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FRISBEE

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2. Introduction

The general quality concept for fish products has not been very well defined over the years. In general, the definition of fish quality has been “What is acceptable for the customer and what they are willing to pay for”. High quality has normally been defined as “when the appearance, the smell, the taste and composition of the fish are equal to and normal for fresh slaughtered fish”.
The accompanying description in the DOW is as follows; “The quality and safety attributes of the selected products to be considered in this subtask are presented in Table 1.” For chilled / superchilled and supercooled salmon this is measurements of texture and specific spoilage organisms. “The following steps are undertaken for each food product. SINTEF will compile existing microbiological and quality kinetic models and data related with salmon under chilled and superchilled conditions. The compilation will include an extended literature search and, with respect to microbiological safety issues for the food products under consideration, the careful exploitation of the public domain microbial data and model based *ComBase* and the commercial predictive environment *Sym’Previus*”.

3. Quality and Safety Models for Chilled Fish

3.1. Introduction

Trends in the food industry have moved from frozen to high quality fresh products during the last years. This requires better control of the cold chain of food products during production, storage and retailing. The FRISBEE project will provide new tools, concepts and solutions for improving refrigeration technologies along the European food cold chain, including fish processing and distribution (GA 245288 FRISBEE DoW 25/05/2010).

Knowledge and research have shown that temperature is the most important parameter affecting quality loss in fish. In practise, the quality of fish decreases approximately linearly with time as long as the temperature and other conditions are constant. Shelf life decreases rapidly as temperature rises, and generally speaking, more or less halves in time if temperature rises 4-6 °C. Figure 1 shows storage time in days due to linear quality deterioration at different storage temperatures. This illustrative quality scale (used in the fish industry in Norway), ranges from nine to zero with a limit for acceptable quality of five.

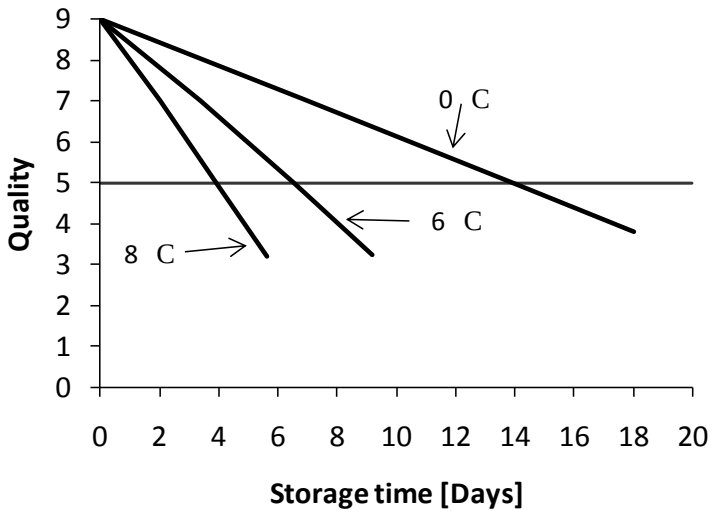

Figure 1 Changes in quality of fish with storage time and temperature

Expected storage life of a chilled fish product depends in addition to storage temperature on initial product quality, processing/handling methods and packaging (the PPP-factors). Figure 2 shows the changes in eating quality of iced cod, in relation to major changes due to autolysis and increased bacterial activity. Here, the limit for acceptable quality limit is 4 for cod stored at 0 °C. Hence, the maximum storage time, based on Figure 2, is 12 days.

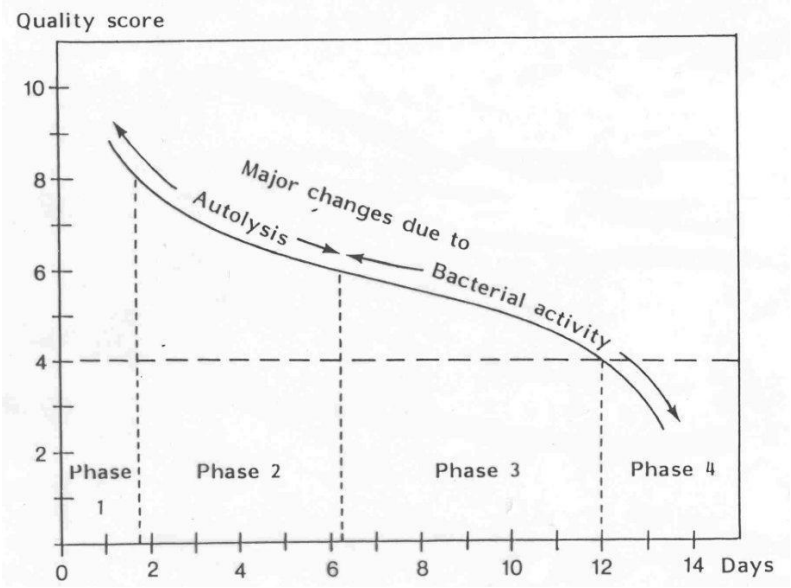

Figure 2 Changes in eating quality of iced (0°C) Cod (1)

In an unbroken cold chain, food products are continuously stored and transported at low and controlled temperatures, all the way from the producer, to the retail dealer and preferably until consumption. To prevent food borne disease outbreaks and to guarantee that the shelf life of the products is in accordance with its use-by dates, the cold chain for fresh foods should not be broken (2).

The regulations for chilled foods have for years been less stringent, with respect to storage conditions, compared with frozen foods. However, national and international legislation and recommendations now seem to be in accordance with the known temperature/shelf life correlation. Most countries have established mandatory temperature limits for the storage, transport and distribution of chilled foods. Maximum temperature limits for foods during transportation between countries are also established (3). The maximum temperature limit for the storage of most fresh foods is in Norway 4 °C (4) and in Europe 5 °C.

In reality, temperatures above these legally binding temperature limits during the cold chain of fresh foods are a major problem. Torstveit et al (1998) investigated temperatures in cabinets in grocery stores during a period of two years from 1995-1997. The results showed that even though regulations are introduced they seem to have no effects on temperature control (5). Similar problems are experienced also in other countries. As lined out in Hemmingsen et al (2002) low education and understanding of temperature load, bacteria growth and product shelf life, may be a reason for the unsatisfactory situation (6).

In order to maintain the recommended temperatures throughout the cold chain, it is important that air and product temperatures are measured correctly. The air outlet temperature in a refrigerated counter is for instance most often different from the product temperatures in the very same counter (7).

It is recommended that the storage temperature is as constant as possible. At fluctuating storage temperatures, foods could undergo unfavourable chemical and biological reactions. These changes might influence the appearance as well as the safety of the product (2).

From a thermal point of view “chilling” and “chilled storage” require different processes and equipment. The differences between the two processes must be understood and used to achieve temperature-controlled cold chains. In a chilling process, the food temperature must be reduced quickly and the required refrigeration capacity is large. The temperature difference between the product to be chilled and the chilling agent is the driving force of the heat removal. This driving force is reduced during the chilling period as the product temperature approaches the temperature of the chilling medium. The heat removal rate also depends on the product surface area, the surface heat transfer coefficients as well as the thickness/geometry and the thermal conductivity of the product. The heat flow from the product’s surface and from within, leads to temperature differences inside the product.

The most commonly used media for food chilling are air (air blast) and water (hydrocooling), but also chilling methods as vacuum chilling, contact chilling or evaporative chilling are used today (3). Refrigerated seawater (RSW) is an effective medium for fish chilling. The choice of a chilling system should be based on product quality requirements as well as economic and thermodynamic limitations.

In chilled storage, transportation and distribution of fresh foods, the refrigeration capacity must be sufficient to maintain a desired product temperature with a minimum of fluctuation. For most foods, mean product temperatures should not be more than 1-2 °C above the desired storage temperature when entering the storage arrangement(3). It is difficult to remove greater amount of heat during the actual storage period of the products in the equipment designed for storage, due to the small temperature difference between the products and the surroundings. If the storage temperature increases, even only for a little while, the product temperature quickly reaches a level where storage life is reduced and several pathogenic bacteria can grow, some of them producing toxins (8, 9), others being infectious by themselves. The storage temperatures should also be as constant as possible during storage, especially at temperatures around 0 °C (3).

3.2. Quality of chilled fish

The shelf life of different fish species varies due to storage temperature. Figure 3 shows practical shelf life for salmon, cod and herring and the dependency on temperature. One should notice, in particular, the relatively large variation in shelf life around 0 °C. As shown in Figure 3, a temperature rise from 0 °C to 2 °C reduces the shelf life of salmon by approximately six days or 25 %. Rapid cooling and maintenance in temperature at between zero and 1 °C during processing and storage of fish is therefore of high importance.

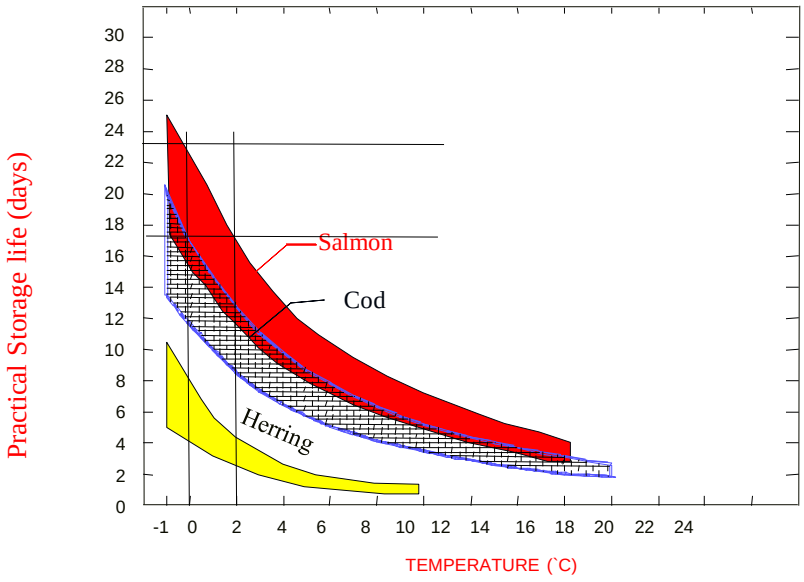

Figure 3 Practical storage life for some important fish species (Nordtvedt, 2009)(10)

3.3. Safety models for chilled foods

In 2006, James et al (11) published a review article on modelling of food transportation systems. The article states that modelling up till now has been used to aid the design and optimisation of food refrigeration systems, and that effort has concentrated on modelling of refrigeration processes that change the temperature of the food (chilling, freezing and thawing). Refrigerated storage systems used to maintain the temperature of the food have attracted less attention from modellers. The review focuses on the modelling of food temperature, microbial growth, and other parameters in the transportation of food.

