III), 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 (Lower left panel in Figure 8). The amount of water-soluble proteins in the superchilled samples was slightly lower and varied from 2.4 to 3.2%. In salmon (Paper II), the values varied between 6 and 10.5%, but the differences were not significant between groups (Upper left panel in Figure 8). The higher extractability of water-soluble proteins in salmon compared to cod is because the sarcoplasmic proteins make up a larger part of the muscle proteins in salmon than in cod (Ofstad et al., 1996b). Salmon is a more active swimmer than cod.

The solubility of sarcoplasmic proteins from wrapped salmon fillets (Paper IV) was investigated by extraction in pure water. The amount of extracted protein from salmon varied between 2.3% and 5.3% of sample wet weight (paper IV). The lower extractability in wrapped than in vacuum packed salmon could be because the extractions were made with water and not buffer. On the other hand, it could also indicate that the sarcoplasmic proteins in wrapped salmon were more denatured than those in the vacuum packed salmon. In addition, the anaerobic conditions in vacuum packed samples would cause slower microbial growth than in wrapped samples. The production of bacterial proteases thus becomes lower and can cause less protein degradation.

In salmon (Paper II), extractability of salt soluble proteins from samples stored at -1.4 °C declined more during the last week of storage compared to the other groups (Upper right panel in Figure 8). This indicated a higher degree of protein denaturation in these samples. Previous reports have proposed that stabilization of myofibril proteins is directly related to better fish quality (Martinez, Friis & Careche, 2001; Rodriguez et al., 2006). Results from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of salt soluble protein fraction in salmon (Paper II) verify a higher degree of protein denaturation in samples stored at -1.4 °C. The band of the myosin heavy chain is weaker in this storage group compared to the corresponding samples stored at -3.6 °C for 20 days and ice chilled (0 days) and frozen (37 days) references.

In cod (Paper III), the amount of extracted salt soluble proteins (Lower right panel in Figure 8) 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 samples (1.1 and 2.2%). In the ice chilled fillets the extractability seems to increase

after 3 weeks, especially for the salt soluble fraction. This was not the case for the superchilled samples, where the values remained low for the whole storage period. Similarly to what was seen in salmon, the low extractability of muscle proteins from cod tissue stored at -2.2 °C indicated freeze denaturation of the proteins.

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 cod fillets can therefore be explained by protein denaturation. In order to maintain good quality of the food product during superchilling, the process has to be optimized with regard to freezing rate and degree of superchilling to minimize protein denaturation and liquid loss. At the same time, the temperature has to be sufficiently low to ensure an increase in microbial shelf life.

A higher degree of freeze denaturation in superchilled vacuum packed salmon fillets can also explain the correlation with drip loss. The extractabilities of water- and salt soluble proteins were also correlated to the liquid loss.

The solubility of sarcoplasmic proteins from rib steaks of pork (Paper IV) was investigated by extraction in pure water. The amount of extracted protein from rib steak of pork was higher at 20/21 days of storage compared to at day 0. This may possibly be attributed to an incomplete resolution of rigor on the first sampling day, which was two days post mortem. At 20/21 days, the extractability was higher in chilled reference samples and in the samples that were deeper superchilled than in samples with a lower degree of superchilling. The values varied between 2.0% and 4.2%.

Proteolytic activity can increase the extractability of sarcoplasmic proteins and lead to the formation of small peptides. Generally, enzymatic activity can be observed by substrate decline or by product formation (Palmer, 1995). Since enzymatic assays are difficult to carry out at subzero temperatures, changes in the amounts of free amino acids can be used to observe the effect of proteolytic activity. Proteases (peptide hydrolases) are enzymes that cleave peptide bonds, either on peptide-terminus (exopeptidase) or internally in a polypeptide (endopeptidase or proteinase) (Bond & Beynon, 1989). These proteolytic enzymes play critical roles in biological systems. Determination of free amino acid content in the fraction of water-soluble (sarcoplasmic) proteins was carried out in vacuum packed salmon (Paper II) and cod (Paper III) and in wrapped salmon (Paper IV).

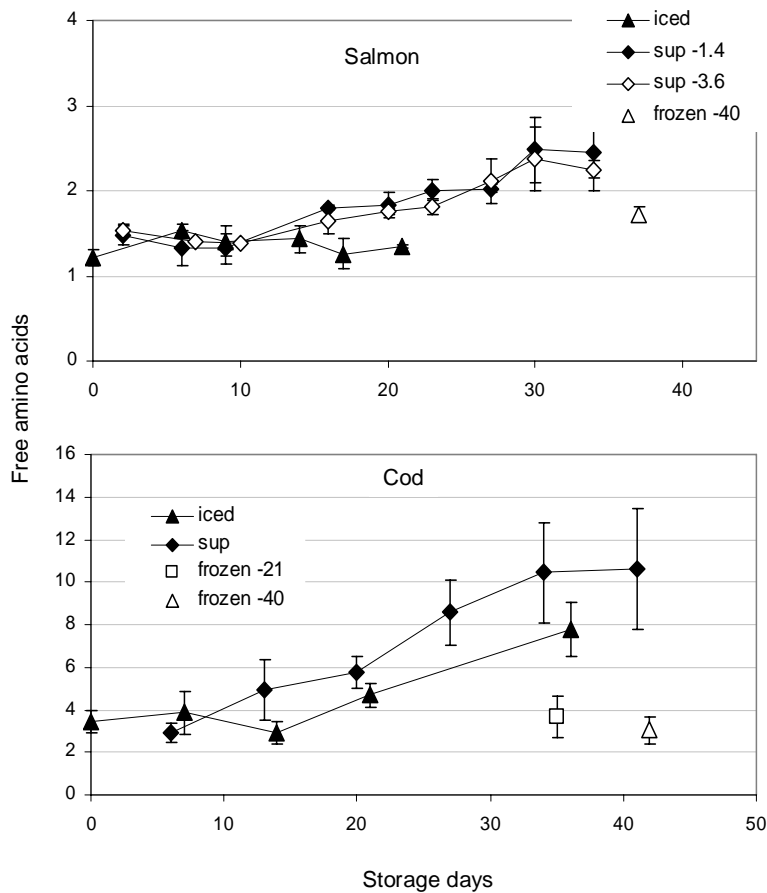


Figure 9: Changes in amounts of free amino acids extracted from vacuum packed cod and salmon fillets subjected to different chilling and storage conditions. Mean values of free amino acids are given in mg/g of wet weight. Y-error bars represent the standard error of the mean.

In cod (Paper III), both ice chilled and superchilled samples had higher levels of free amino acid compared to frozen samples after about five weeks (Lower panel in Figure 9). The total level of free amino acids was initially 3-4 mg/g wet weight. After 5-6 weeks of superchilling, it had increased to around 10 mg/g wet weight, while for ice chilled samples the value was about 8 after 37 days. This agrees with the findings of Sotelo, Franco, Aubourg and Gallardo (1995a), who state that the content of free amino acids in hake increases with storage time at -5 °C, but not at -20 °C.

In the iced or frozen salmon fillets and during the first two weeks of superchilled storage (Paper II), the content of free amino acids varied between 1.2 and 1.8 mg per g wet weight (Lower panel in Figure 9). This is in accordance with the findings in iced salmon (Hultmann & Rustad, 2004b) but lower than for superchilled cod (Paper III). Between two weeks and one

month, the values increased by up to 2.4-2.5 mg per g wet weight for the superchilled samples. Increases in the content of free amino acids indicate exoproteolytic activity during storage, which can be due to increased enzyme concentrations when part of the water is frozen. The free amino acid content in fish normally ranges from about 0.5% to 2.0% of muscle weight and is lower in farmed than in wild fish (Haard, 1992a).

The results of these two experiments indicate one or more of the following alternatives. The enzymes in cod may be more active than those in salmon or the proteins may be more susceptible to proteolysis. The former is supported by findings that show higher cathepsin B activity in cod (Hultmann & Rustad, 2007) than in salmon (Hultmann et al., 2004b).

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

Water-soluble proteins were extracted with pure water from wrapped salmon fillet and from vacuum packed rib steak of pork (Paper IV). Precipitations of the extracts with trichloroacetic acid (TCA) or ethanol were performed to evaluate the amount of peptides resulting from degradation of the water-soluble (sarcoplasmic) proteins. The intention was to relate the extent of protein degradation, and thereby enzymatic activity, to the process and storage treatment.

All proteins of the water extract are soluble in water, and their solubility in TCA decreases with increasing acid concentration and increasing peptide length (Yvon, Chabanet & Pélissier, 1989). The experiment was performed with final acid concentrations of 2.5%, 5% and 12% in the extracts. As expected, both in samples from salmon and from pork, the solubility of proteins decreased with increasing acid concentration. An exception was found for the

superchilled salmon samples on the last experiment days (day 24/25), which had a higher solubility at acid concentration of 5% compared to of 2.5%. This was not observed at 17/18 days. In the reference samples of rib steak of pork, the amount of acid soluble peptides increased with storage and was higher at 20/21 than at 0 days of storage. The amount of acid soluble peptides was higher in pork compared to salmon, while the amounts of free amino acids were the same.

The solubility of proteins decreases with increasing ethanol concentration (Rohm, Jaros, Rockenbauer, Riedler-Hellrigl, Uniacke-Lowe, Fox, 1996). The experiment was performed on the extracts with alcohol concentrations of 30% and 70% on storage day 0, and days 20/21. As expected, both in samples from salmon and from pork, the solubility of proteins decreased with increasing alcohol concentration.

4.4 Textural properties

The texture of fish is an important quality characteristic, and soft fillets are a problem for the fish industry (Hultmann et al., 2004b; Haard, 1992a; Sigholt et al., 1997). The hardness values were significantly higher in superchilled salmon fillets stored at -3.6 °C compared to superchilled fillets stored at -1.4 °C, ice chilled fillets and frozen fillets (Paper II). Results showed that the hardness values of salmon superchilled at -3.6 °C decreased during the first two weeks of storage. During the entire storage period, the hardness values of superchilled fillets stored at -1.4 °C were significantly higher than for the ice chilled or frozen fillets. Textural hardness values of ice chilled and frozen salmon were in the same range or higher than found in earlier studies (Hultmann et al., 2004b; Mørkøre, Hansen, Unander & Einen, 2002; Skjervold, Fjæra, Østby, Isaksson, Einen & Taylor, 2001). An increase in the maximum penetration force in ice stored post rigor filleted salmon has also been observed (Skjervold et al., 2001), which agrees with the present results. After three weeks of superchilled storage, the textural hardness values stabilized. At the end of storage, the hardness values of the frozen references were lower compared to at 0 days, while the values of superchilled samples were higher. From these results the conclusion is that softening of fillets is not found to be a problem in superchilled salmon. Textural analyses were not performed in the cod vacuum packed cod fillets (Paper III). However, superchilled cod fillets appeared less soft but also somewhat flakier than the ice chilled fillets.

4.5 Appearance

Color of Atlantic salmon is influenced by storage and the concentration of astaxanthin decreases with frozen (Regost, Jakobsen & Rørå, 2004) and chilled storage (Gobantes, Choubert & Gomez, 1998). In paper II, no differences in red or yellow color were found between samples stored at different temperatures.

However, in superchilled fillets of salmon (Paper II and IV), white spots emerged on the product surface (Figure 10). These spots were not present immediately after the superchilling, but appeared after equalization of temperature (i.e. first few days of storage). Superchilled fillets stored at the lowest temperature showed more intense spots than fillets stored at the higher superchilling temperature. Lightness values of vacuum packed fillets stored at $-3.6\text{ }^{\circ}\text{C}$ (Paper II) were generally higher than of those stored at $-1.4\text{ }^{\circ}\text{C}$. The higher values might be due to the more intense white spots at fillets stored at $-3.6\text{ }^{\circ}\text{C}$.



Figure 10: Spots developed on salmon fillet surface during superchilled storage.

