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Doctoral Thesis

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Dedicated to my lovely husband John and my two sons Allen and Aden

Abstract

The superchilling process is defined as a method of preserving food by partial ice-crystallization. The product quality of superchilled food is very promising, and has nearly the same sensorial attributes and nutritional value as the original product. However, more research is required to make the technology more suitable to the preservation of food. The optimal degree of superchilling and information on the development of the ice crystals during the superchilling process and storage are needed because of their large influence on the quality of the final superchilled food.

The main objective of this thesis was modelling and studying of ice crystallization/recrystallization of food during the superchilling process and storage. In order to fulfil the objective the following research activities have been carried out: A one-dimensional model for predicting partial freezing time necessary to achieve an optimal degree of superchilling in foods was developed. The degree of superchilling is the amount of free water frozen (5 -30%) inside the food and is among the most important parameters which influence the quality of superchilled product. The study of the ice crystallization/recrystallization of food in superchilling technology was studied based on the superchilling rate, and the state of food muscle. The relationship between the development of ice crystals in salmon and quality parameters during the superchilled storage was also studied. The final research activity in this thesis was to study differences in the superchilling storage methods, shell freezing and non- shell freezing.

The developed model was sufficient to study the thermal behaviour of food, and had the advantage that it was simple, very fast and detailed enough to estimate the superchilling time and behaviour of food. The model was validated experimentally using salmon, and there was good agreement between the numerical and experimental results. Further study to quantify the model using other food products is recommended.

The characteristics of ice crystals have a large influence on the quality of the final superchilled food. At a high superchilling rate (227 $W/m^2.K$, -30 ℃ and 2.1 min), smaller and well distributed ice crystals within and outside the cell were formed compared to a slow superchilling rate (153 $W/m^2.K$, -20 ℃ and 4.2 min), where larger and extra-cellular ice crystals were formed. The state of muscle also has an influence on the characteristics of ice crystals. In pre-rigor muscle, the ice crystals were formed inside the cells regardless of the superchilling rate. However, at a slow superchilling rate the ice crystal size was larger than at a high superchilling rate. The formation of the ice crystals inside the cells, regardless of the superchilling rates, is the most important factor for reducing the damage of food muscles and hence maintaining the quality.

New information was discovered in this work on the development of ice crystals during the superchilling process and storage of salmon. There was a significant increase in ice crystal size between the superchilling process (day 0) and superchilled storage (after 1 day of storage). The ice crystals formed in the surface layer were 4 times larger after only 1 day of storage than those formed at day 0. Prior to temperature equalisation, ice crystals growth progressed from the surface to the centre of the superchilled food. Different layers with different sizes of ice crystals within the superchilled salmon were also observed. This was due to thermal behaviour within the superchilled sample, and because we have both ice at the surface and water at the centre, the diffusion process should occur. The recrystallization at this time (between day 0 and 1) is unavoidable however, after temperature equalization (after 1 day of storage) and control of temperature during storage there was no significant growth of ice crystals for the entire storage time.

The development of ice crystals in red salmon muscle was also studied during the superchilling process and storage. The size of the ice crystals formed in the red salmon muscle was smaller than those formed in the white salmon muscle. In addition, the ice crystals formed in the pre-rigor red muscle was smaller than that formed in the post-rigor red salmon muscle. These findings are significant for the industry because small ice crystals indicate better quality.

Quality changes have been studied with a focus on physical measurements, water holding capacity (WHC) and drip loss. The disappearance of liquid water is a major factor affecting the protein changes during superchilled storage. It was observed that the drip loss was lower in superchilled salmon compared to conventional chilled salmon, and frozen salmon between 1 and 14 days of storage. No significant differences were found in WHC and drip loss between 1 and 14 days of storage in superchilled salmon.

The two superchilling storage methods showed differences in the development of ice crystals within the superchilled salmon. In non-shell frozen samples, the ice crystals were mainly formed in the extracellular spaces. Fine and well distributed ice crystals were formed in both the intracellular and extracellular spaces in shell frozen samples.

Generally, the results found in this study have given more information in the superchilling area. The developed model which can be scaled-up to the industrial level, together with information on the development of the ice crystals, which has a large influence on the quality of the final superchilled food are useful for the industry in estimating the refrigeration requirements for a superchilling system and designing the necessary equipment. In addition, the quality study revealed that the superchilling is practicable if the product is partially freezing fast, with an optimal degree of superchilling (5 - 30 %), good packaging and a strict control of the temperature during superchilled storage.

Preface

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) at Norwegian University of Science and Technology (NTNU). The work was carried out at the Department of Energy and Process Engineering from March 2010 – February 2014.The work was supervised by Professor Trygve Magne Eikevik and co-supervised by Professor Turid Rustad.