Microbiological safety of food relies on microbial examination of raw materials and final products, coupled with monitoring process parameters and hygiene standards. Lebert and Lebert (2006) reviewed the concept of predictive microbiology to evaluate the effect of processing, distribution and storage operations in food safety (12). The review article summarizes the basis for integration of modelling. In Table 1 classification of some models are given.

Table 1 Classification of some models used in Lebert and Lebert (2006)

Primary models	**Secondary models**	**Tertiary models**
(modified) Gompertz function	Response surface models (modified)	Pathogen Modeling Program
Logistic model	Arrhenius model	Growth Predictor
Baranyi model	Square root models	*Pseudomonas* Predictor
Rosso model	γ-models	Seafood Spoilage and Safety Predictor
(modified) Monod model	Z values	ComBase
D values of inactivation		Sym'Previus

The predictive microbiology models describe the growth, survival, and/or inactivation of a microorganism. Primary models describe the change of bacterial numbers over time under environmental conditions and generate information about the microorganism such as generation time and lag phase duration. Secondary models describe the evolution of one or more parameters of a primary model in relation to environmental conditions. The tertiary models take modelling to its final form. Applications which incorporate primary and secondary models into user-friendly computer software packages (12). The article gives a fairly good explanation of all models given in Table 1. The challenges to obtain accurate predictions seem to be related to difficulties in describing the mechanisms that govern microbial metabolism and food properties, and in deducing the actual factors that effect microbial growth from the variety of food characteristics.

Predictive modelling is a scientific discipline that permits assessment of the impact of process stage deviation when integrated in a stage of the food chain, and is more recently being used to assess the exposure of consumers to the presence of hazards due to the occurrence of deviations in the food chain. Predictive models do not capture failures, e.g. failure of process conditions, related to food safety. To address both deviations and failures, predictive modelling must be combined with other techniques. A new approach based on a combination of traditionally predictive modelling and event/fault tree analysis techniques is proposed by Doménech et al (2010) (13). The modelling permits representation of normal and abnormal (i.e. failures) variations of parameters throughout the food chain for better estimation of the real impact of such deviations/failures on consumer safety. A combination of event tree and fault tree analysis techniques is adopted to represent a failure anywhere in the food chain, also including failures in the processing parameters in the food industry. An application example is presented in the paper, considering pasteurized milk.

More advanced predictive models formulate a stochastic process representing the presence of a microbiological, chemical or physical load in foods, which depends on a set of intrinsic parameters (e.g. pH, water activity, acids, salt and preservatives), extrinsic parameters (e.g.

chilling, modified atmosphere) or processing parameters (e.g. heat treatment, pressurization and irradiation (13).

Predictive microbiology integrates knowledge of microbial response to environmental conditions into a mathematical model, allowing for the quantifications of the effect of processing, distribution and storage parameters on quality and safety of foods (14). Time -Temperature Integrators or indicators (TTI) are tools that can potentially be used to monitor the integrated time/temperature impact on fish quality, offering a cost-effective and user friendly way to detect any problematic points in the chill chain. TTI are defined as simple, inexpensive devices that can show an easily measurable time- and temperature-dependent change that cumulatively indicates the time-temperature history of the product from the point of manufacture to the consumer (14). Giannakourou et al (2005) mention that several studies have been published on modelling microbial growth of a number of spoilage bacteria in fish, but that only a few focuses on the effect of fluctuating temperature control conditions that could potentially reflect the actual distribution chain. TTI have been proposed to monitor the time/temperature histories of refrigerated salmon, catfish fillets, Mediterranean fish boque, fresh seafood and of air-packed sea bass. The article assesses the applicability of time-temperature integrators as effective tools of chill chain. The conducted field tests showed the applicability and usefulness of TTI monitoring in the fish chill chain, also elucidating the practical difficulties and limitations that need to be addressed for expanding TTI use as a reliable management tool.

4. Quality and Safety Models for Superchilled Fish

4.1. Introduction

Fresh foods demands good methods to keep them at an acceptable /low temperature all through the production line, transport and storage. Storage temperature is important in all stages of the products shelf life, including storage by producer, retailers and consumers. The market opinion is still that fresh foods are better than frozen foods. Thus, methods for keeping the food fresh are actively sought, and keeping the right temperatures is essential.

Research and development of new and improved methods for chilling have resulted in the concept of superchilling. Literature reports several names to describe superchilling, including deep chilling, partial chilling, partial freezing and even supercooling (15).

Superchilling is a conservation method for foods where some of the water in the food product is frozen. The product is then held at a temperature between -0.5 and -4 °C (16). Previous research on the superchilling method assumed that the ice fraction has significant importance for the superchilling process due to process control and product quality (17). Recent research however, has concluded that an ice fraction between 5-20 % is acceptable and that an ice fraction of more than 30% will cause higher drip loss in fish products (18).

The superchilling process was described as early as 1920 by le Danois (19), even though he did not actually use the terms “superchilling” or “partial freezing”. In the 1970’s and 1980’s most research was done regarding transportation of fish and the effect of using low temperature to increase shelf life of fish during water fishing trips. Waterman and Taylor (20) used the term superchilling instead of partial freezing when fish was first chilled by ice and then the temperature was lowered by the use of the Portuguese method or the Cold Air method (cold air is ducted into fish room and then blown between shelves or boxes of fish and ice). The improvement was seen only after about 12 days storage, and the method gave most value when keeping the earliest caught fish on a three-week distant water fishing trip. Thawing was necessary before any processing because 50% of the water within the fish was frozen. The advantage of the methods was prolonged duration of a fishing trip made by ordinary wet fishing trawlers.

The concept of superchilling has been under continuous development for the last 10-20 years. Even today, superchilling of foods is performed in different ways; superchilled storage of foods without any pre treatment (21-23) and superchilled storage after initial surface freezing followed by temperature equalization (24-29). Practical superchilling methods reported in literature are refrigerated sea water (RSW), air blast tunnels and contact chilling (17). The advantage of initial surface freezing of fresh foods is that the ice fraction on the surface of the product acts as a cold buffer during further storage and transportation. During storage, the ice distribution equalizes and the product obtains a uniform temperature at which it is maintained during storage and distribution (26). When the temperature is kept at superchilling storage temperatures, there is no need for additional crushed ice to keep the temperature low. Chilled fresh fish is normally packed in boxes filled with approximately 30 %

ice (equivalent to approximately 130 trucks per week in 2008 from Norway) to keep the temperature low during transport and storage.

Several review articles on superchilling have been published during the years. Nordtvedt (2003) (17) summarizes earlier literature surveys given by Carlson (1969) (30) and Einarsson (1988) (31), and experiences in superchilling of salmon at SINTEF and Norway in the period 1988-2003. The review of Carlson (1969) is about superchilling of fish in the 1930s and the process used was called the Bellefon-Falliot process. Fish was stored in airtight containers filled with brine at temperature -2 °C and -3 °C. The process was favourable according to less weight loss and at least 30% reduction of deteriorated fish. In spite of the good results the process did not gain universal acceptance (17).

In 1988, Einarsson (31) made a literature review in superchilling or deep chilling, as he calls it. Two parts of the article give the basis for the today's superchilling process, namely (i) freezing point of various foods, and (ii) reaction kinetics at temperature just above freezing temperature. According to Einarsson (1988), superchilling normally provides better food quality than ordinary chilled food after some time in storage. The main disadvantages with superchilling are according to (31), the growth of ice crystals that might break all structure and often increased drip loss during storage and preparation (17). Based on previous review articles and work Nordtvedt (2003) states that superchilling is a method for increasing the shelf life of food products. Several different methods for superchilling have been tested on an experimental basis, and the main effort now is to use the research knowledge on an industrial scale in the food industry.

4.2. Quality of superchilled fish

Lowering the temperature during production and storage of fresh foods will in any case result in increased shelf life regarding microbiological quality. The growth and propagation of bacteria are likely to occur in the temperature range between 5°C and 37°C, although the minimum temperatures for psychrophilic and psychrothrophic bacteria are approximately -15°C and -5°C, respectively, so these types of bacteria may grow, albeit slow, at lower temperatures.

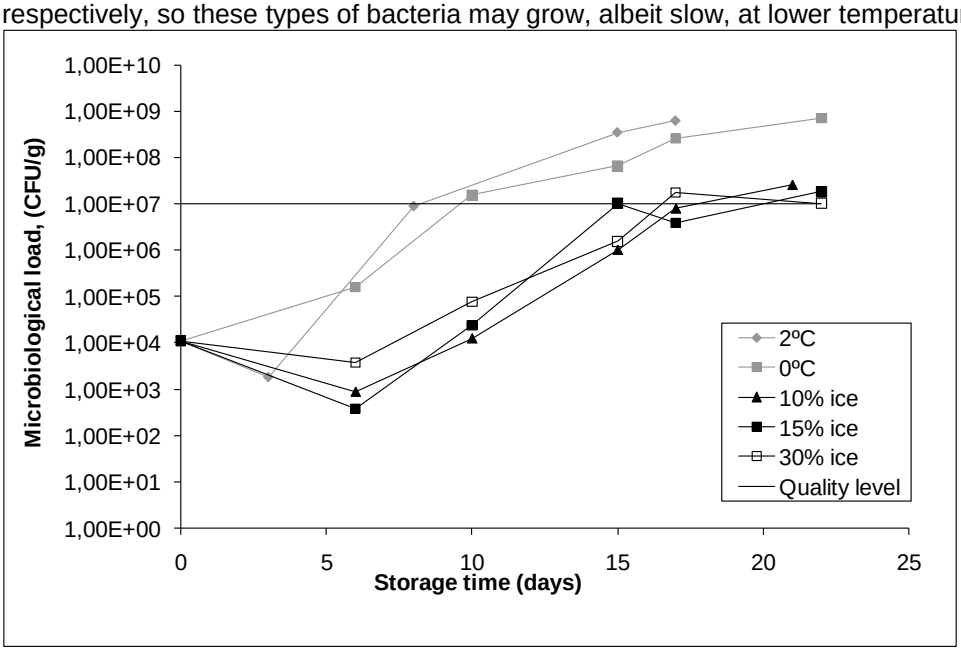

Figure 4 Microbiological load (total count) for unpacked chilled and superchilled salmon fillets (32).

For the last 10 years a lot of experiments have been carried out regarding storage and quality of superchilled fish. Only a few years ago, scientists were convinced that the amount of ice stored inside a superchilled product is a major contributor to the shelf life of the end product (Haugland et al, 2005, Magnussen et al, 2008). It was also expected that higher ice fractions could increase the drip loss of the fillets due to destruction of cell membranes (Foegedinger, 1996).