Similar white spots were also detected on surface of cod fillets (Paper III) and rib steaks of pork (Paper IV) after about one week of superchilled storage. These spots were also 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 naked eye. Leakage of common amino acids is unlikely, since preliminary tests confirmed no difference in amino acid amount or composition on surface and non-surface muscle tissue (results not shown). However, further analysis should be made to clarify whether there are other low-molecular compounds present on the surface that are likely to cause this effect (Tanimoto, Okazaki, Morimoto & Yoneda, 2000).

We would suggest that this effect could be due to diffusional effects through drip channels from inside of the muscle to the 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, Koochmaraie & Goll, 1995). Degradation of the transmembrane protein integrin is also found to coincide with opening of drip channels (Lawson, 2004). However, in all species observed, the groups of samples which had the most spots did not correspond to the groups with the higher drip loss. One theory is that this may indicate that some of the proteins in the drip liquid denature in the drip channels. Nevertheless, an explanation still remains to be found. Effort should be put into reducing this spot effect, because of the negative effect on the appearance of the products.

4.6 Microbiology

Microbial examinations were performed in all experiments. In the fish samples, the growth of H₂S-producing bacteria was the main objective of the microbial studies. In the pork experiments, both psychrotrophic plate counts and total plate counts were examined. The total plate counts alone can not be used as an indicator of spoilage flora on food products (NFSA, 2002). However, total plate counts were performed to observe the differences in general growth during various storage conditions on the surfaces of the pork roasts (Paper I), the rib steaks of pork (Paper IV), and the wrapped salmon fillets (Paper IV).

According to the supplier, with the existing chilled storage procedures at temperatures around 2-4 °C, the shelf life of vacuum packed pork roasts is 14 days. This is consistent with our results on microbiological quality of the chilled references (Paper I). On the other hand, the bacterial counts in the superchilled samples remained at lower values for a significantly longer period of time. After 55 days of storage, the surface of these samples had 4.52 log units of CFU. At day 111 the value had increased to 6.13 log units. Both the chilled references and the superchilled samples with an interrupted superchilling process exceeded a value of 6 log units before 13 days of storage (Lower panel in Figure 11).

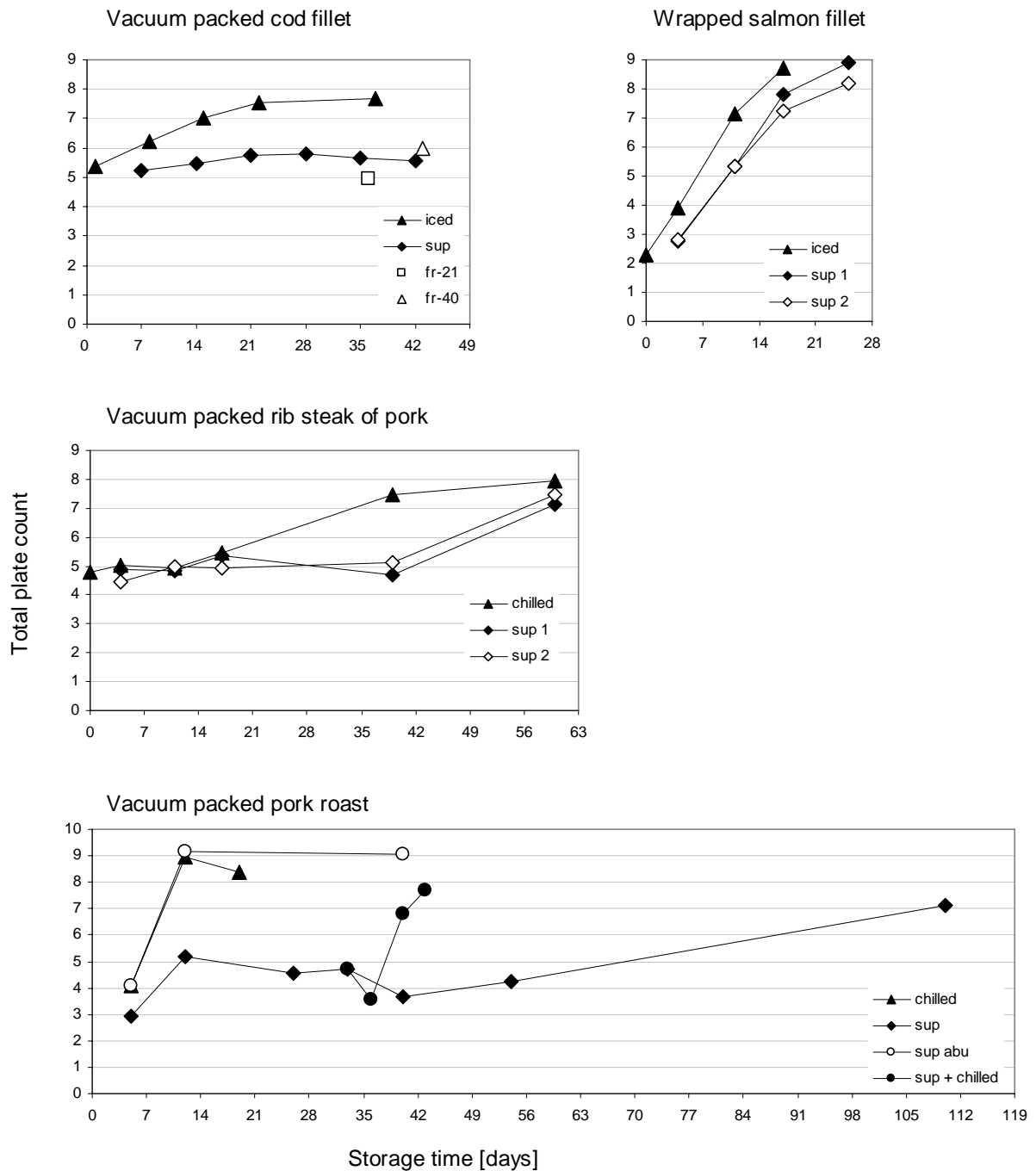


Figure 11: Changes in total plate counts on surface of vacuum packed cod, wrapped salmon fillets, vacuum packed rib steak of pork and vacuum packed pork roast subjected to different chilling and storage conditions. Mean values of plate counts are given as the number of colony forming units (CFU) per cm² of product surface for cod and for pork roasts, and as CFU per g for salmon and rib steak of pork.

The total plate count of the reference chilled samples of rib steak of pork (Paper IV) passed 6 log units of CFU between sampling day 17 and 39, while both groups of superchilled samples

reached this level between sampling day 39 and 60. The microbial level on the traditionally chilled samples was thus acceptable for at least 17 days, which is longer than the corresponding pork roast samples (Paper I). The superchilled samples were still at an acceptable level after 39 days (Middle panel in Figure 11).

The trend in the psychrophilic plate count on pork roasts (Paper I) was similar to that of the total plate count. The superchilled roasts had the lowest bacterial numbers, their bacterial count were low during the first 55 days of the storage period, but increased significantly between day 55 and 111. After 6-13 days the psychrotrophic counts of the chilled references reached high levels. The temperature abused samples showed the same development as the references. The long period of interruption in the superchilled storage (for 4 days up to +3.4 °C) allowed the microorganisms to grow equally well as in the ordinary chilled references. The samples that were transferred to +3.5 °C after 34 days at -2.0 °C showed a large increase in the psychrotrophic plate count after only three days. The values showed large variations and were lower than the chilled references after 13 and 20 days.

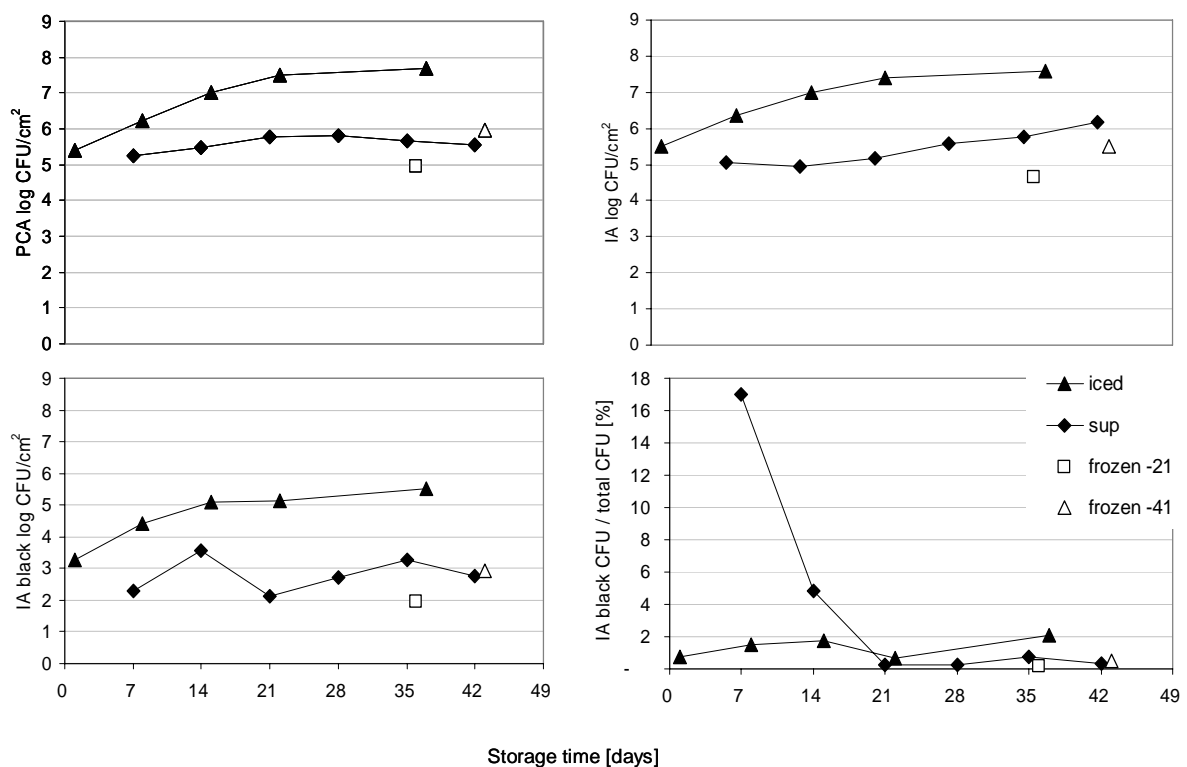


Figure 12: Changes in total plate (PCA) counts and iron agar (IA) counts on surface of vacuum packed cod fillet subjected to different chilling and storage conditions. Mean values

of plate counts are given as the number of colony forming units (CFU) per cm² of product surface.

The total plate count numbers (Upper left panel in Figure 12) on surface of ice chilled references of vacuum packed cod fillets increased from 5.4 log units CFU on day 1 to 7.5 and 7.7 log units after 22 and 37 days of storage, respectively (Paper III). Values above 5.7 log units are considered as high and 6.7 log units represents a limit for what is recommended for consumption, although total plate count number alone cannot be used as an absolute limit (NFSA, 2002). Superchilled samples generally had lower plate count numbers (5.2-5.8 log units of CFU) during the 6-week storage.

After two weeks, the total number of colonies on iron agar (Upper right panel in Figure 12) was generally much higher in ice chilled cod fillets compared to superchilled fillets. A maximum value of 7.6 log units CFU was reached after five weeks of ice chilled storage.

In the ice chilled samples, the black counts on iron agar (Lower left panel in Figure 12) increase during storage and the value is always higher than for the superchilled samples. After 7 days of superchilled storage, the fraction of H₂S-producing colonies on cod fillet surface is 17% (Lower right panel in Figure 12). 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.

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. The lower total plate count in superchilled compared to ice chilled cod fillets corresponded to the lower pH values in the superchilled fillets.