The PhD study was funded by NTNU and the Research Council of Norway through the project Competitive Food Processing in Norway (RCN project number: 178280). The study developed a model which predicts the partial freezing time (time spent in the freezers) necessary to achieve an optimal degree of superchilling in food. The characteristics of ice crystals during the superchilling process and storage were also studied. The degree of superchilling and characteristics of ice crystals are very important parameters because of their strong influence on the quality of the final superchilled food. The study concluded that the superchilling is a method for preserving the freshness and high quality of food. In order to fulfil with the demand for short processing times, a technique for quick/fast shell freezing food products which result in better production yields, improved product quality and a longer shelf life is required. Good packaging and a strict control of temperature throughout the cold chain is important.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Professor Trygve Magne Eikevik for his excellent guidance, enthusiasm and patience during my PhD study and research. I extend my appreciation to my co-supervisor Professor Turid Rustad for her expertise on quality analyses and always being available for discussions, assistance and valuable corrections of my writing. I am grateful to all who assisted me with laboratory work, as this work has been largely experimental. My sincere thanks go to laboratory senior engineer Tora Bardal and Professor Elin Kjørsvik, at NTNU SeaLab, for giving opportunities and guiding me to work in their exciting laboratory. I would also like to thank SINTEF laboratory engineer, Per Egil Gullsvåg, for his assistant with the Impingement freezer and calorimetric experiments. Inge Håvard Rekstad, Martin Bustadmo, Erik Seehuus and Marius Østnor Døllner are also thankful for their laboratory assistance.

I would also like to thank the food engineering staff from NTNU and SINTEF for their patient and valuable corrections.

I owe special thanks to my parents, Mary Kaale and Daniel Kaale for their parental guidance and care.

I am deeply grateful to my lovely husband John and to my wonderful children Allen and Aden for their patience, love and support.

Table of Contents

List of papers

This thesis is a collection of 8 journal papers and 1 manuscript. They are referred to in the text by their Roman numerals or name and year. The papers are listed in appendices 1-IX

I. Kaale, L. D., Eikevik, T. M., Rustad, T., & Kolsaker, K. Superchilling of food, a review. Journal of food engineering, 107(2), 141-146. 2011

II. Kaale, L. D., Eikevik, T. M., Kolsaker, K., & Stevik, A. M.. Modelling and simulation of food products in superchilling technology. Journal of Aquatic Food Product Technology http://dx.doi.org/10.1080/10498850.2012.721160. 2013

III. Kaale, L. D., Eikevik, T. M., Bardal, T., Kjorsvik, E., & Nordtvedt, T. S. The effect of cooling rates on the ice crystal growth in air-packed salmon fillets during superchilling and superchilled storage. International journal of refrigeration, 36(1), 110-119. 2013.

IV. Kaale, L. D., Eikevik, T. M., Rustad, T., Nordtvedt, T. S., Bardal, T., & Kjorsvik, E. Ice crystal development in pre-rigor Atlantic salmon fillets during superchilling process and following storage. Food Control, 31(2), 491-498. 2013.

V. Kaale, L. D., Eikevik, T. M., Bardal, T., & Kjorsvik, E. A study of the ice crystals in vacuum-packed salmon fillets (*Salmo salar*) during superchilling process and following storage. Journal of food engineering, 115(1), 20-25. 2013

VI. Kaale, L. D., & Eikevik, T. M. A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (*Salmo Salar*) fillets during superchilling process and storage. Journal of food engineering, 114(2), 242-248. 2013.

VII. Kaale, L. D., & Eikevik, T. M. A study of the ice crystal sizes of red muscle of pre-rigor Atlantic salmon (*Salmo salar*) fillets during superchilled storage. Journal of Food Engineering 119 (3), 544 – 551. 2013.

VIII. Kaale, L. D., Eikevik, T. M., Rustad, T., & Nordtvedt, T. S. Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage. LWT - Food Science and technology, 55(2), 528-535. 2014.

IX. Kaale, L. D., & Eikevik, T. M. The influence of superchilling storage methods on the characteristics of ice crystal (distribution/ location) during storage of Atlantic salmon (*Salmo salar*). In: The European Federation of Food Science and Technology (EFFoST) conference. Bologna, Italy, 2013.

The author's contribution

The PhD student (Lilian Daniel Kaale) has been the main author of all nine articles included this thesis. The work has been done in the following manner.

Paper I: The PhD student (Lilian Daniel Kaale) did the literature review, and wrote and published the paper in the Journal of Food Engineering.