Several studies were performed to calculate and develop models for time – temperature - ice fraction during superchilling of different products.

Figure 4 show average maximum storage time for unpacked chilled and superchilled salmon fillets, which is approximately 8-10 days and 15-17 days respectively. Analyses of drip loss and liquid loss showed no advantage in the fillets containing less ice, on the contrary fillets with high ice fraction showed the best scores for physical quality during storage. As shown in Figure 4, the extent of superchilling (ice fraction) seems not to influence microbiological shelf life.

In Stevik et al (2010), the coherence between ice fraction in superchilled foods and physical and microbiological changes during storage was investigated. The achieved ice fraction after superchilling is presented in Table 2.

Table 2: Ice fraction of superchilled salmon (n=3)

Product	Superchilling method	Superchilling time [minutes]	Target ice fraction [%]	Achieved ice fraction [%]
Salmon fillets	LIC cabinet	5	20	29 ± 3
Salmon fillets	Air Tunnel	10	10	15 ± 3
	Air Tunnel	20	20	38 ± 4

Superchilling by means of LIC and air both gave an acceptable ice fraction within a short period of chilling time. The internal reproducibility of ice fraction in each batch was satisfactory for salmon, related to a more even weight and geometrical distribution among the salmon fillets compared with other foods. The results presented in Table 2 reveal the challenge of hitting the intended level of ice when a large number of pre-trials are not possible. A slight increase in drip loss could be seen for salmon fillets during storage. The drip loss increased from approximately 1.7% to 4% during four weeks storage, see Figure 5.

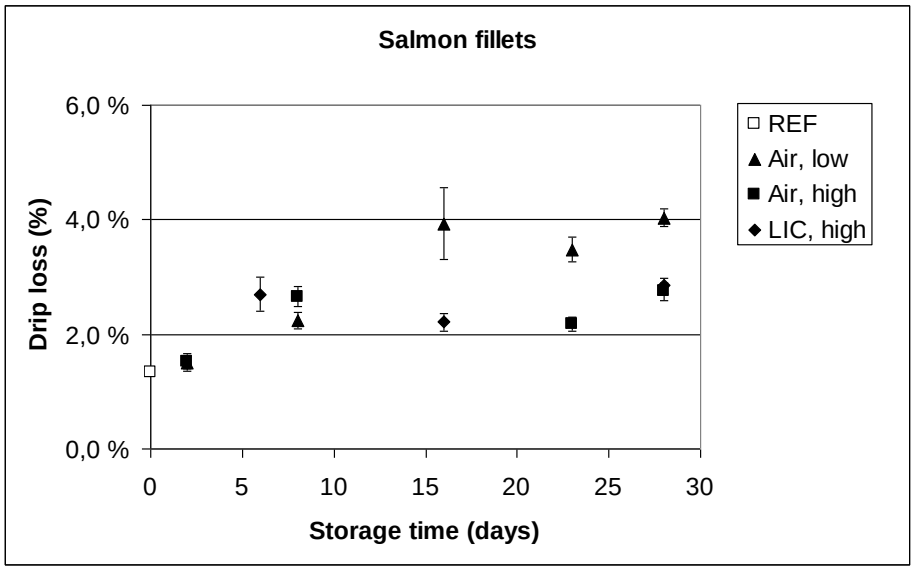

Figure 5 Drip loss for salmon during 30 storage days. *Air, low*: air tunnel giving low ice fraction. *Air, high*: air tunnel giving high ice fraction. *LIC, high*: liquid CO_2 cabinets giving high ice fraction (Stevik et al, 2010).

The quantification of drip loss is done by weighing the liquid left in the packaging after removing the sample. Liquid loss (LL), however, is determined on minced muscle by low-speed centrifugation. LL is expressed as percentage of weight of the mince lost during centrifugation of approximately 2 gram of sample for five minutes (33).

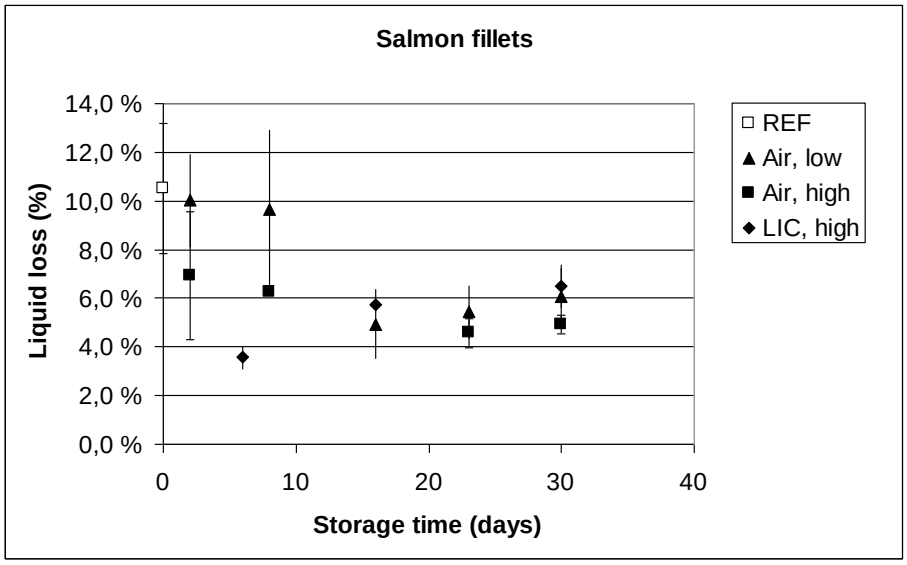

Figure 6 Liquid loss for salmon during 30 storage days. *Air, low*: air tunnel giving low ice fraction. *Air, high*: air tunnel giving high ice fraction. *LIC, high*: liquid CO_2 cabinets giving high ice fraction (Stevik et al, 2010).

The liquid loss for salmon fillets during storage is presented in Figure 6.

No trends for the liquid loss can be seen for salmon fillets, but the levels are generally low, and the results correspond to former findings. The microbiological quality of superchilled fillets of salmon was considerably prolonged compared to the stated shelf life of chilled fillets, see Figure 7.

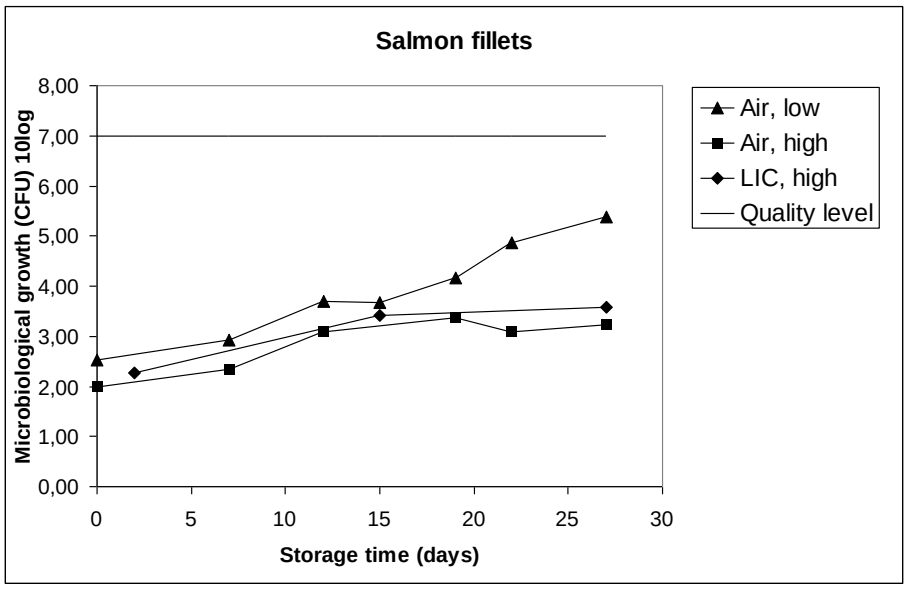

Figure 7 microbiological loads (total count [cfu/g]) in salmon during 30 storage days. *Air, low*: air tunnel giving low ice fraction. *Air, high*: air tunnel giving high ice fraction. *LIC, high*: liquid CO_2 cabinets giving high ice fraction (Stevik et al, 2010)

The quality limit of 10^7 CFU/g reflects a usual microbiological quality measure, above which food is regarded as unfit for human consumption (34). The salmon fillets reached a CFU level of 10^5 by the end of the storage period (4 weeks). For salmon fillets, the lower ice fraction seemed to give a shorter shelf life than the higher ice fraction.

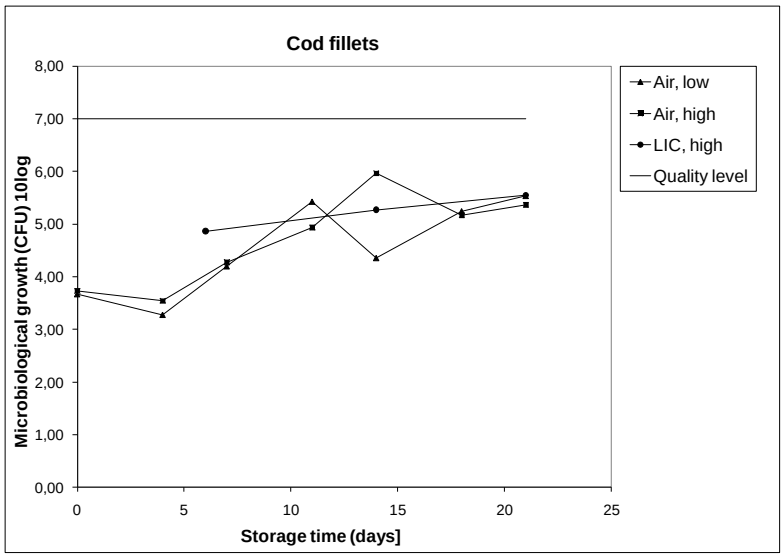

Figure 8 Microbiological growths (total count [cfu/g]) in cod during 20 storage days. *Air, low*: air tunnel giving low ice fraction. *Air, high*: air tunnel giving high ice fraction. *LIC, high*: liquid CO_2 cabinets giving high ice fraction (Unpublished work, Stevik et al, 2010)

Unpublished work of Stevik et al (2010) shows that the microbiological shelf life of superchilled cod fillets is good, even after 20 days of cold storage, as seen in Figure 8. Duun and Rustad (2007) (29) investigated quality changes during superchilled storage of cod (*Gadus morhua*) fillets at -2.2 °C. Superchilled cod showed increased shelf life with respect to reduced growth of sulphide-producing bacteria compared to ice chilled samples. Drip loss was also lower in superchilled cod. Figure 9 shows the main results regarding development of bacterial growth during storage time.