The total plate counts of the ice chilled reference samples of wrapped salmon fillets (Upper right panel in Figure 11) were significantly higher than for the other storage groups during the whole storage period (Paper IV). In the second half of the storage period (17 and 25 days), the bacterial growth on the samples at the lighter level of superchilling was higher than on samples at the deeper level. Between sampling days 4 and 10, the bacterial level of the

reference samples passed 6 log units of CFU. The superchilled samples were still below 6 log units of CFU after 10 days, but were above this level at day 17. This means that there was a 4-6 day delay in the total microbial growth on salmon samples, which may contribute to a corresponding increase in shelf life.

Our results (Paper II and III) confirm low growth of H₂S-producing bacteria during superchilled storage (Sivertsvik et al., 2003). Both ice chilled and superchilled salmon fillets stored at -1.4 °C held good or very good quality with respect to growth of sulphide-producing bacteria. Sulphide-producing bacteria were not detected in superchilled fillets stored at -3.6 °C or in frozen fillets. This confirms that the raw material was of high microbial quality.

The appearance of white spots of the surface of superchilled products did not correspond to the higher microbial growth on surface of these samples. On the contrary, the total plate counts of superchilled samples were lower than of the other storage groups. This observation also applies to the iron agar count on cod fillets. These findings are interpreted as a strong indication of that the spots most likely were not of microbial origin.

4.7 Other observations

For the main part, the temperature of the superchilled roasts (Paper I) was kept at -1.8 to -2.4 °C. These small variations seem to be tolerated, which was not the case for severe fluctuations as in the temperature interrupted storage. Superchilled samples with temperature abuse resembled chilled references with respect to drip loss and microbial levels. The superchilling storage improved shelf life from 2 to 16 weeks and drip loss was less than in traditional chilled storage. Shelf life might even be further prolonged if temperature is kept more constant.

This study shows that superchilling prolongs shelf life, but the optimal degree of superchilling (i.e. amount of ice formed) has not been identified for the chosen products. The amount of ice is very sensitive to alterations in temperature and thus both the process and storage conditions are crucial in order to obtain an optimal degree of superchilling. There is no satisfactory fast evaluation method for superchilling degree immediately after the initial chilling process, and time consuming calorimetric methods has to be used to classify the degree of superchilling. An uncontrolled process might lead to excessive drip loss and reduced water-holding capacity. Further actions should be taken to develop faster prediction and evaluation tools for

the degree of superchilling. Optimal process and storage conditions have to be established for each product type and quality.

Immediately after superchilling, the amount of water in the vacuum packed salmon samples to be stored at temperatures of $-1.4\text{ }^{\circ}\text{C}$ and $-3.6\text{ }^{\circ}\text{C}$ was $32 \pm 2\%$ and $49 \pm 5\%$ (mean \pm SEM), respectively (Paper II). The high ice content of the samples to be stored at $-1.4\text{ }^{\circ}\text{C}$ may be explained by a slightly too long duration of the chilling procedure since the ice fraction curve is very steep in this temperature range. The fillets stored at $-3.6\text{ }^{\circ}\text{C}$ had a firm, frozen appearance.

5 Conclusions

Superchilling has a potential for prolonging the shelf life of the chosen products. Based on both sensory and microbial evaluations, the superchilling storage improved shelf life of pork roasts from 2 to 16 weeks, and shelf life might even be further prolonged if temperature is kept more constant. The hygiene of the raw materials is important and initial bacterial counts on rib steaks of pork were high, but remained stable for twice as long (41/42 days) as for the chilled samples (20/21 days).

The H₂S-producing bacteria in superchilled cod fillets did not exceed the limit of consumption during the whole storage period of six weeks, while the microbial shelf life of the ice chilled fillets was estimated to be 8 days after processing.

The microbial counts were higher in ice chilled than in superchilled wrapped salmon fillets. However, this can not be directly related to shelf life. H₂S-producing bacteria were not detected in vacuum packed salmon during 34 days of superchilled storage at the lower temperature level.

Despite the fact that fish proteins are less stable than those of meat are, meat generally lost more of its weight as drip during storage and thawing in the present experiments than fish lost. Drip loss was less in superchilled pork roasts than in traditional chilled storage. Within the fish species studied, cod lost more drip than salmon. From the present results it can be concluded that superchilling did not lead to increased drip loss in the raw materials studied.

Superchilled storage resulted in reduced protein stability and higher liquid loss, especially in cod. In addition to affecting cooking and eating qualities, the liquid loss influences post storage processing of the products.

Textural analyses showed that softening did not occur in salmon fillets during the superchilled storage. However, cathepsin activities were relatively stable during the superchilled storage. This means that these enzymes are not deactivated at the selected storage temperatures and may therefore still lead to softening during subsequent chilled storage.

For the main part, the temperature of the superchilled pork roasts was kept at -1.8 to -2.4 °C. These small variations seem to be tolerated, which was not the case for severe fluctuations as in the temperature interrupted storage. Superchilled samples with temperature abuse resembled chilled references with respect to drip loss and microbial levels.

In superchilled samples of all species studied, white spots emerged on the product surface. However, the groups of samples which had the most spots did not correspond either to higher drip loss or to higher microbial growth on surface of these samples. This is interpreted as a strong indication of that the spots most likely were not of microbial origin.

There is a need to improve the process conditions, and effort should be made in order to obtain an optimal process for each product studied.

6 Further work

The next step would be to optimize the superchilling process and storage conditions of the products. In order to maintain good quality of the food product during superchilling, the process has to be improved with regard to chilling rate and storage temperature (i.e. the ice fraction) 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.

It has been found that the activity of some enzymes increases at temperatures just below 0 °C (Fennema, 1975). Due to concentration of solutes in the liquid when some of the water freezes out, the activity of these enzymes has to be clarified. In paper IV, some investigations on the products of proteolytic activity have been performed. Peptide solubility of fractions of water-soluble proteins has been studied. Further analyses should be conducted in order to identify the peptides and the cleavage pattern. This would enable classification of the enzymatic activity and the possibility to control it.

A high hygienic status of the raw material is essential for shelf life. Processing and further storage affects the number of bacteria and the bacterial flora, such as growth of specific spoilage bacteria and possibly food poisoning bacteria. More specific studies on the microbial flora should be pursued to determine shelf life more exactly and to ensure good quality and proper safety of the products.

Most experiments conducted in this work are performed on vacuum packed products. It can be relevant for further studies to combine superchilling with other techniques, e.g. MAP to obtain possibly positive synergistic effects and to help maintain a stable product temperature. The addition of cryoprotectants should be explored in combination with superchilling. It is reported that different cryoprotectants (amino acids and sugars) can prevent protein aggregation which is promoted by formaldehyde (Sotelo & Mackie, 1993).

There is no satisfactory fast evaluation method for measurement of the ice content after the initial chilling process, and time consuming calorimetric methods has to be used. Further actions should be taken to develop faster prediction and evaluation tools for the ice content of

the products. Each product still has to be evaluated on its own, due to compositional and geometrical differences.

For the food industry to use this technology it is also important to know that suitable equipment for handling, storage and transport is available. These aspects are currently being worked on.

7 Literature

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Quality changes during superchilled storage of pork roast

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Abstract

Quality parameters (sensory, physical, biochemical and microbiological) of superchilled vacuum packed boneless pork roasts were studied during storage for 16 weeks. Superchilling of vacuum packed pork roast at a temperature of $-2.0\text{ }^{\circ}\text{C}$ improved shelf life significantly compared to traditional chilled storage at $+3.5\text{ }^{\circ}\text{C}$. Superchilled pork roasts maintained good sensory quality and low microbiological counts during the whole storage period. The drip loss in superchilled samples was lower and showed less variation than in the references and the temperature abused roasts. The consequences of an interrupted cold chain during storage were also investigated. Superchilled samples with temperature abuse resembled chilled samples with respect to drip loss and microbial levels. To take advantage of all the benefits of superchilling and successfully implement the process in the food industry a stable, controlled temperature during storage is critical.

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Keywords: Superchilling; Pork; Sensory analysis; Bacterial count; Drip loss

1. Introduction

With the large increase in demand for fresh food, focus is on shelf life and food safety. The shift from frozen storage with a shelf life of months or years to fresh products with a shelf life of days or weeks has substantially changed the demands for chilling and the cold chain. A long shelf life is required in order to achieve sufficient time for distribution and sale. Hence, a huge effort is put into improving shelf life by a variety of techniques, such as modified atmosphere packaging (MAP), new additives and other hurdle technologies. However, the most important factor for improving shelf life is the product temperature. The actual storage temperature of chilled fresh foods is normally $4\text{--}10\text{ }^{\circ}\text{C}$ and in the Norwegian distribution chain the storage temperatures also vary a lot (Hemmingsen, 2002). At these temperatures, the activity of many spoilage microorganisms is still high and pathogenic bacteria are able to grow and produce toxins (Marth, 1998). Even if some pathogenic bacteria are able to grow at temperatures below $0\text{ }^{\circ}\text{C}$

(Sutherland, 2005), growth and toxin production of these bacteria are significantly reduced at lower product temperatures. Due to the long shelf life of superchilled products, the growth of pathogens needs to be further studied. To minimize the growth of spoilage and pathogenic bacteria, the storage temperature must be reduced as much as possible, without affecting the product quality.

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 $1\text{--}2\text{ }^{\circ}\text{C}$ below this. According to Fennema, Powrie, and Marth (1973), the initial freezing points (which is the temperature where ice starts to form in the product) of most foods are between -0.5 and $-2.8\text{ }^{\circ}\text{C}$. Since the initial freezing point of the pork roast is about $-1\text{ }^{\circ}\text{C}$, this indicates a superchilling range from -1 to $-3\text{ }^{\circ}\text{C}$. The surrounding temperature is set below the initial freezing point of the product, 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 heat load is assumed. In this temperature range, a drop in temperature of about $1\text{ }^{\circ}\text{C}$ will often result in doubling the amount of

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water frozen out (Power, Morton, & Sinclair, 1969). With the high latent heat of ice (335 kJ/kg), this will give a certain degree of after cooling of the product if the cooling is interrupted. A low product temperature can thereby be ensured even with a short cooling time.

For many food products, superchilling results in better quality than conventional chilling and superchilling is therefore an attractive compromise between freezing and conventional chilling. Sivertsvik, Rosnes, and Kleiberg (2003) found that superchilled storage increased the shelf life of Atlantic salmon three times while McMullen and Stiles (1994) found that the shelf life of retail ready pork cuts stored at -1.5°C was 7 weeks. Jeremiah, Gibson, and Argnoza (1995) found a shelf life of 9 weeks at the same temperature. Jeremiah and Gibson (2001) concluded that storage life could be more than doubled at -1.5°C compared to at $+2$ and 5°C .

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. This in turn may result in increased drip loss, loss of water holding capacity and textural changes. Freezing damage to the tissue can increase the enzymatic activity (Foegeding, Lanier, & Hultin, 1996). Many enzymes also have significant activity in partially frozen systems (Fennema, 1975). Increased rates of glycolysis and hydrolysis of phospholipids have been observed in the temperature range of -2.5 to -6°C (Fennema, 1975; Ohshima, Wada, & Koizumi, 1985). Changes in pH and salt concentration during freezing can activate, stabilize or inactivate enzymes depending on the enzymes and other conditions in the food (Fennema, 1975). The shelf life of superchilled food is far shorter than of 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 fewer changes in microstructure, which in turn may result in a lower degree of freeze denaturation and less drip loss.

In order to prolong product shelf life further, superchilling can be used in combination with several preservation factors or techniques (hurdles) such as vacuum packaging, MAP, appropriate HACCP systems, microbial inhibitors or combinations of these processes. MAP in combination with superchilling has shown promising results (Rosnes et al., 1998).