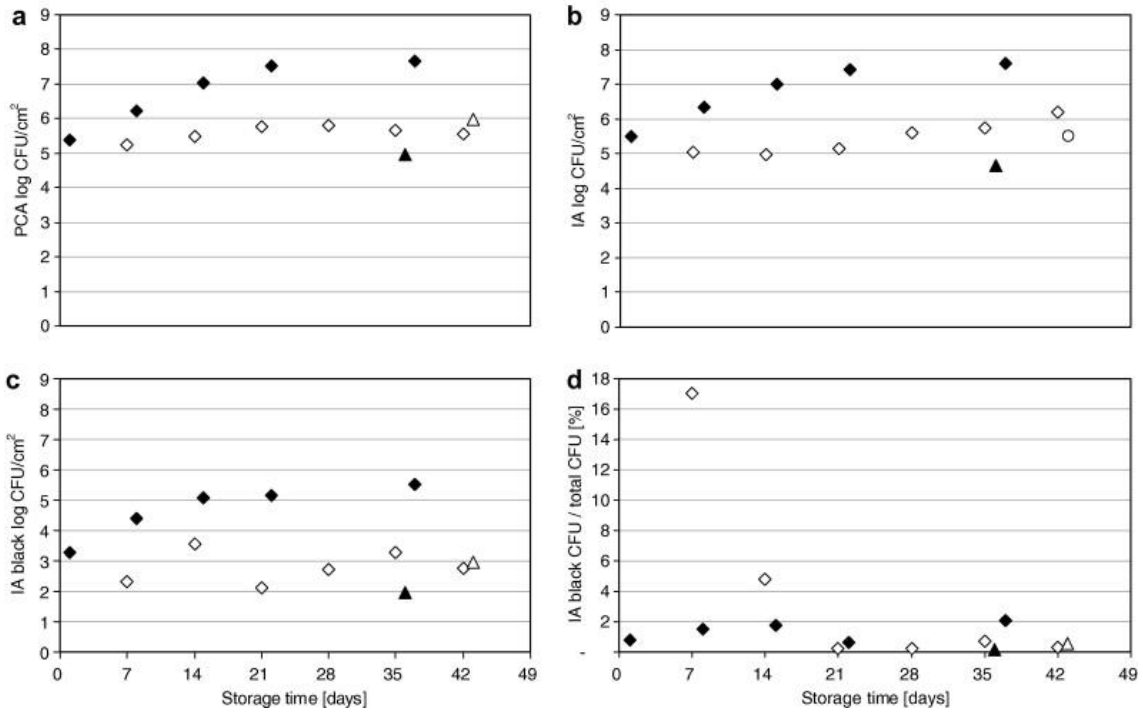

Figure 9 Development in PCA (total plate count) and IA (*S. putrefaciens*-like organisms) counts on the surface of vacuum packed portions of cod fillet at different storage conditions. (a) PCA after incubation for 72 ± 3 h at 25 °C, and (b)-(d) total counts, black counts and the percentage of black counts on IA after incubation for 72 ± 3 h at 20 °C, respectively. Open diamonds

indicates superchilled storage at -2.2 °C, filled diamonds indicate ice chilled storage, filled triangles indicate frozen storage at -21 °C and open triangles indicate frozen storage at -40 °C. All values are means of six samples (29).

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. In order to maintain good quality of the cod fillets during superchilling, there is a need for process optimization to minimize protein denaturation and liquid loss (29).

Figure 10 shows the sensory test of superchilled salmon compared to traditionally chilled salmon. The samples were stored for 21 days and sensory tests were performed during the whole storage time. After 14 days of storage the sensory score of chilled salmon was unacceptable (14 days on truck + 7 days in traditional packages on ice). This is shown by taking out the blue bars at day 14 and 21 in Figure 10. The superchilled salmon, on the other hand, shows high quality score after 21 days storage (35).

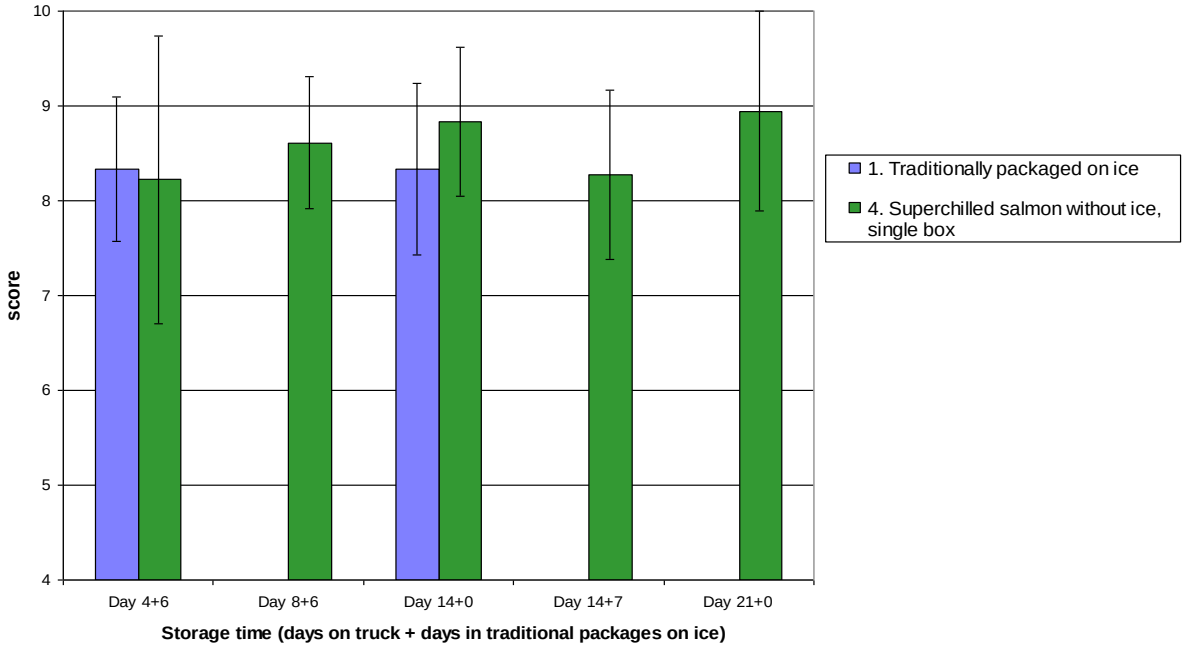

Figure 10 Sensory test of superchilled salmon and chilled salmon stored for 21 days, without and with ice, respectively (35).

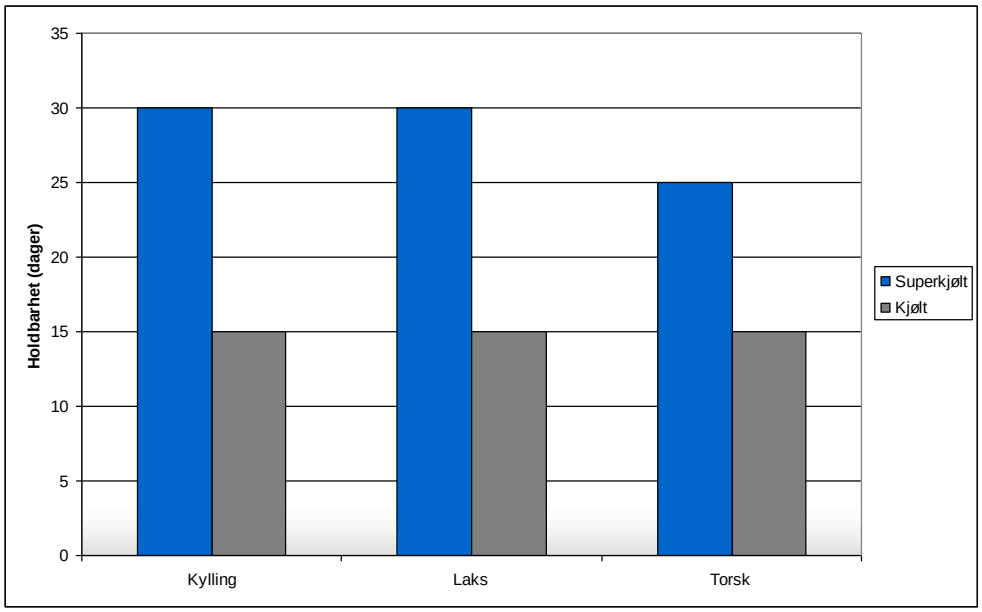

Figure 11 Microbiological shelf life based on total bacterial count for superchilled and chilled products of chicken (=kylling), salmon (=laks) and cod (=torsk). Y-axis gives shelf life in days. The blue columns are superchilled products and the grey columns are chilled products (36).

Beaufort et al (2009) (21) investigated the microbiological and organoleptic properties of cold-smoked salmon before retail display. The effect of storage at -2 °C was found to reduce the rate of development of *L. monocytogenes* in naturally contaminated cold-smoked salmon. After superchilling storage for 14 days and 28 days, all samples were contaminated with less than 100 CFU/g (which is the maximum limit given by the European regulations on microbiological criteria for food safety, EU Regulation 2073/2005). After storage at -2 °C/14d + 4 °C/10d + 8 °C/18d, 12 % of the samples were above the 100 CFU/g limit. After storage at -2 °C/28d + 4 °C/10d + 8 °C/18d, 21% of samples were above the 100 CFU/g limit (21).

Bahuaud et al (2008) evaluated the impact of superchilling on the quality of Atlantic salmon (*salmon salar*) *pre-rigor* fillets. Superchilling resulted in higher liquid leakage and increased myofibre breakage in the fillets, while texture values of fillets measured instrumentally were not affected by superchilling one week after treatment. The samples were stored on ice at room temperature +5 °C for seven days before analysis. Measurements of samples showed 25% ice fraction in the product after the end of the superchilling treatment (24). Microscopical analysis detected several freezing imperfection in the upper layer of all superchilled fillets. This is probably due to the development of large intra- and extra- cellular ice crystals during superchilling.

One of the challenges due to superchiling and transport is the common understanding within the market that boxes of fresh fish should include a specific amount of ice at arrival by the consignee. Aune and Nordtvedt (1999) (37) investigated the temperature during transportation of superchilled salmon fillets from Norway to France. Core temperature of the salmon fillets and storage temperature was continuously measured, and the results are given in Figures 12 and 13.

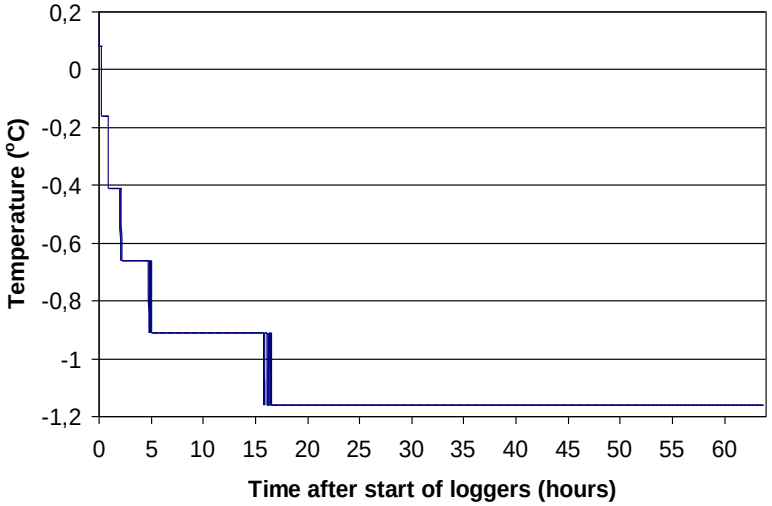

Figure 12 Core temperatures of salmon of weight class 3-4 kg during transportation (Aune and Nordtvedt, 1999).

From Figure 13, it can be seen that the air temperature was as low as -6.3 °C during transport, and that the air temperature fluctuated very much during transportation. This may indicate a poor regulation system of the truck chilling system.

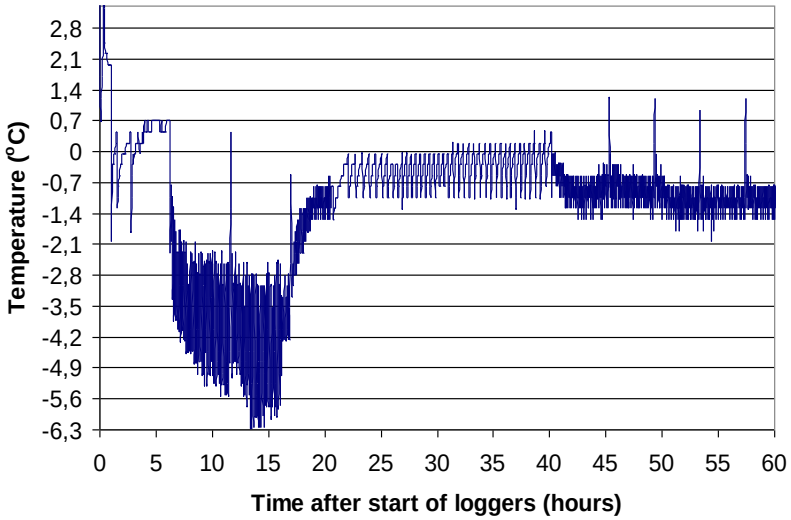

Figure 13 Air temperatures in the cargo compartment of the truck during transportation (Aune and Nordtvedt, 1999).

The temperature measurements of salmon fillets at arrival were -1.4 °C. The desired value for the consignee in France was between -0.5 °C and 0.5 °C. Despite the low temperature, overall quality of the salmon fillet was satisfactory.

4.3. Safety models for Superchilled Foods

A mathematical empirical safety model for estimation of remaining shelf-life for selected meat products after different retention periods in the refrigeration chain was developed (38), based on bacterial growth of *E. coli*, *Salmonella spp* and *Listeria monocytogenes*. The plot diagram is a log (CFU) versus days plot. Maximum value of CFU (colony forming unit) is 10^7 CFU/g. Some assumptions are made for the model; (a) no bacteria growth when the product is stored at -1.1 °C, (b) the equations also valid for temperatures just below zero degrees Celsius, (c) the product temperature is stable for six hours into a new period before it changes instantaneously to the new temperature, followed by the bacteria *lag-time,* (d) calculation of shelf life assumes

that the product will remain at the last temperature and show remaining retention time, and (e) the drip-loss premise storage temperature of -2 °C.The safety model requires some modifications before it can be put to use, and until today only valid for a few selected meat products (38). The model is based on shelf life experiments of superchilled meat products, and may be used as basis for development of a safety model for salmon.

5. Shelf life Extension

Chilled foods are often preserved by low temperature alone and their shelf life depends on the achieved storage temperatures. In order to prolong product shelf life further, several preservation factors or techniques (hurdles) could be used, such as vacuum packaging, modified atmospheric packaging (MAP), microbial inhibitors, partial freezing or combinations of these processes together with an appropriate HACCP system. Modified atmosphere packaging in combination with partial freezing/superchilling has shown promising results (39).

5.1. Modified atmosphere packaging

The principle of modified atmosphere packaging (MAP) is the replacement of air in the packaging with different, fixed gas mixtures. Carbon dioxide (CO_2) is the most important gas used in MAP since it has bacteriostatic and fungistatic properties (40). Under MAP conditions, the growth of common spoilage microorganisms is inhibited and the fish shelf life can be extended by a factor of 1.5-2 compared with chilled storage in air (40). This was intensively looked into by Fernández et al (2009) where the effects of natural additives, superchilling, and MAP on the shelf-life of Atlantic Salmon (*Salmon salar*) fillets were investigated (41). The use of superchilling and MAP (90% CO_2 g/p =2.5) increased salmon shelf life from eleven days (control sample) to 22 days. The extension period was confirmed with aerobic mesophile counts and sensory attributes (texture and odour), although the shelf life estimated from sensory analysis differed from the microbiological analysis. During the storage time, the physiochemical properties (drip loss, pH, Total volatile nitrogen (TVN) and *k*) were under the acceptable limits (41). The extent of ATP degradation is expressed as the k-value, which is defined as the ratio of the sum of inosine and hypoaxanthine concentrations to the total concentration of ATP metabolites. A fresh fish will have a low k-value (42). TVN is often used as a spoilage indicator for fresh seafood held on ice. The effect of different MAP atmospheres (with and without additives or preservatives) on the aerobic mesophiles counts for Salmon salar fillets is given in Figure 14.

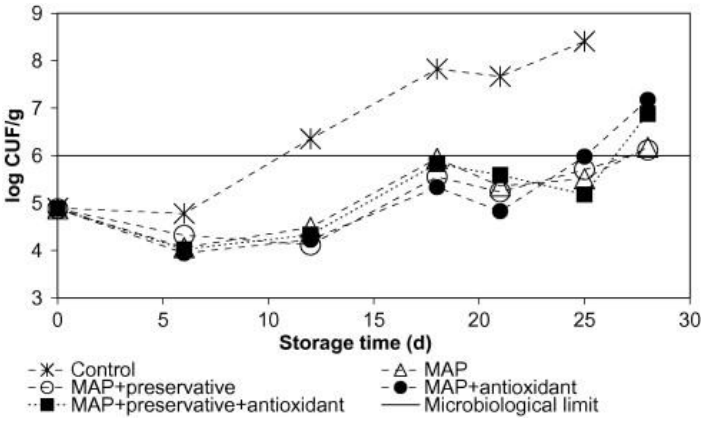

Figure 14 Effect of different MAP atmospheres on the aerobic mesophile counts for *Salmon salar* fillets. Fernández et al. (2009).

MAP is a mild preservation process with many benefits. It extends the products shelf life and allows the products to be transported longer distances (2).

Fresh fish are traditionally stored on ice or under refrigeration during distribution and marketing, and the normal shelf life in Norway ranges from 5 to 14 days, depending on species, harvest location and season. The limited shelf-life and large geographical distances, enforces a larger amount of freezing, and only a small percentage of fisheries products are therefore marketed as fresh at the retail level. High carbon dioxide levels in MAP have been shown to inhibit the normal spoilage flora of seafood (see (41)), thereby doubling or tripling product shelf life. In addition to prolonged shelf life, the elimination of ice in modified atmosphere packages result in economic and sanitation advantages. The shelf life extension expands the market potential, reduces waste during distribution and retail display, gives processors pre-packaging opportunities and benefits consumers with dry, high quality, ready-to-cook fresh products (2).

The main concern of modified atmosphere packed (MA-packed) fish products is the growth and the toxin formation of non-proteolytic strains of *Clostridium botulinum* type E, and the growth of *Listeria monocytogenes*. *C. botulinum* is an obligate anaerobic bacteria, it is widely distributed in aquatic and marine environments and it can grow down to a temperature at 3.3 °C (2). The bacterial toxin production is very temperature dependent. At 4 °C the time before the bacteria start to produce toxin is 60 days and at 0 °C, no growth or toxin production occurs. *L. monocytogenes* may grow at 0 °C but the growth is very limited at this temperature (2).

Even if most microbial activity is prevented by using modified atmospheres with moderate to high levels of CO_2, the aerobic spoilage organisms, which usually warn consumers of spoilage, are also inhibited. At the same time, the growth of pathogens may be allowed or even stimulated. The extended shelf life of MAP products allows extra time for these pathogens to reach dangerously high levels in the food products. Since the growth of the pathogenic bacteria is reduced as the product temperature is lowered, it is extremely important that low temperatures are kept in the production and distribution of all MAP products. In addition, a strict processing hygiene is necessary (2).

The quality of chilled and superchilled MA-packed salmon fillets was investigated by Torstveit et al (2002). The product samples were studied during storage for seventeen days. Quality changes were based both on biochemical and microbiological parameters. The water holding capacity (WHC) was used to describe the ability of muscle foods to hold water under specific conditions. WHC influences the sensory quality of food products such as juiciness, texture and taste, and the functional properties of the muscle protein (43). Due to the insulating atmosphere layer in the MA-packages, the bottom layers of the salmon fillets freeze faster compared to the top layer. The main results from Torstveit et al (2002) are summarized in Table 3 below.