Today, the principle of superchilling is to a large extent used in commercial chilling processes. It is used to reduce residence time in equipment during cooling of pork carcasses – as so-called shock cooling. At commercial pork abattoirs, a considerable amount of the production that is cut and pending for sale is currently frozen. This practice represents increased costs and loss of commercial value. The costs increase both directly by freezing and thawing and indirectly by drip loss and general loss of quality. An additional cost of approximately 10 NOK (1.7 USD)/kg for freezing vs. chilling is the economical motive for using superchilling. Superchilling is cheaper than freezing because the amount of heat that has to be removed in superchilling is less than half the amount that has to be removed during freezing. In

superchilling, there is no need for further removal of heat after the superchilling process. During a standard chilling process, the products typically have a temperature of around 12°C , reducing the temperature down to 4°C requires removal of heat, and is also a time consuming process due to the small temperature differences. Superchilling can be a tool in shifting from frozen to chilled intermediate storage. The share of production that is not ordered for sale at the time of production can be superchilled and stored at superchilled temperature. This storage will also work as a buffer to the market, thereby simplifying the operations at the plant and improving the flexibility towards the market.

The aim of this study was to investigate the effects of a superchilling process on selected quality parameters of pork in order to prolong shelf life without freezing the meat. The consequences of an interrupted cold chain during storage were also investigated.

2. Materials and methods

2.1. Raw materials and processing

Boneless roasts from leg of pork of the Norwegian breed Noroc (LYLD), progeny of the crossing of a LY sow and a LD boar from the races Norwegian Landrace (L), Finnish Yorkshire (Y) and Norwegian Duroc (D), were used for the storage experiment. The average product weight of the 96 vacuum packed (Dixievac[®]) pork roasts (taken from the same number of pigs) was 1076 ± 259 g.

Two similar experiments were performed. In one experiment an adequate superchilling process was conducted. In the other experiment samples were superchilled in exactly the same manner, but were stored at fluctuating temperature. Two days after superchilling, the temperature in the storage room was raised from -2.0 to $+3.4^{\circ}\text{C}$ for 4 days and then returned back to -2.0°C (Fig. 1). The frequency of sampling from this experiment was however somewhat lower than for that of the correctly stored group. In addition, an experiment to investigate the subsequent cold chain from producer to

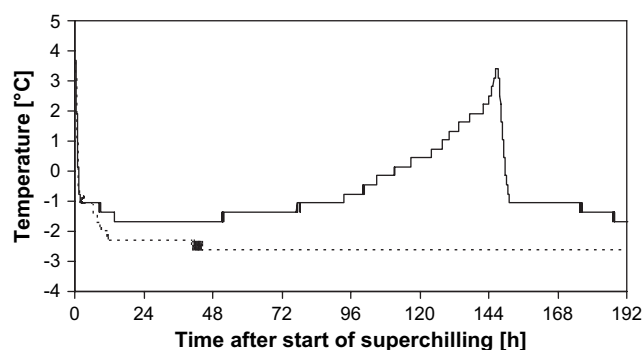


Fig. 1. Temperature in the center of pork roasts during cool down in a freezing tunnel of -30°C with air velocity of 2 m/s for 1 h 35 min and subsequent temperature equalization in storage at -2.0°C . The solid-drawn line shows temperature interrupted storage and the dotted line indicates the proper temperature progress. The temperature was detected every 2 min using logs with external sensors of the type StowAway Tidbit X.

consumer was performed by transferring superchilled samples to cold storage after 34 days. Chilled samples were used as references. All samples were from animals of the same age and size, and were equally treated with regard to slaughtering, hanging etc. prior to superchilling and storage. The experimental set-up is shown in Table 1.

The pigs were slaughtered at the production plant. After cutting and packing, the products were transported by refrigerated truck to the main storage. In the morning 2 days after slaughter, the products were delivered by refrigerated truck to the lab, where they were weighed and superchilled. The pork roasts were cooled down for 1 h 35 min in a freezing tunnel at a temperature of $-30\text{ }^{\circ}\text{C}$ and an air velocity of 2 m/s. The duration of the cooling was based on previous experiments and simulations on similar products (Hardarson, 1996; Hardarson & Magnussen, 2001). After cooling the samples were immediately transferred to a cold storage room at $-2.0 \pm 0.4\text{ }^{\circ}\text{C}$ for temperature equalization and storage. The day of chilling was defined as day 0; 15–17 h prior to analysis the samples were transferred to $+5\text{ }^{\circ}\text{C}$ for thawing.

2.2. Sampling for microbiological and sensory analyses

Analyzes of microbial and sensory quality were done at days 6, 13, 20, 27, 34, 41, 55 and 111 for the superchilled samples. Chilled references were analyzed after 6, 13 and 20 days and temperature abused samples were analyzed at days 6 and 13, and at day 41. Samples that were transferred to chilled storage subsequent to superchilled storage were analyzed at 34 + 3, 34 + 7 and 34 + 10 days. Two roasts were analyzed at each sampling time and used both for microbial and sensory analyses. The packages were opened and samples for microbial analyses taken as described below. The rest of the samples were then used for sensory analyses. The panel used was an internal, trained panel at the production plant. They were trained in quality evaluation of meat products.

2.3. Sensory analyses

A panel of three trained judges used an analytical quality control test to evaluate appearance and odor of raw products and the flavor and texture of roasted products. The products were roasted using a standardized procedure in a Teflon coated pan without the addition of fat. The properties were evaluated

on a scale from 1 to 9, where scores of 1–3 represented rejection. The judges were also giving comments in addition to the scores. The total score is the sum of the scores for appearance, flavor and texture. If one of the properties was rejected, the whole product was rejected. By scores of 7–9, the sample was characterized as good. The samples were coded and served in randomized order.

2.4. Microbiological analyses

Samples were collected as described by NMKL (2002). A 1–2 mm thick piece of surface was aseptically cut using a 10 cm² template, weighed in a sterile Petri dish and transferred to a Stomacher filter bag. Nine millimeters of sterile sodium chloride (9 g/l) was added and the sample was homogenized for $2 \times 30\text{ s}$ at 230 rpm. The homogenate was defined as dilution 10^{-1} . Dilution series of the homogenate were made in tubes with sterile NaCl (9 ml, 9 g/l). The same samples were used for both total plate count and psychrotrophic plate count. Two samples were cut from each pork roast.

Total plate count analysis was performed according to NMKL (1999). One milliliter of three dilutions was transferred in triplicate to empty Petri dishes; 9 ml of tempered ($46 \pm 2\text{ }^{\circ}\text{C}$) medium (Plate Count Agar (PCA), Difco) was added. Colonies were counted after $72 \pm 3\text{ h}$ incubation at $25\text{ }^{\circ}\text{C}$.

Psychrotrophic bacteria were determined by a modification of the NMKL (2000) method (plates were incubated at a lower and a uniform incubation temperature). The samples were distributed on top of PCA medium in Petri dishes and incubated at $5\text{ }^{\circ}\text{C}$. Colonies were counted after $168 \pm 3\text{ h}$.

2.5. Sampling for physical and biochemical analyses

Physical and biochemical analysis was performed in quadruplicate at days 1, 8, 15, 22, 29, 43, 57 and 113. References were cold stored ($+3.5\text{ }^{\circ}\text{C}$) and sampled in duplicate at 1, 8, 15 and 22 days and frozen stored ($-36\text{ }^{\circ}\text{C}$) and sampled in duplicate after 147 days. Temperature abused samples were analyzed in quadruplicate at days 1, 8, 22, 43 and 90. After determining the drip loss, samples were cut from the middle of the product. Connective tissue and surface were avoided in order to obtain more homogeneous samples. The meat was minced twice in a small food mill and kept on ice.

The vacuum packed bag was opened and all liquid was decanted into a graded cylinder for quantifying the drip loss. Squeezing of the samples was avoided. Approximately 2 g of the drip loss was weighed accurately and dried at $105\text{ }^{\circ}\text{C}$ for 24 h to determine the water fraction of the drip. This was done in duplicate.

pH was determined in duplicate in approximately 2 g of minced sample mixed with an equal amount of KCl (0.15 moles/l) (Bendall, 1973).

Liquid loss (LL) was determined on minced muscle by low-speed centrifugation as described by the water holding capacity method of Eide, Borresen, and Strom (1982). A centrifugal force of 210 g was used instead of 1500 g (Hultmann &

Table 1
Experimental set-up

Trial	Storage conditions	Number of samples for		Sum
		Sensory/microbial analyses	Physical/biochemical analyses	
1	Superchilling	16	32	54
	Superchilling followed by chilling	6		
2	Superchilling with temperature abuse	6	20	42
	Chilling	6	8	
	Freezing	2	2	

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 quadruplicate. 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.

During the superchilled storage the temperature was logged in the products and in the surrounding air. Logs with external sensors of the type StowAway TidbiT XT were used to perform measurements as close to the core of the product as possible. Air temperatures were logged by logs of the type StowAway-IS Temp with internal sensor. To determine necessary residence time of the products in the freezing tunnel, pilot experiments were performed. Results from these experiments indicated that the roasts needed to stay in the freezing tunnel for 1 h 35 min at a temperature of -30°C and air velocity of 2 m/s, which equals a convection coefficient to $20\text{ W/m}^2\text{K}$. This was sufficient to reduce the temperature in the center of a pork roast from the initial 3 to -1.1°C .

Microsoft Excel was used for data processing and statistical analyses. Assuming the uncertainties to be independent and random, the uncertainty in the difference between two numbers (means) was calculated as the square root of the sum of the uncertainties (standard deviation) in the numbers added in quadrature. The ratio between the difference divided by the uncertainty in the difference was used to find the percentage probability of obtaining this ratio if the numbers are not significantly different (Taylor, 1997). The significance level was set at 95%.

3. Results and discussion

3.1. Product temperatures

After equalization in superchilled storage at -2.0°C the temperature reached -2.3°C after 13 h and -2.6°C after

44 h (Fig. 1). The temperature in the products during storage was $-1.9 \pm 0.7^\circ\text{C}$. The decrease in temperature during equalization shows the importance of controlling the freezing process to obtain the right amount of ice in the products and also the importance of a stable controlled storage temperature.

3.2. Microbial analysis

According to the supplier, with the existing chilled storage procedures at temperatures around $2\text{--}4^\circ\text{C}$, the shelf life of vacuum packed pork roasts is 14 days. This is consistent with our results on microbiological quality of the chilled references. For the products stored at $+3.5^\circ\text{C}$, the total plate count increased rapidly (Fig. 2). On the other hand, the bacterial counts in the superchilled samples remained at lower values for a significantly longer period of time. The bacterial counts in the superchilled samples stored for 111 days were significantly ($p < 0.05$) lower than in the chilled samples stored for 19 days. This is in agreement with recent reports (Holley, Peirson, Lam, & Tan, 2004). After 55 days of storage, these samples had 4.52 log units colony forming units per gram of surface. At day 111, the value had increased to 6.13 log units. Both the chilled references and the superchilled samples with an interrupted superchilling process exceeded a value of 6 log units before 13 days of storage.