Table 3: pH, water holding capacity (WHC), moisture content (MC) and bacterial concentration of chilled, superchilled and frozen MA-packed salmon fillets at different storage days

Storage day	Treatment	Mean values with standard deviation			
		pH (n=3)	WHC [%] (n=12)	MC [%] (n=6)	Plate count [cfu/g] (n=9)
7th	Chilled	-	73.41 ± 6.48	70.15 ± 1.66	$1.6*10^6 \pm 5.8*10^5$
	S-chilled	-	82.15 ± 3.20	66.20 ± 0.65	$2.2*10^6 \pm 1.2*10^6$
14th	Chilled	6.09 ± 0.10	83.20 ± 0.65	66.35 ± 1.51	$1.1*10^8 \pm 2.1*10^7$
	S-chilled	6.04 ± 0.10	81.93 ± 3.59	67.25 ± 2.97	$5.9*10^7 \pm 2.1*10^7$
17th	Chilled	6.16 ± 0.02	79.49 ± 7.63	66.22 ± 1.66	$4.6*10^8 \pm 2.1*10^8$
	S-chilled	6.12 ± 0.08	81.90 ± 3.92	66.22 ± 1.11	$3.2*10^8 \pm 1.0*10^8$
	Frozen	6.26 (n=1)	75.36 (n=2)	66.50 (n=2)	-

The experimental results gave somewhat equivalent bacterial growth on superchilled product and chilled products. The biochemical quality of superchilled MA-packed salmon (water content and WHC) fillets changed little during storage and was as good as, or even better than the ordinary chilled salmon fillets (43).

5.2. Vacuum packaging

The quality of superchilled vacuum packed Atlantic Salmon (*salmon salar*) fillets was investigated by Duun and Rustad (2008) (27). Texture, drip loss, cathepsin activities and protein extractability were investigated during storage and compared to ice chilled and frozen references. The storage time of vacuum packed salmon fillets can be doubled by superchilling and storage temperature between -1.4 °C and -3.6 °C compared to ice chilled storage salmon fillets.

6. Quality and Safety Models for Supercooled Fish

The definition of supercooling is taken from James et al (2009) (44). They state that the phenomenon known as supercooling (also referred to as “undercooling” or “subcooling”) is where the temperature of a solution or material is reduced below its freezing point without crystallization occurring due to an energy barrier that must be surmounted before nucleation starts. When ice crystallization occurs, there is a corresponding increase in temperature(due to the latent heat of ice crystallization) from the supercooled temperature to the freezing point, and the point at which nucleation is initiated may be referred to as the “nucleation point” or “metastable limit temperature”.

As also pointed out in James et al (2009), there are few published data on supercooling of foods. There are a few references to supercooling in fruits and vegetables, but none relating to supercooling in fish. The IIR Recommendations for the Processing and Handling of Frozen Foods state that supercooling in most foods is negligible but can be used in the manufacture of ice creams (45). The FRISBEE project will examine the potential for using supercooling in the chilling process in fish manufacturing.

7. Predictive modeling – predictive microbiology

Predictive microbiology modeling is about growth, death, and survival of microorganism due to the shelf-life of foods (quality and safety). Predictive microbiology focuses on quantitative conditions and mathematical modeling, in such a way that one can predict how preservation, process conditions, storage time and hygiene influence microbiological growth.

Mathematical models and databases within predictive modeling have been generated during recent years with information on several different bacteria, and the models are today included in computer software. Not all models can be used for all types of foods, and care must be taken to check that the model can be used for a specific food product (46).

Examples of model software are:

i. **Pathogen Modeling Program (PMP)** www.arserrc.gov/mfs/pathogen.htm

The program was developed in the USA (USDA-ARS Eastern Regional Research Centre, Philadelphia, Pennsylvania), and contains 37 models for growth or death of 11 different species of pathogenic bacteria. PMP does not contain information regarding testing on different foods, and results may be uncertain when used on fish products.

ii. **COMBASE** www.combase.cc

The program was developed by several countries (England, USA, Australia and EU), and the *ComBase Predictor* program contains models for growth and death of 12 different species of pathogenic bacteria including the food tainting bacteria *Brochothrix thermosphacta*. Information regarding testing on different foods is not included, but the *ComBase browser* give access to approximately 35.000 growth/ death curves for food relating bacteria.

iii. **Seafood Spoilage and Safety Predictor (SSSP)** www.difres.dk/micro/sssp

The program was developed in Denmark (Danish Institute for Fisheries Research, Copenhagen) and contains models of shelf-life and growth of food tainting bacteria and *Listeria monocytogenes* in fish products. Results of trials of models for specific fish products are included in the program, and contribute to make SSSP a user friendly tool regarding fish. SSSP can forecast the effect of fluctuating storage temperature on shelf-life of different fish products.

iv. **Sym'Previus tool** www.symprevius.org

A stochastic modelling approach was developed to describe the distribution of *Listeria monocytogenes* contamination in foods throughout their shelf life. This model was designed to include the main sources of variability leading to a scattering of natural contaminations observed in food portions: the variability of the initial contamination, the variability of the biological parameters such as cardinal values and growth parameters, the variability of individual cell behaviours, the variability of pH and water activity of foods as well as portion size, and the variability of storage temperatures. The publication concern cold smoked salted salmon and cheese curds. This tool is also applicable in spoilage bacteria (47).

There are two pronounced different types of predictive mathematical models, (*a*) Models for relative deterioration rate, which are used to predict the effect of storage temperature on the shelf-life of fish products, and (*b*) Models which describe growth, death or survival of specific bacteria, also called kinetic models (46).

7.1. Equations

Newly caught fish contain a diversity of micro flora. Total viable counts (TVC) of 10^2-10^6 cfu/g are usual on whole fish and cut fillets. During chilled storage, psychrotolerant microorganisms are selected; thus, differential counting of these microorganisms is suggested as a measure of fish quality, so-called specific spoilage organism (SSO). Owing to the selection of microorganisms in chilled fish, the correlation between SSO and freshness is usually higher than between TVC and freshness (42) (48).

For salmon, the microbial flora are dominated by psychotropic Gram negative, bacteria species of *Pseudomonas, Moraxella, Acinetobacter, Shewanella, Flavobacterium, Vibrionaceae* and *Aeroemonadeceae*, but also Gram positive bacteria like *Bacillus, Micrococcus, Clostridium, Lactobacillus* and *Corynebacterium* can be found in various quantities. Mainly, the Gram negative fermentative bacteria (*Pseudonomas ssp*. and *Shewanella ssp*.) are the most dominant species in fresh, iced fish. For vacuum packed fresh fish, *Photobacterium phosphoreum* and lactic acid bacteria dominates the growth of microorganisms (49).

7.1.1. *A user-friendly software for food safety*

Halder et al (2011) have integrated a process model (that provides spatial temperature-time histories) with existing predictive microbiological models to provide for comprehensive prediction concerning food safety for many food processing situations. Databases of microbial growth and inactivation kinetic parameters for various food types were developed through extensive regression analysis on decades of experimental data from the literature to facilitate predictive modeling and make "user-friendly" software (50). The sequential stages of bacterial growth are the lag phase, the exponential phase, the stationary phase and the death phase. The lag phase is the stage at which the microorganisms adapt to a new environment and the lag time (L) is the time taken by the microorganisms to adapt to that environment prior to exponential growth.

The predictive model for the lag phase and the exponential phase using a first-order growth model is given for two different situations, isothermal (constant temperature) and non-isothermal. A first order model cannot predict the stationary phase. Lag times (L_T) are available for various temperatures from published literature. In a first order model, for isothermal

situations, the growth rate is zero until the lag time is over and k_T, the growth rate at temperature T, is used after the lag time has elapsed. The model is given as:

$$\frac{dN}{dt} = \begin{cases} 0 & t < L_T \\ k_T N & t > L_T \end{cases} \quad (1)$$

Prediction of microbial growth under fluctuating temperature conditions is given in Equation 2 (50);

$$\frac{dA}{dt} = \frac{1}{L_t} \quad (2)$$

where A is total adaption during the lag phase. An adaption rate can be defined as the reciprocal of the lag time. When A equals 1, adaption is complete and growth starts. The growth is thus, given by Equation 3;

$$\frac{dN}{dt} = \begin{cases} 0 & A < 1 \\ k_T N & A > 1 \end{cases} \quad (3)$$

7.1.2. *The Seafood Spoilage and Safety Predictor*

The Seafood Spoilage and Safety Predictor (47) is a well-recognised tool for predicting safety and quality of seafood products. It relies on many years of research at the Danish Institute of Fisheries Research and is widely recognised for its high quality level.

Therefore, it was decided to select appropriate models incorporated and documented in this SSSP tool as for use in FRISBEE, more precisely, for use as kinetic models to describe the Quality Indicator "Specific spoilage organisms" in salmon (GA 245288 FRISBEE DoW 25/05/2010, p. 53).

The SSSP has five main categories of models:

1. relative rate of spoilage models (RRS)
2. microbial spoilage models
3. histamine formation models
4. *Listeria monocytogenes* in chilled seafood
5. *Listeria monocytogenes* and lactic acid bacteria.

Only the first two categories will be discussed below, as they are the most important in relation to what is needed for FRISBEE.

7.1.2.1. Relative rate of Spoilage models (RSS)

RSS models are given as square root spoilage model for fish (including salmon). The model was initially developed based on growth characteristics for psychrotolerant microorganisms isolated from seafood. These psychrotolerant microorganisms have a theoretical minimum growth temperature (T_{min}–value) of -10°C, and are used in the square-root RRS model in Equation 5 below. The definition of RRS is the shelf life at 0°C divided by the shelf life at t °C. Estimation of temperature characteristics is given in Equation 5;

$$\sqrt{RS} = k_3 \times (T - T_{min}) \quad (5)$$

where RS is rate of spoilage, k_3 is a constant and T is temperature in [°C].

The shelf-life of fresh fish is predicted at different temperatures, given by Equation 6:

$$Shelf - life\ at\ T°C = \frac{Shelf-life\ at\ T_{ref}}{\left[\frac{T-T_{min}}{T_{ref}-T_{min}}\right]^2} \quad (6)$$

The RRS model is valid for fresh seafood from temperate waters stored between -3 °C and +15 °C. The model is applicable for aerobically stored as well as vacuum packed and modified atmosphere packed fresh seafood.

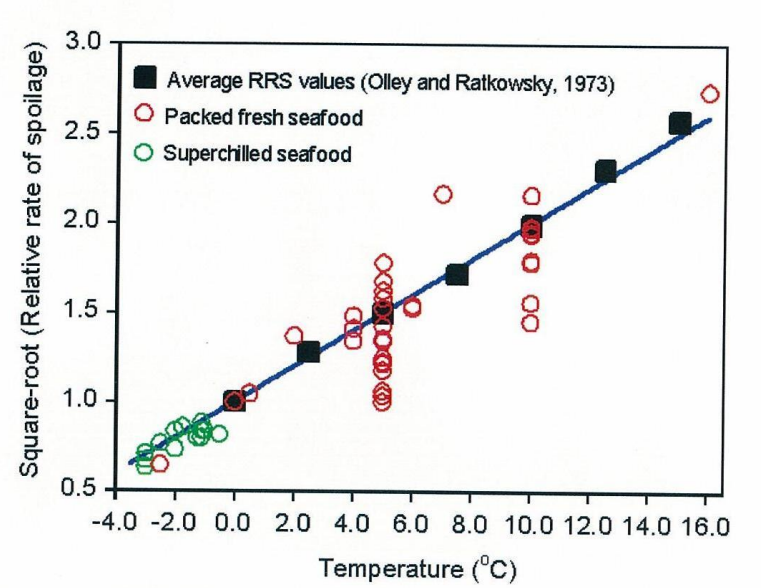

Figure 15 Comparison of the square-root spoilage model (Equation 4 used with a reference temperature of 0°C) and different sets of RRS data for fresh seafood from temperate waters.