The trend in the psychrophilic plate count (Fig. 3) was similar to that of the total plate count. The superchilled samples had the lowest bacterial numbers, their bacterial count were low during the first 55 days of the storage period, but increased between days 55 and 111. After 6–13 days the psychrotrophic counts of the chilled references reached high levels. The temperature abused samples showed the same development as the references. The long period of interruption in the superchilled storage (for 4 days up to $+3.4^\circ\text{C}$) allowed the microorganisms to grow equally well as in the ordinary chilled references. The samples that were transferred to $+3.5^\circ\text{C}$ after 34 days at

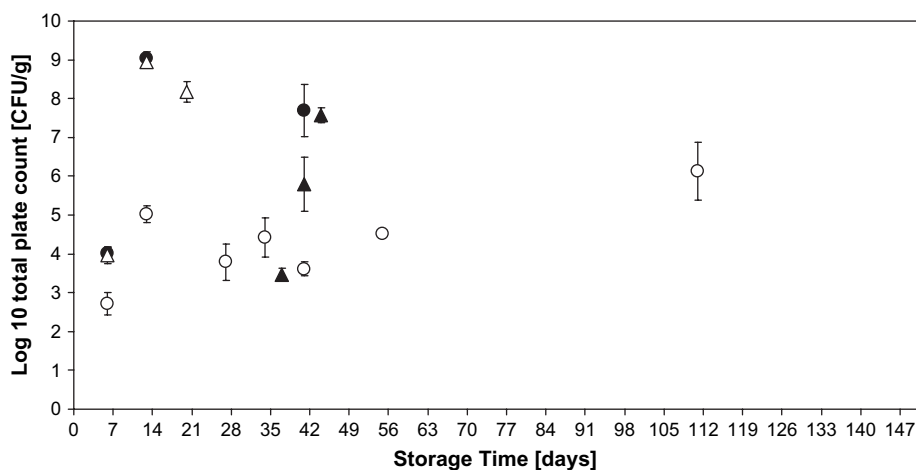


Fig. 2. The total plate count on the surface of vacuum packed pork roasts at different storage conditions after incubation for 72 ± 3 h at 25°C . Open circles indicate superchilled storage at -2.0°C , filled circles indicate superchilled storage at -2.0°C with temperature abuse for 4 days up to $+3.4^\circ\text{C}$, open triangles indicate chilled storage at $+3.5^\circ\text{C}$ and filled triangles indicate chilled storage at $+3.5^\circ\text{C}$ after 34 days of superchilled storage at -2.0°C . Standard error of the mean is shown as y-error bars. $N = 2$.

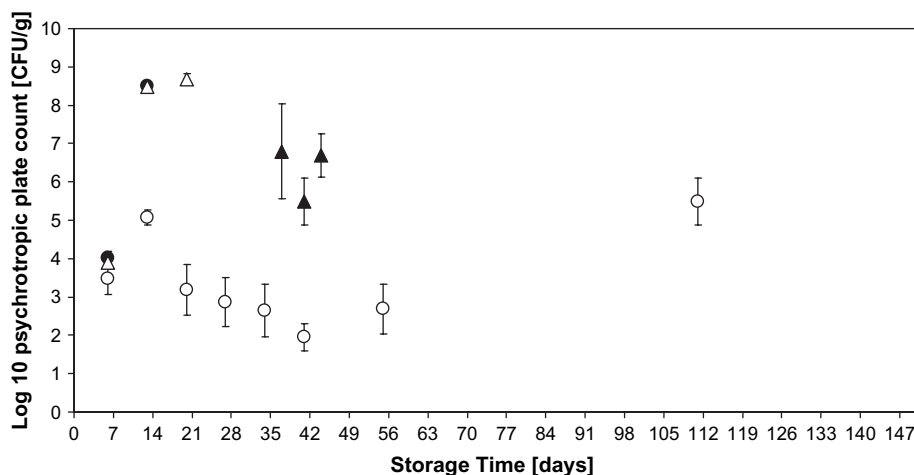


Fig. 3. The psychrotrophic plate count on the surface of vacuum packed pork roasts at different storage conditions after incubation for 168 ± 3 h at 5°C . Open circles indicate superchilled storage at -2.0°C , filled circles indicate superchilled storage at -2.0°C with temperature abuse for 4 days up to $+3.4^\circ\text{C}$, open triangles indicate chilled storage at $+3.5^\circ\text{C}$ and filled triangles indicate chilled storage at $+3.5^\circ\text{C}$ after 34 days of superchilled storage at -2.0°C . Standard error of the mean is shown as y-error bars. $N = 2$.

-2.0°C showed a large increase in the psychrotrophic plate count after only 3 days. The values showed large variations and were lower than the chilled references after 13 and 20 days.

3.3. Sensory analysis

The sensory quality of the superchilled pork roasts was acceptable during the entire storage period (Table 2). This

Table 2
Sensory scores of roasted vacuum packed pork roast during storage time at different storage conditions

Processing and storage method	Storage time [days]	Total score	Odor/taste score	Appearance score	Consistency score
Superchilled	6	8.0 ± 1.0	9.0 ± 0.0	8.0 ± 0.0	6.0 ± 1.0
	13	7.5 ± 0.5	8.0 ± 0.0	8.0 ± 0.0	7.5 ± 0.5
	20	7.5 ± 0.5	8.0 ± 0.0	7.0 ± 1.0	8.0 ± 0.0
	27	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	7.5 ± 0.5
	34	5.0 ± 2.0	5.5 ± 2.5	8.0 ± 0.0	7.0 ± 0.0
	41	8.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	7.5 ± 0.5
	55	7.5 ± 0.5	8.0 ± 0.0	7.5 ± 0.5	7.5 ± 0.5
	111	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	7.0 ± 0.0
Temperature abused	6	6.5 ± 0.5	7.0 ± 0.0	6.0 ± 0.0	7.0 ± 0.0
	13	7.5 ± 0.5	8.0 ± 0.0	7.5 ± 0.5	7.5 ± 0.5
superchilled					
Superchilled + chilled	34 + 3	7.5 ± 0.5	8.0 ± 0.0	7.0 ± 1.0	7.0 ± 0.0
	34 + 7	7.5 ± 0.5	7.5 ± 0.5	8.0 ± 0.0	7.0 ± 0.0
	34 + 10	7.0 ± 0.0	7.5 ± 0.0	8.0 ± 0.0	7.0 ± 0.0
Chilled	6	6.5 ± 0.5	7.0 ± 0.0	6.0 ± 0.0	7.0 ± 0.0
	13	8.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0
	20	3.0 ± 0.0	3.0 ± 0.0	—	—

Appearance and odor were evaluated on raw products while flavor and texture were evaluated on roasted products. The properties were evaluated on a scale from 1 to 9, where scores of 1–3 represented rejection. The total score is the sum of the scores for appearance, flavor and texture. Processing and storage methods of the pork roasts were as following: superchilled and stored at -2.0°C up to 111 days, superchilled and stored at -2.0°C with temperature abuse for 4 days up to $+3.4^\circ\text{C}$ for up to 13 days, superchilled and stored at -2.0°C for 34 days and subsequently at $+3.5^\circ\text{C}$ for additional 3, 7 and 10 days and stored at $+3.5^\circ\text{C}$ up to 20 days. Values are given as mean \pm SEM. $N = 2$.

supports the findings of others, who found the shelf life of vacuum packed pork loins stored at -1.5°C to be 15 weeks based on appearance (Jeremiah et al., 1995). Vacuum packed pork chops stored at -4°C which were still acceptable based on both appearance and off-odor after 7 weeks (Lee, Simard, Laleye, & Holley, 1985). An exception was observed at day 34, but this was probably due to individual variations in the raw material. Pork roasts transferred to chilled storage after 34 days of superchilled storage were of good sensory quality for an additional period of 10 days at the new temperature. This is in accordance with the development in total plate count numbers, but not with the count of psychrotrophic bacteria, which reached a high level after 3 days. References stored at $+3.5^\circ\text{C}$ held good quality for 13 days, but were of poor quality after 20 days. Sensory tests indicate that the quality of the superchilled roasts is not reduced by high numbers of psychrotrophic bacteria, neither in chilled references stored for 13 days nor in samples stored for 10 days at $+3.5^\circ\text{C}$ subsequent to superchilled storage. This is in accordance with Liu, Yang, and Li (2006) who found that maximum growth rate and lag-phase of spoilage bacteria had a better correlation with shelf life of chilled and superchilled pork than maximum cell number.

Canadian researchers stored pork under vacuum or modified atmosphere at -1.5 , 2 and 5°C , but the chilling procedure was not described (Greer, Dilts, & Jeremiah, 1993; Jeremiah & Gibson, 1997; Jeremiah et al., 1995). The effects on microbiological, sensory and color properties and weight loss were investigated. At -1.5°C , storage life based on appearance of CO_2 or vacuum packaged pork loin was greater than 16 weeks and slightly over 12 weeks, respectively. Nevertheless, due to off-flavor development coinciding with high numbers of lactic acid bacteria, actual storage life was restricted to 9 weeks in both packaging treatments (Jeremiah et al., 1995). Jeremiah and Gibson (2001) concluded that storage life can be more than doubled by storage at subzero temperatures (-1.5°C) compared to at 2 and 5°C .

3.4. Physical and biochemical analysis

At day 1, all roasts had a pH value of 5.6 ± 0.1 . The pH values of the temperature abused samples and the chilled references increased more than the superchilled roasts (results not shown). After about 4 weeks, the values of the superchilled samples decreased from 5.9 to 5.6. Holley et al. (2004) found that the pH started at 5.7 and remained relatively stable at pH 6.0 from week 6 to 8. The variation in the pH values of the superchilled samples was smaller than the variation in the other sample groups. During storage the mean water content of the pork roast samples varied from 75% to 77% (w/w).

The drip loss in superchilled samples was lower and showed less variation than in the chilled references and the temperature abused roasts (Fig. 4). After 7 and 21 days, the drip loss was significantly ($p < 0.05$) lower in the superchilled samples than in the chilled and temperature abused samples. The values varied between 30 and 55 ml/kg of product for the superchilled roasts, while the values of temperature abused superchilled, the chilled and the frozen references ranged between 54 and 110, 30 and 95, and 30 and 35 ml/kg of product weight, respectively. According to the manufacturer, frozen and thawed roasts lose around 100 ml/kg of product under industrial conditions. The results of the superchilled group were comparable to the results of others, who found values of drip loss from vacuum packed pork loins to be of 2–4.75% (Jeremiah et al., 1995). The results of the chilled references were also similar to the findings of others (Cayuela, Gil, Banon, & Garrido, 2004). Product weight losses due to drip loss can be as high as 1–3% in normal fresh retail cuts (Offer & Knight, 1988) and as high as 10% in pale soft exudative (PSE) products (Melody et al., 2004). In addition to loss of saleable weight, drip can contain more than 100 mg protein per millimeter fluid, mostly water-soluble sarcoplasmic proteins (Savage, Warriss, & Jolley, 1990). In our experiment the amount of dry matter of the lost fluid was between 11% and 14% (w/w) in fresh samples before stabilizing at

10–11% (w/w) after 1 month of storage (results not shown). A majority of the water lost as drip belongs to the entrapped (immobilized) water and preservation of this is a main goal in meat processing. The retention of entrapped water is affected by the net charge of the myofibrillar proteins and is therefore related to the muscle pH. The structure of the muscle cell, the properties of the myofibrillar proteins and the permeability of the membrane as well as the extracellular space are also influencing water mobility. It is likely that the gradual mobilization of water from intrafibrillar spaces to extrafibrillar spaces may be a key factor as a source of drip (Huff-Lonergan & Lonergan, 2005).

Water holding capacity or liquid loss influences the profitability of fresh pork products by affecting processing yields and palatability (Melody et al., 2004). The references and the temperature abused roasts showed better liquid retention than the superchilled ones (Fig. 5). Low drip loss will imply that more loosely bound water still remain in the product when the liquid loss analysis is conducted. The muscle pH decreased with storage time, while the liquid loss increased. When pH decreases, the net charge of the proteins is reduced (pI of myosin is 5.4) and hence the proteins can hold less free water. The standard deviations are high. This is probably due to raw material variations, since standard variations of individual samples were small.

For the main part, the temperature of the superchilled roasts was kept at -1.8 to -2.4 °C. These small variations seem to be tolerated, which was not the case for severe fluctuations as in the temperature interrupted storage. Superchilled samples with temperature abuse resembled chilled references with respect to drip loss and microbial levels. The superchilling storage improved shelf life from 2 to 16 weeks and drip loss was less than in traditional chilled storage. Shelf life might even be further prolonged if temperature is kept more constant.