The average RRS of fresh seafood is appropriately predicted by the square-root model Equation 5. It is interesting to observe that data from superchilled seafood follow the general linear trend between the square root of the relative rate of spoilage and temperature.

As shown in Figure 15, considerable deviation was obtained in this study. The most deviating results were obtained in experiments with the fish species flathead, hake, salmon, snapper and trout. As salmon is part of the deviating results, it was concluded not to select the RSS models for inclusion in FRISBEE.

7.1.2.2.Microbial spoilage models

Microbial spoilage models (MSM) from (51) can be used to predict the influence of a SSO, quality index as defined in the DoW.

The concept of SSO is as follows: “Seafood spoilage is dynamic with changes in spoilage reactions and changes between different groups of spoilage micro-organisms depending on product characteristics and storage conditions (Dalgaard 2000, Dalgaard 2006). This dynamic nature of seafood spoilage complicates the development of microbial spoilage models and the

application of these models for shelf-life prediction. However, the concept of specific spoilage organisms (SSO) has allowed the formulation of microbial spoilage models (Dalgaard 2002). SSO has been defined as the part of the total micro-flora responsible for spoilage of a given product and the spoilage domain as the range of product characteristics and storage conditions within which a given SSO causes product rejection (Dalgaard, 1995)." (SSSP – help file on microbial spoilage models).

The predictions can only be performed after product and environmental characteristics have been entered, i.e. (i) initial concentration of bacteria in CFU/g, (ii) storage temperature and (iii) possible other factors like the level of CO_2, pH and a_w.

For fresh seafood from temperate/cold waters stored aerobically at chill temperatures the SSO is H_2S-producing *Shewanella* bacteria. In SSSP, the primary model for this SSO consists of a log-transformed 3-parameter logistic model.

$$\log(N_t) = \log\left(\frac{N_{max}}{1+\left(\frac{N_{max}}{N_0}-1\right)\times\exp(-\mu_{max}\times t)}\right)$$

with N_t (cfu/g) the level of the SSO at a certain time *t*, N_0 (cfu/g) the level of the SSO at time zero (i.e., the moment the spoilage organisms contaminate the fish product), N_{max} (cfu/g) the maximum population level attained by the SSO, and μ_{max} the maximum specific growth rate (1/h).To enable the use of this model under dynamic temperature conditions in a realistic cold chain, its dynamic version (i.e., under the form on a differential equation) is needed:

$$\frac{dN}{dt} = \mu_{max}\left(1-\frac{N}{N_{max}}\right)N$$

The secondary model in the SSSP microbial spoilage model includes the effect of temperature

$$\sqrt{\mu_{max}} = 0.0299\times(t^{o}C + 7.08)$$

This model for growth of H_2S-producing *Shewanella* bacteria has been validated by comparison of observed and predicted growth rates in different fresh seafoods, namely cod, haddock, hoki, orange roughy, smooth oreo dory, sea bream and snapper (Dalgaard, 1999). The model predicted growth rates accurately in these fresh fish stored aerobically at low temperatures.

In SSSP, there is also a more elaborate secondary model available, see Equation (7).

$$\begin{aligned}\sqrt{\mu_{max}} = {} & b \\ & * (T - T_{min}) \\ & * \sqrt{(a_w - a_{w,min})/(a_{w,ref} - a_{w,min})} \\ & * \sqrt{(pH - pH_{min})(pH_{ref} - pH_{min})} \\ & * (\%CO_{2,max} - \%CO_2)/\%CO_{2,max}\end{aligned} \qquad (7)$$

The secondary growth model is used with user-defined parameter values and can be applied for all bacteria where an estimate of the constant *b* and the cardinal parameter T_{min} can be obtained. Estimates of the other cardinal parameter values are not needed, but clearly allow for a more flexible use of the model when available, especially under MAP conditions (given the factor related with CO_2 concentration).

To predict shelf-life, the initial concentration of H_2S-producing *Shewanella* bacteria is required and it can be determined using Iron agar Lyngby (Gram et al., 1987) or Peptone Iron agar (Levin, 1968). For H_2S-producing *Shewanella* bacteria, minimal spoilage levels from 10^6 cfu/g to 10^8 cfu/g has been reported in the literature and SSSP uses a minimal spoilage levels of 10^7 cfu/g. The following equation is used to calculate product shelf-life.

$$\text{Shelf life (days)} = \frac{[\log(10^7) - \log(N_0)] \times \ln(10)}{\mu_{max}(h^{-1}) \times 24} \quad (8)$$

In FRISBEE, there is no specific need to calculate the shelf-life, but it could be an added value if assuming a certain initial value for the H_2S-producing *Shewanella* bacteria.

What is important before this model can be used in FRISBEE is the validation of the primary and secondary model in salmon. We will therefore perform dedicated experiments in autumn 2011.

Listeria monocytogenes growth and growth boundary models:
This model includes the effect of 12 environmental parameters on growth and on the growth boundary of *L. monocytogenes*. Information on the lag time of *L. monocytogenes* in naturally contaminated lightly preserved seafood is still limited. Therefore, the growth model for *L. monocytogenes* can be used without lag time (fail safe predictions) or with lag time (more realistic predictions for naturally contaminated products). SSSP uses a relative lag time of 4.5 for *L. monocytogenes*. SSSP can predict growth of *L. monocytogenes* for both constant and changing storage temperatures.

Primary model for growth of *Listeria monocytogenes*:
The logistic model with delay (t_{lag}) is given in Equation 9 and 10 and is used to predict changes on concentrations of *Listeria monocytogenes* during storage at constant or changing storage temperatures.

$$logN_t = logN_0 \quad (9)$$

$$logN_t = log\left[\frac{N_{max}}{\left(1+\left(\left(\frac{N_{max}}{N_0}\right)-1\right)exp\left(-\mu_{max}\times\left(t-t_{lag}\right)\right)\right)}\right] \quad (10)$$

Measurements of Listeria monocytogenes in superchilled and chilled salmon are being considered in agreement with FRISBEE partner ACTIA within the near future.

7.1.3. *Texture and Odour*

The softening of muscle is due to proteolytic digestion of minor cell components that link the major structural units together. The highly unsaturated lipids of fish easily become oxidized, resulting in alterations in smell, taste, texture, colour and nutritional value. Oxidation starts immediately after catch, but becomes particularly important for shelf-life only at temperatures >0°C, when oxidation rather than microbial activity becomes the major spoilage factor.

The extent of lipid oxidation can be measured using different methods regarding either the reactants or products (gas chromatography (GC), high-performance liquid chromatography (HPLC), thiobarbituric-acid-reactive substances (TBARS) test, fluorescence spectroscopy and others). To monitor the progression of lipid oxidation, it is important to use more than one method, especially when comparing different types of fish products.

Odour is one of the most important parameters to evaluate fish freshness. The volatile compounds contribution to fish odour can be divided into three groups based on their origin as shown in Figure 16.

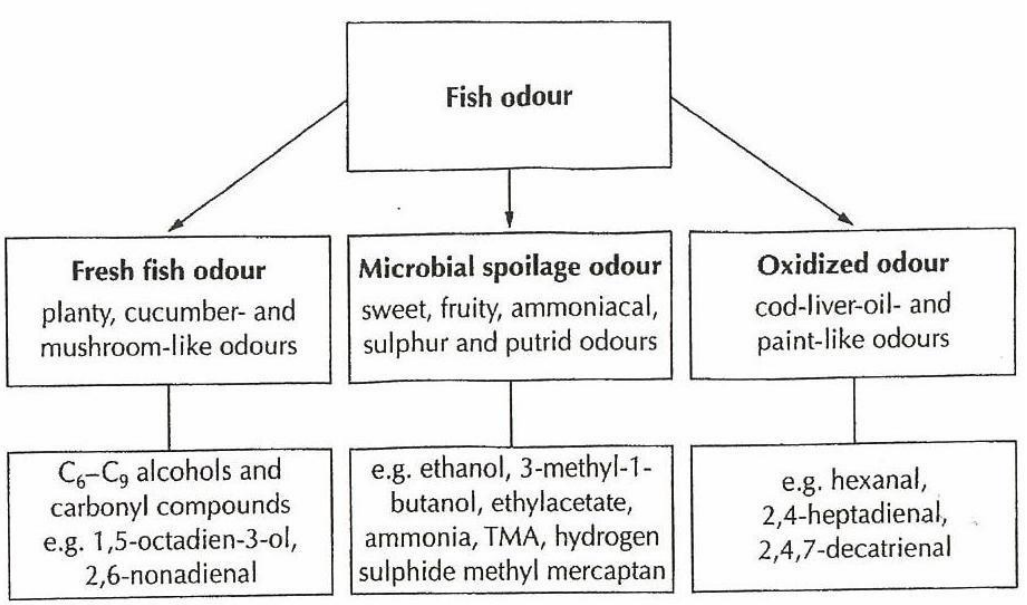

Figure 16 Categorization of fish odour and the volatile compounds that contribute to the characteristic odour of fresh, spoiled and oxidized fish (TMA, trimetylamine) (42).

To predict remaining shelf life, a combination of different standard methods that use rapid measurement techniques with mathematical model must be developed also for texture and odour measurements (42).

8. Conclusion / Remarks

In WP5, SINTEF will identify optimum preservation process conditions for superchilling of pork and salmon, including ice content, temperature level and cooling rates, and assess effect of process conditions on quality. (SINTEF, D5.1.1.2). During autumn 2011, experimental work in lab- and pilot scale will be preformed on salmon. Planned activities are measurements of temperature, ice content, microbial load (spoilage bacteria) and quality measurements due to different chilling technologies through cold chain to retail cabinets for;

- Traditional chilled salmon.
- Superchilled salmon in impingement freezer.
- Superchilled salmon in CBC (Contact Blast Chiller).

The results from these experiments will be used to attempt to complete final models for safety and quality aspects of chilled and superchilled salmon as functions of time and temperature.