This study shows that superchilling prolongs shelf life, but the optimal degree of superchilling (i.e. amount of ice formed) has not been identified for the chosen product. The superchilling

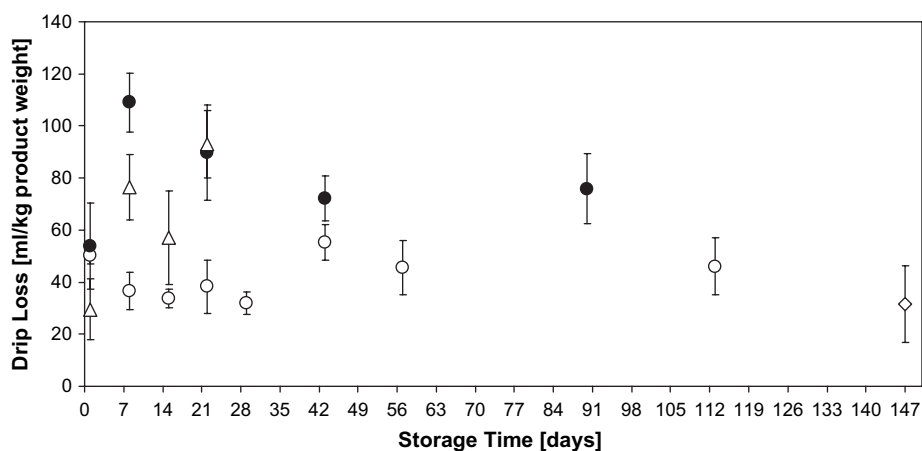


Fig. 4. Development in drip loss from vacuum packed pork roast during storage time at different storage conditions. Open circles indicate superchilled storage at -2.0 °C, filled circles indicate superchilled storage at -2.0 °C with temperature abuse for 4 days up to $+3.4$ °C, open triangles indicate chilled storage at $+3.5$ °C and open diamonds indicate frozen storage at -36 °C. Standard error of the mean is shown as y-error bars. $N = 4$ for the former two series and $N = 2$ for the latter two series.

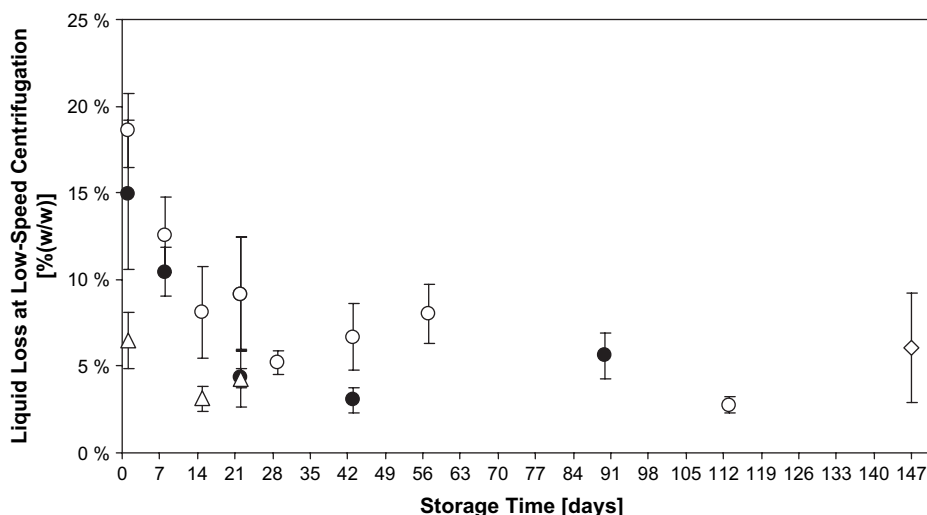


Fig. 5. Development in liquid loss at low-speed centrifugation of vacuum packed pork roast during storage time at different storage conditions. Open circles indicate superchilled storage at $-2.0\text{ }^{\circ}\text{C}$, filled circles indicate superchilled storage at $-2.0\text{ }^{\circ}\text{C}$ with temperature abuse for 4 days up to $+3.4\text{ }^{\circ}\text{C}$, open triangles indicate chilled storage at $+3.5\text{ }^{\circ}\text{C}$ and open diamonds indicate frozen storage at $-36\text{ }^{\circ}\text{C}$. Standard error of the mean is shown as y-error bars. $N = 4$ for the former two series and $N = 2$ for the latter two series.

degree is very sensitive to alterations in temperature and thus both the process and storage conditions are crucial in order to obtain an optimal degree of superchilling. There is no satisfactory fast evaluation method for superchilling degree immediately after the initial chilling process, and time consuming calorimetric methods have to be used to classify the degree of superchilling. An uncontrolled process might lead to excessive drip loss and reduced water holding capacity. Further actions should be taken to develop faster prediction and evaluation tools for the degree of superchilling. Optimal process and storage conditions have to be established for each product type and quality.

4. Conclusions

Superchilling pork roasts at $-2.0\text{ }^{\circ}\text{C}$ improved shelf life significantly compared to traditional chilling at $+3.5\text{ }^{\circ}\text{C}$. The superchilled roasts maintained good sensory quality and low microbiological counts during the whole storage period (16 weeks) while the shelf life of chilled samples was 14 days. Sensory tests indicate that the quality of the superchilled roasts is not reduced by high numbers of psychrotrophic bacteria.

The drip loss in superchilled samples was low and showed less variation than in the chilled references and the temperature abused samples. The temperature abused and the chilled samples had lower liquid loss, measured by centrifugation, than the superchilled samples.

Temperature fluctuations during superchilled storage are unfortunate and should be avoided. The amount of ice in the products is highly dependent on the temperature and this has a large influence on the quality changes during storage.

There is a need to develop faster and improved prediction and evaluation tools to control the amount of ice that needs to be formed. This knowledge will be critical in order to

gain a deeper understanding of the relations between the superchilling mechanism and the identified benefits.

This study shows that superchilling prolongs shelf life, but the optimal degree of superchilling has not been identified for the chosen product.

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

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Abstract

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

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

1. Introduction

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

The most important factor for increasing shelf life is the product temperature, and since fish is more highly perishable than meat, the temperature is even more important. Superchilling is often used to describe a process, where

food products are stored between the freezing point of the products and $1-2$ °C below this. The surrounding temperature is set below the initial freezing point of the food, which is between -0.5 °C and -2.8 °C (Fennema, Powrie, & Marth, 1973), and depending on the method used some ice is formed in the outer few millimeters. For many food products, superchilling results in better quality when compared to conventional chilling (Einarsson, 1988).

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

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

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

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

2. Materials and methods

2.1. Raw materials and processing

Twenty-one Atlantic salmon (*Salmo salar*) from a fish farm in mid-Norway, were slaughtered (stunned by a blow

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

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

2.2. Sampling

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

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

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

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

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

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

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

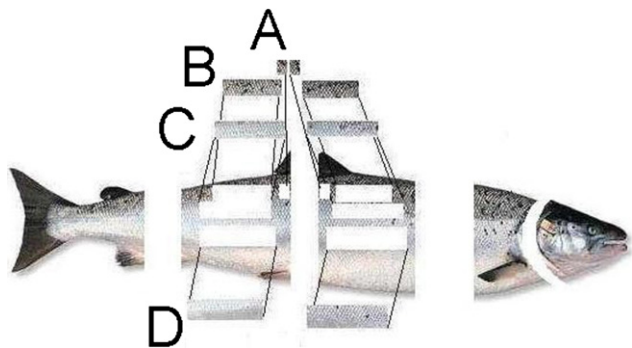


Fig. 1. Sampling locations. A: sulphide producing bacteria, B: water holding capacity, protein and moisture content, C: texture analysis and D: protein extractability and enzymatic activity. All analyses were performed on ventral side of fillet.

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

Liquid loss (LL) was determined on minced white muscle by low-speed centrifugation as described by the water holding capacity method of Eide, Borresen, and Strom (1982). A centrifugal force of 210g was used instead of 1500g (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 quadruplicate. Water content in the mince was determined by drying minced sample of ~ 2 g at $105^\circ C$ for 24 h. The analyses were run in duplicate.

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

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

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

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

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

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

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

2.3. Data analysis

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

3. Results and discussion

3.1. Estimates of ice content of superchilled samples

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

3.2. Drip loss

The drip loss was found to be larger, and to vary more in samples stored at -1.4 °C than in the other storage

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

3.3. Muscle pH and liquid loss

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

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

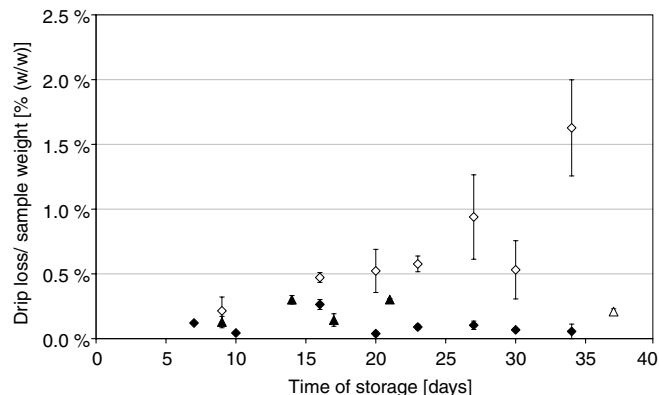


Fig. 2. Relative quantity of drip loss in farmed salmon during storage. Iced and frozen references and superchilled samples at -1.4 and -3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Values (percentage of initial sample weight) are given as mean \pm SEM.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

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

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

Values are given as mean ± SEM.

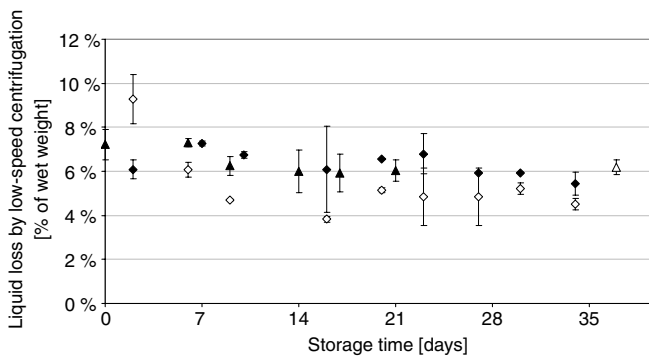


Fig. 3. Changes in liquid loss in farmed salmon during storage. Iced and frozen references and superchilled samples at -1.4 and $-3.6\text{ }^{\circ}\text{C}$ are represented by filled and open triangles and open and filled diamonds, respectively. Values (percentage of sample weight) are given as mean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

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

In ice chilled salmon fillets, the liquid loss (5.9 – 7.3%) was lower than that of cod fillets (8.6 – 14.5%). In superchilled salmon fillets, the liquid loss was generally much lower (3.8 – 9.3% at $-1.4\text{ }^{\circ}\text{C}$ and 5.4 – 7.3% at $-3.6\text{ }^{\circ}\text{C}$) than in cod fillets (20.5 – 29.8% at $-2.2\text{ }^{\circ}\text{C}$) (Duun & Rustad, in

press). These results are supported by Ofstad, Kidman, Myklebust, and Hermansson (1993), who found the liquid loss in coarsely chopped cod muscle to be more than twice as high as for salmon muscle.

3.4. Bacterial status

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

3.5. Fillet colour

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

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

Storage time (days)	Ice chilled storage	Superchilled storage at $-1.3\text{ }^{\circ}\text{C}$	Superchilled storage at $-3.6\text{ }^{\circ}\text{C}$	Frozen storage at $-40\text{ }^{\circ}\text{C}$
0	Very good	–	–	–
9	Good	Very good	–	–
10	–	–	Very good	–
17	Good	–	–	–
20	–	Good	Very good	–
21	Very good	–	–	–
30	–	Very good	Very good	–
34	–	Very good	Very good	Very good

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

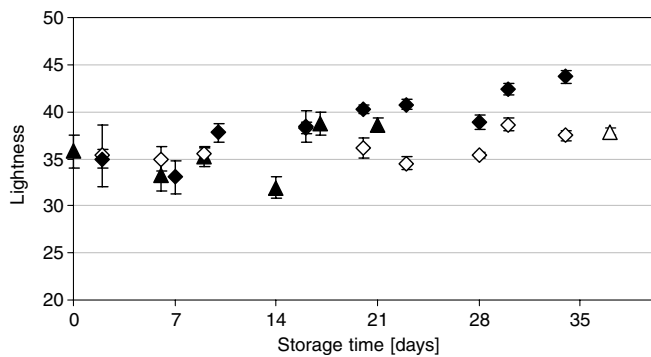


Fig. 4. Colour changes on surface of farmed salmon fillets during storage. Iced and frozen references and superchilled samples at -1.4 and $-3.6\text{ }^{\circ}\text{C}$ are represented by filled and open triangles and open and filled diamonds, respectively. Lightness is given as mean CIE L -values of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

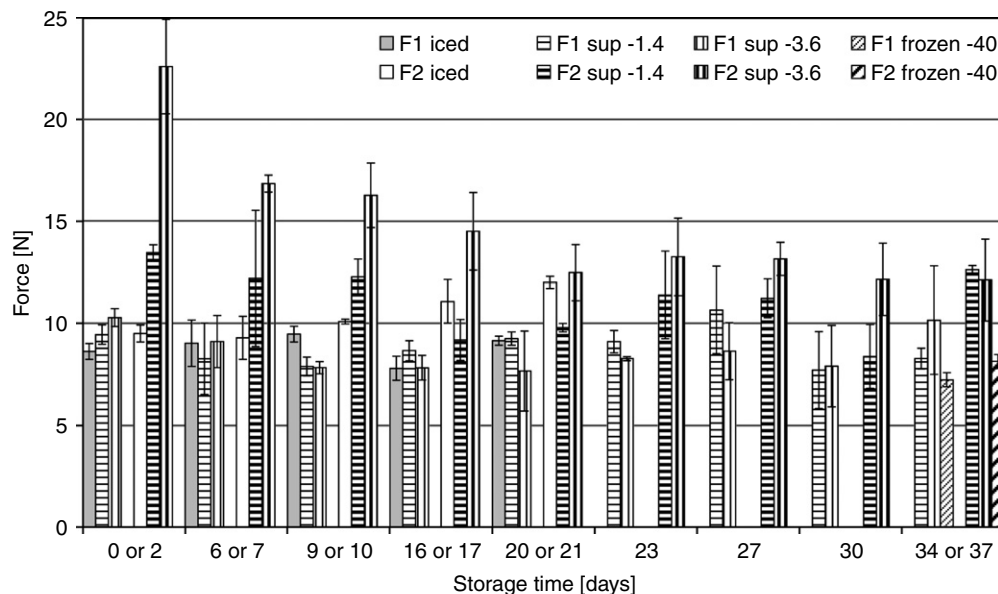


Fig. 5. Changes in fillet fracturability (F1) and hardness (F2) in farmed salmon during storage. Values are given as mean force (N) \pm SEM. $N = 2$.

due to the more intense white spots of fillets stored at $-3.6\text{ }^{\circ}\text{C}$. The origin of similar white spots on cod fillet surface may be caused by drip channels (Duun & Rustad, in press).

3.6. Textural profile analyses

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

3.7. Protein stability

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

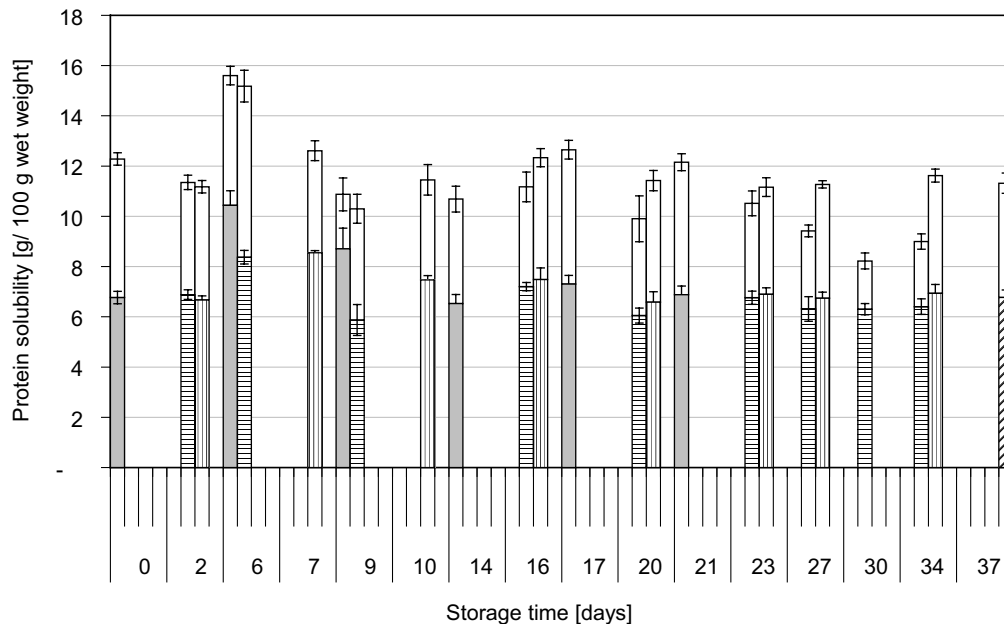


Fig. 6. Changes in extractability of water soluble and salt soluble protein in farmed salmon at 4 °C during storage are shown as lower (shaded) and upper bars (white), respectively. Iced and frozen references and superchilled samples stored at -1.4 °C or -3.6 °C are represented with gray bars, bars with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

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

3.8. Cathepsin activities

Cathepsin B is a lysosomal cysteine protease that hydrolyses a wide range of proteins and has an important role in the hydrolysis of tissue proteins (Barrett & Kirschke, 1981). Cathepsin B + L activity is the combined activity of cathepsin B and cathepsin L. Activities of cathepsins B and B + L at 5 °C and, 20 °C were relatively stable during the storage period (Fig. 8) in all storage groups. This means that these enzymes are not deactivated at the selected storage temperatures and may, therefore, lead to softening dur-

ing subsequent chilled storage. As expected, the activity values of cathepsins B + L was higher than that of cathepsin B, and higher at 20 °C than at 5 °C. The cathepsin B activities of ice chilled samples were similar to those of Hultmann and Rustad (2004).

3.9. Amount of free amino acids

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

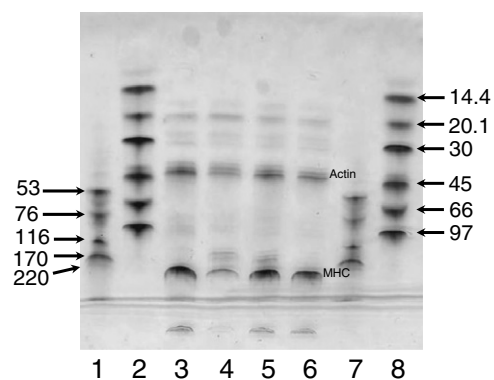


Fig. 7. Composition of salt soluble proteins in farmed salmon during storage using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples of ice chilled (0 days), superchilled at -1.4 °C (20 days) and -3.6 °C (20 days) and frozen (37 days) salmon fillet are represented in well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

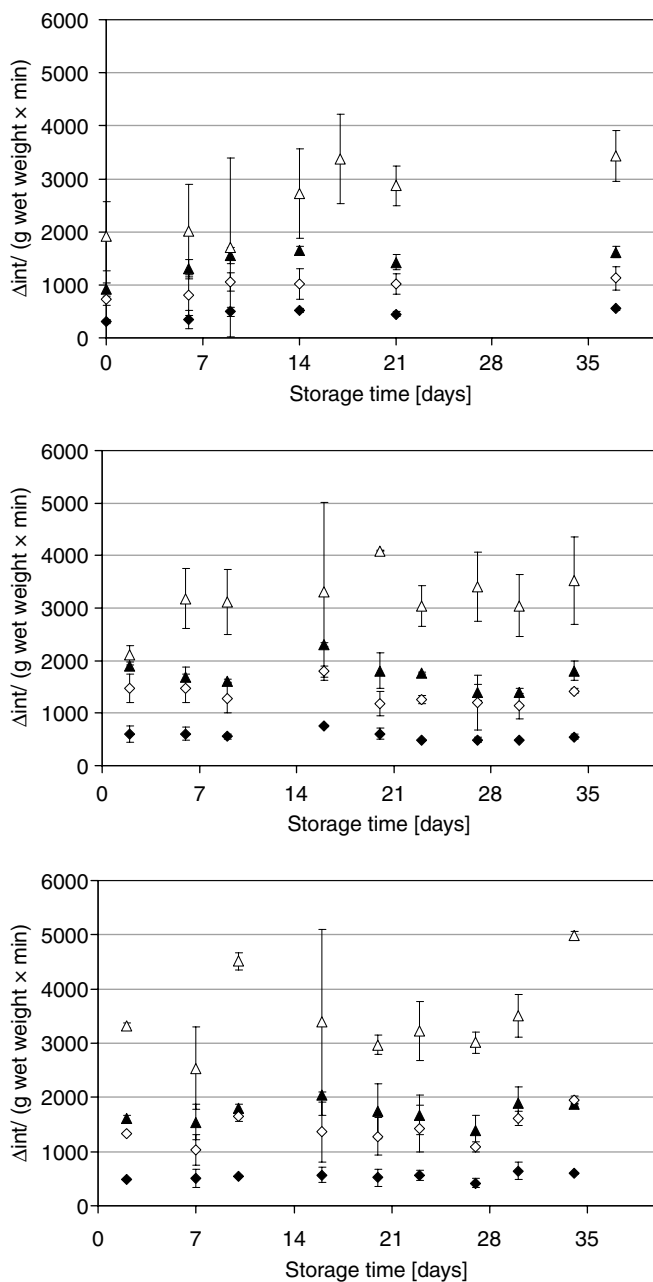


Fig. 8. Cathepsin B and B + L activities in farmed salmon during storage. The panels display activities in iced or frozen references (day 0–21 or day 37, respectively) (upper), superchilled at -1.4 °C (middle) and superchilled at -3.6 °C (lower). Cathepsin B + L activity at 20 °C and 5 °C and cathepsin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

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

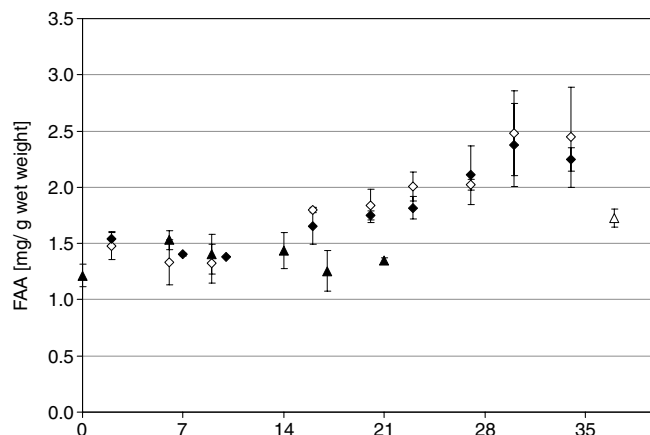


Fig. 9. Changes in the amount of free amino acids in farmed salmon during storage. Iced and frozen references and superchilled samples at -1.4 and -3.6 °C are represented by filled and open triangles and open and filled diamonds, respectively. Values are given as mean \pm SEM of mg/g wet weight. $N =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

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

4. Conclusion

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

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

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

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

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

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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 $-2.2\text{ }^{\circ}\text{C}$ 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.