In the case of aerobically stored salmon, the focus will be on the H_2S producing bacteria, i.e. *S. putrefaciens* like organisms. Existing SSSP models are providing a solid basis for these modeling efforts. Also, when using mathematical models in calculating shelf-life of foods, the effect of varying product composition of the newly slaughtered fish or animal product is not considered in the modeling. For farmed fish, it is likely that the quality is somewhat equal, but for wild fish the quality varies due to sea temperature (season), access to feed,

processing/filleting and continent. This is however, out of the scope of the FRISBEE project and will not be examined further.

9. References

1. Huss, H. 1976. Konsumfisk - biologi, teknologi, kvalitet og holdbarhet. *Dansk Get. Tidsskrift* 59:165-175.
2. Hemmingsen, A.K.T. 2002. Quality of fresh Foods. In Department of Energy and Process Engineering. Norwegian University of Science and Technology, Trondheim. 34.
3. IIR. 2000. Recommendations For Chilled Storage of perishable produce. In Paris, France. 2019.
4. Lovdata. FOR 2008-12-22 nr 1623. Forskrift om næringsmiddelhygiene (næringsmiddelhygieneforskriften). In L.-L.-o.m. FKD - Fiskeri og kystdepartementet, HOD - Helse- og omsorgsdepartementet, editor Lovdata, Oslo.
5. Torstveit, A.K., Magnussen, O.M. 1998. Temperature conditions in refrigerated counters in Vest Agder, Norway. In IIF-IIR. Nantes, France.
6. Hemmingsen, A.K.T., Aune, E.J., Magnussen, O.M. 2002. Challenges in the distribution of fresh foods.Manuscript. In Trondheim.
7. Sandbakk, M. 1999. Plassering av temperaturfølere i fler-plans kjøledisker. In SINTEF Energy Research AS, Trondheim. 14.
8. Marth, E.H. 1998. Extended shelf life of refrigerated foods: Microbiological quality and safety. *Food Technology* 52:57-62.
9. Hemmingsen, A.K.T., Aune, E.J., Magnussen, O.M. 2001. Calculations of chilling and warming times of a fish patè product. In Trondheim. 13.
10. Nordtvedt, T.S. 2009. Superkjøling av fisk - en litteraturstudie og prosjektoversikt. In SINTEF Energiprosesser AS, Trondheim. 20.
11. James, S.J., James, C., Evans, J.A. 2006. Modelling of food transportation systems - a review. *International Journal of Refrigeration* 29:947-957.
12. Lebert, I., Lebert, A. 2006. Quantitative prediction of microbial behaviour during food processing using an integrated modelling apporoach: a review. *International Journal of Refrigeration* 29:968-984.
13. Doménech, E., Escriche, I., Martorell, S. 2010. Exposure Assessment based on a combination of event and fault tree analysis and predictive modelling. *Food Control* 21:1338-1348.
14. Giannakourou, M.C., Koutsoumanis, K., Nychas, G.J.E., Taoukis, P.S. 2005. Field evaluation of the application of time temperature integrators for monitoring fish quality in the chill chain. *International Journal of Food Microbiology* 102:323-336.
15. Winther, U., Ziegler, F., Hognes, E.S., Emanuelsson, A., Sund, V., Ellingsen, H. 2009. Carbon footprint and energy use of Norwegian seafood products. In SINTEF Fisheries and Aquaculture, Trondheim. 89.
16. Fennema, O.R., Powrie, W.D., Marth, E.H. 1973. Low-Temperature Preservation of Foods and Living Matter. Dekker, New York.
17. Nordtvedt, T.S. 2003. Super chilling - State of the art review. In SINTEF Energy Research, Trondheim. 11.
18. Stevik, A.M., Claussen, I.C:. 2010. Superkjøling - horisont. In Workshop, Competetive Food Processing in Norway. Stamsund, Norway.
19. le Danois, E. 1920. Nouvelle mèthode de frigorification du poisson. In French Patent No. 506.926. France.
20. Waterman, J.J., Taylor, D.H. 1967. Superchilling. In Torry Advisory Note. Torry Research Station, Ministry of Technology, Aberdeen. 10.
21. Beaufort, A., Cardinal, M., Le-Bail, A., Midelet-Bourdin, G. 2009. The effect of superchilled storage at -2ºC on the microbiological and organoleptic properties of cold-smoked salmon before retail display. *International Journal of Refrigeration* 32:1850-1857.
22. Harrington, R. 2009. New method for bleeding and superchilling cod fillets could cut costs. *Breaking News on Food Processing & Packaging*
23. Gallart-Jornet, L., Rustad, T., Barat, J.M., Fito, P., Escriche, I. 2007. Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon (*salmon salar*) fillets. *Food Chemistry* 103:1268-1281.

24. Bahuaud, D., Mørkøre, T., Langsrud, Ø, Sinnes, K., Veiseth, E., Ofstad, R., Thomassen, M.S. 2008. Effects of -1.5 ºC Super-chilling on quality of Atlantic salmon (*Salmon salar*) pre-rigor Fillets: Cathepsin activity, muscle histology, texture and liquid leakage. *Food Chemistry* 111:329-339.
25. Stevik, A.M., A.S. Duun, T. Rustad, M. O'Farrell, H. Schulerud, and S. Ottestad. 2010. Ice fraction assessment by near-infrared spectroscopy enhancing automated superchilling process lines. *Journal of Food Engineering* 100:169-177.
26. Magnussen, O.M., Haugland, A.,Hemmingsen, A.K.T., Johansen, S., Nordtvedt, T.S. 2008. Advances in superchilling of food - Process characteristics and product quality. *Trends in Food Science & Technology* 19:418-424.
27. Duun, A.S., Rustad, T. 2008. Quality of superchilled vacuum packed Atlantic salmon (Salmo salar) fillets stored at -1.4 and -3.6 degrees C. *Food Chemistry* 106:122-131.
28. Duun, A.S., Hemmingsen, A. K. T., Haugland, A., Rustad, T. 2008. Quality changes during superchilled storage of pork roast. *LWT-Food Science and Technology* 41:2136-2143.
29. Duun, A.S., and T. Rustad. 2007. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chemistry* 105:1067-1075.
30. Carlson, C.J. 1969. Superchilling of Fish- A Review. In Freezing and Irradiation of Fish. R. Kreuzer, editor Fishing News Ltd, London. 101-103.
31. Einarsson, H. 1988. Deep Chilling (Superchilling, Partial Freezing) a literature survey. Chalmars University of Technology, Gøteborg, Sweden. 30 pp.
32. Stevik, A.M. 2007. Microbiological level of unpacked, chilled and superchilled salmon fillets. In SINTEF Energy Research, Trondheim.
33. Stevik, A.M., Hemmingsen, A.K.T., Duun, A.S. 2010. Superchilling, icefraction and quality. In 1st IIR Cold Chain and Sustainability Conference. Cambridge.
34. Gram, L., Dalgaard, P. 2002. Fish spoilage bacteria - problems and solutions. Current Opinion. *Biotechnology* 13:262-266.
35. Nordtvedt, T.S. 1998. Use of partial freezing - superchilling - in fish processing and distribution. In IceTech. Reykjavik, Iceland.
36. Claussen, I.C. 2010. Superkjøling - Fremtidens metode for fersk fisk til forbruker. In Norfishing 2010. Trondheim, Norway. 22.
37. Aune, E.J., Nordtvedt, T.S. 1999. An environmentally transport of salmon from Norway to France by means of super-chilling and a new packaging method. In 20th International Congress of Refrigeration, IIR/IIF. Sydney, Australia. 6.
38. Olsen, Ø. 2008. Modelling and calculation of shelf life. In SINTEF Energy Reserach Trondheim. 4.
39. Rosnes, J.T., Sivertsvik, M., Skipsnes, D., Nordtvedt, T.S., Corneliussen, C., Jakobsen, Ø. 1998. Transport of superchilled salmon in modified atmosphere. In IIR Symposium Commission C2. Nantes, France. 12.
40. Fernández, K., Aspé, E., Roeckel, M. 2010. Scaling up parameters for shelf-life extension of Atlantic Salmon (Salmon salar) fillets using superchilling and modified atmosphere packaging. *Food Control* 21:857-862.
41. Fernández, K., Aspé, E., Roeckel, M. 2009. Shelf-life extension on fillets of Atlantic Salmon (*Salmon salar*) using natural additives, superchilling and modified atmosphere packaging. *Food Control* 20:1036-1042.
42. Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J., Nilsen, H. 1997. Methods to evaluate fish freshness in research and industry. *Trends in Food Science & Technology* 8:258-265.
43. Torstveit, A.K., Osvik, A.H., Stevik, A.M., Rustad, T. 2002. Quality of chilled and superchilled MA-packed salmon fillets. In 32nd WEFTA meeting. Galway, Irland. 10.
44. James, C., Seinemartin, V., James, S.J. 2009. The freezing and supercooling of garlic (*Allium sativum L.*). *International Journal of Refrigeration* 32:253-260.
45. IIR. 2006. Recommendations for the Processing and Handling of Frozen Foods. In IIR, Paris, France.
46. Dalgaard, P., Mejlholm, O. 2006. Prædiktiv mikrobiologi - et vigtigt redskab til forudsigelse og styring af fiskeprodukters holdbarhed og sikkerhed. In Fisk & Hav. Danmarks Fiskeriundersøgelser, Afdeling for Fiskeindustriel Forskning, København. 41.

47. Couvert, O., Pinon, A., Bergis, H., Bourdichon, F., Carlin, F., Cornu, M., Denis, C., Gnanou Besse, N., Guillier, L., Jamet, E., Mettler, E., Stahl, V., Thuault, D., Zuliani, V. 2010. Validation of a stochastic modelling approach for *Listeria monocytogenes* growth in refrigerated foods. *International Journal of Microbiology* 144:236-242.
48. Mejlholm, A.G., Borggaard, C., Bolm-Hansen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., Dalgaard, P. 2010. Predicting growth rates and growth boundary of *Listeria monocytogenes* - An international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology* 141:137-150.
49. Holmvåg, T.R. 2007. Mikrobiologisk kvalitet og vekttap hos islagrede laksefileter produsert *pre- og post rigor*. In Institutt for marin bioteknologi. Norges Fiskerihøgskole, Tromsø. 39.
50. Halder, A., Dhall, A., Datta, A.K., Black, D.G., Davidson, P.M., Li, J., Zivanovic, S. 2011. A user-friendly general-purpose predictive software package for food safety. *Journal of Food Engineering* 104:173-185.
51. Dalgaard, P. 2009. Seafood Spoilage and Safety Predictor (SSSP) software v. 3.1. In Technical University of Denmark, Lyngby. http://sssp.dtuaqua.dk/.