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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 $1\text{--}2\text{ }^{\circ}\text{C}$ below this. The initial freezing points of most foodstuffs are between $-0.5\text{ }^{\circ}\text{C}$ and $-2.8\text{ }^{\circ}\text{C}$ (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, Oehlenschläger, 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 gutted fish weight, was $13.9 \pm 1.7\%$. 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 cm² 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 230g was used instead of 1500g (Hultmann & Rustad, 2002). The LL is expressed as the percentage of weight of the mince lost during centri-

fugation 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 KH₂PO₄, pH 7.0) at 4 °C using an Ultra Thurrax and centrifuged (20 min, 10,400g, 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 KH₂PO₄, 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 sulphosalicylic acid (10%) and leaving overnight at 4 °C (Osnes & Mohr, 1985). The samples were centrifuged (10 min, 600g, 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*-phthalaldehyde (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/ cm² 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/cm²) 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 ferric citrate and cysteine, *S. putrefaciens*-like organisms forms H₂S resulting in a black precipitate of FeS. The growth of H₂S 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 H₂S 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 H₂S-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 H₂S-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

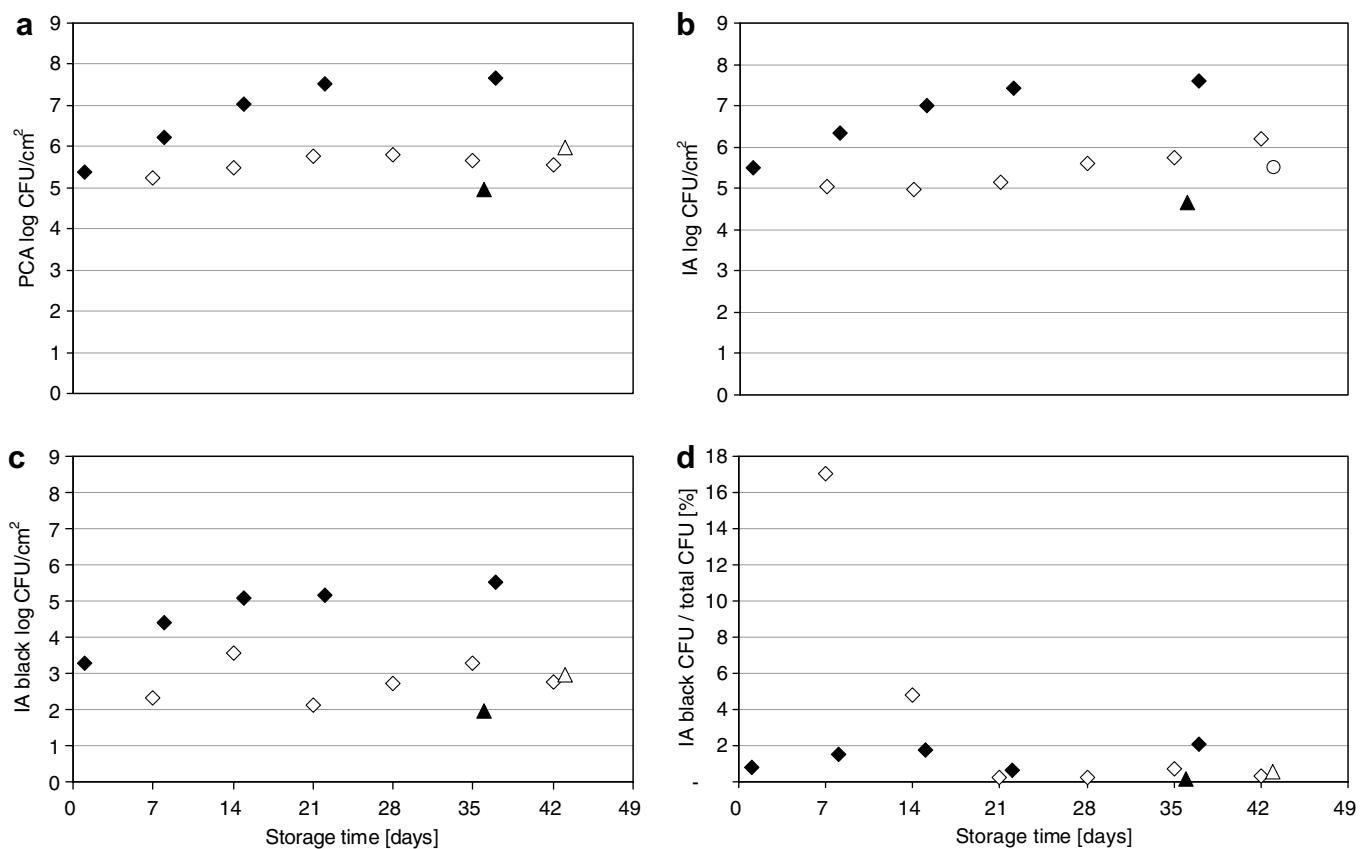


Fig. 1. Development in the total plate counts (PCA) and iron agar (IA) counts on the surface of vacuum packed portions of cod fillet at different storage conditions. (a) Total plate counts after incubation for 72 ± 3 h at 25 °C, and (b)–(d) total counts, black counts and the percentage of black counts 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

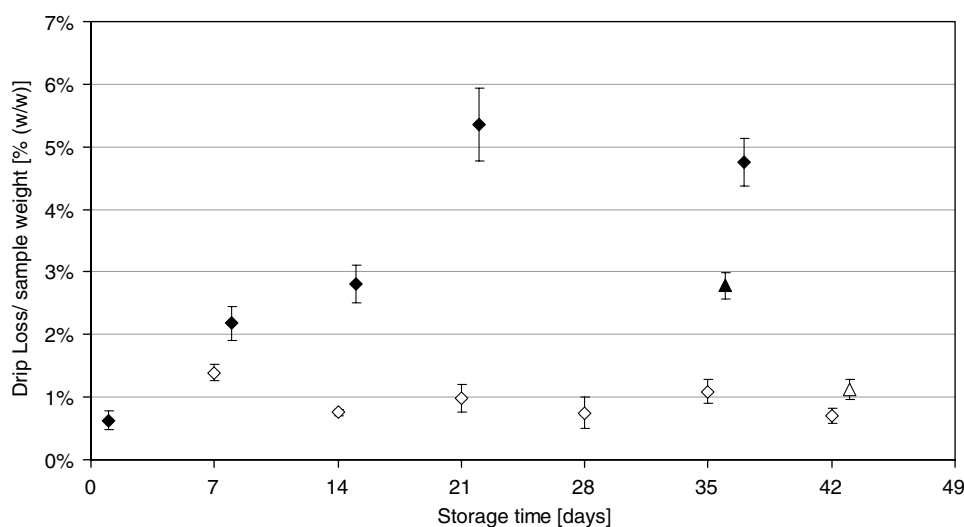


Fig. 2. Development in drip loss from vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at -2.2°C , filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at -21°C and open triangles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

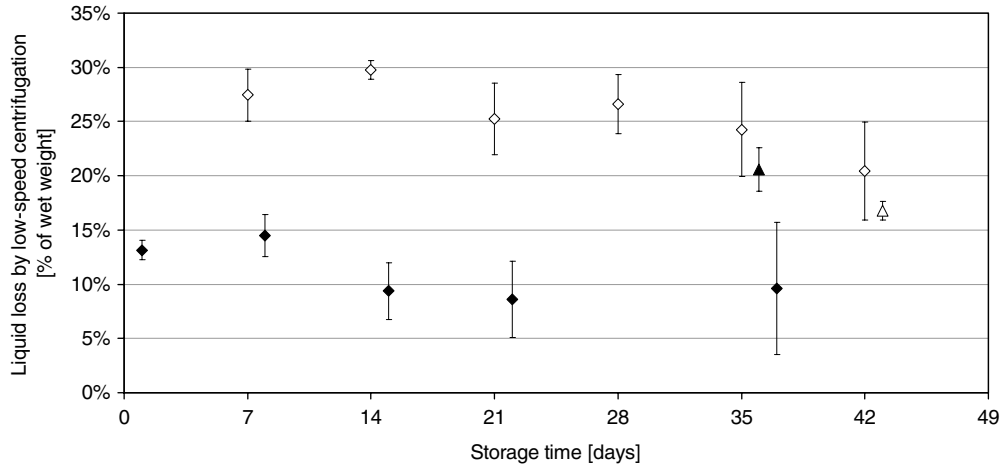


Fig. 3. Development in water holding capacity expressed as liquid loss (LL) of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at -2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen 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

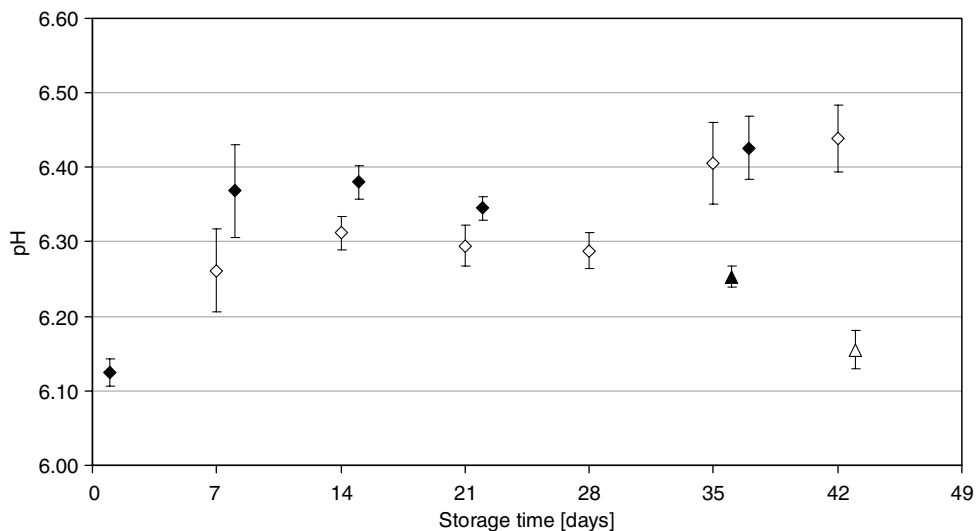


Fig. 4. Development in pH of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at -2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at -21 °C and open triangles indicates 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

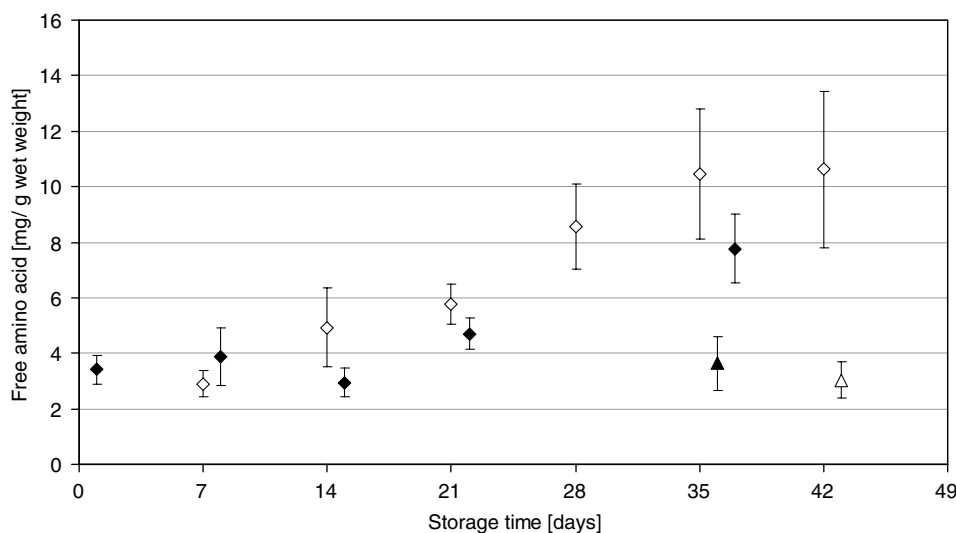


Fig. 5. Development in free amino acid content of vacuum packed portions of cod fillet during storage time at different storage conditions. Open diamonds indicates superchilled storage at –2.2 °C, filled diamonds indicates ice chilled storage, filled triangles indicates frozen storage at –21 °C and open 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 H₂S 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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