Paper II: The PhD student (Lilian Daniel Kaale) was involved in developing the model using Matlab software supervised by Professor Kjell Kolsaker. The PhD student (Lilian Daniel Kaale) planned and performed the experiments for validating the model, interpreted of the results, and wrote and published the paper in the Journal of Aquatic Food Product Technology.

Paper III: Professor Elin Kjorsvik and engineer Tora Bardal trained the PhD student (Lilian Daniel Kaale) how to use different equipment for carrying out tissue processing and microscopic analysis experiments in their laboratory. The PhD student (Lilian Daniel Kaale) did the literature survey, discovered the method, planned and performed the experiments, interpreted of the results, and wrote and published the paper in the International Journal of Refrigeration.

Paper IV: The PhD student (Lilian Daniel Kaale) did the literature survey, planned and performed the experiments, interpreted of the results, and wrote and published the paper in the Food Control.

Paper V: The PhD student (Lilian Daniel Kaale) did the literature survey, planned and performed the experiments, interpreted of the results, and wrote and published the paper in the Journal of Food Engineering.

Papers VI and VII: The PhD student (Lilian Daniel Kaale) did the literature survey, planned and performed the experiments, interpreted of the results, and wrote and published the papers in the Journal of Food Engineering.

Paper VIII: The PhD student (Lilian Daniel Kaale) did the literature survey, planned and performed the experiments, interpreted of the results, and wrote and published the paper in the LWT – Food Science and technology.

Paper IX: The PhD student (Lilian Daniel Kaale) did the literature survey, planned and performed the experiments, interpreted of the results, and wrote and presented the paper to the European Federation of Food Science and Technology (EFFoST) conference, Bologna, Italy.

List of Figures

Nomenclature

Roman Symbols

$C\ i$	Element heat capacity (ρ*cp*L)	J /m^2 K
C_p	Specific heat capacity	J /kg K
h_a	Convective heat transfer coefficient	W /m^2 K
i	Indicate position in the X direction	-
k	thermal conductivity	W /m K
L	Thickness of the slab	m
N	Number of subdivisions	-
R	Thermal resistance	m^2K/W
t	Time	s
T'	unknown temperatures at time (t+Δt) at a given node	℃
T_∞	air temperature	℃
T_i	Initial temperature	℃
X	Position	m

Greek Symbols

Δt	Time step	s
ρ	Density	kg/m^3
δ	Element thickness	m

Chapter 1

Introduction

1.1. Background

Superchilling is a technology used to preserve the freshness and high quality of food by partial crystallization. The technology was described as early as 1920 by Le Danois. Various definitions have been used to describe the process 'superchilling', 'deep-chilling' light freezing, supercooling or 'partial ice formation' (Einarsson, 1988). The main aim is to extend the shelf life of foods compared to conventional chilling and to maintain quality and freshness of foods. Today the volume and value of fresh, refrigerated foods is increasing along, with the flow of these products between countries, and superchilling appears to be a better mode for their preservation. This has increased the interest of the food processing companies and research institutes based on the research in the superchilling technology. However, most of these studies have focused on the chemical, microbiology and physical analyses of foods in superchilling. Nevertheless, successful implementation of superchilling in the food industry will depend on an efficient method for defining the optimal degree of superchilling. The current method to measure the degree of superchilling is calorimeter, which is labour-intensive and time-consuming (Ottestad et al., 2009; Stevik et al., 2010). To ensure that superchilling achieves its objectives, there is a need to establish a tool which is better, quick and more efficient to define the degree of superchilling in foods. In addition, information, on the development of ice crystals during the superchilling process and storage, and to control temperature during storage/distribution, is needed. Therefore, this work focuses on the modelling and ice crystallization/recrystallization of food products in superchilling technology. There is a need to develop a tool for predicting the partial freezing time necessary to achieve the optimal degree of superchilling in food products which will increase shelf life, and maintain quality of food products. The characteristics of ice crystals (crystallization/recrystallization) which have a large influence on the quality of final superchilled food should be investigated. On the other hand, the temperature should be stable enough to avoid significant levels of ice crystal growth that can cause structural damage during the storage of the superchilled product.

1.2. Status of knowledge

The first reference concerning industry interest in the commercial use of the superchilling storage of food products appeared in the trade journal fishing Gazette 1935 (Carlson, 1969). The

method has been used aboard a number of Portuguese trawlers working in the warmer parts of the Atlantic, and has also been tried on a German vessel (Waterman and Taylor, 2001). The past 10 – 15 years, superchilling technology has increased significantly and many advantages of using superchilling to preserve food products have been shown (Kaale et al., 2011). On the negative side of superchilling, the main concern is the formation and growth of ice crystals (Einarsson, 1988). However, the structural changes due to ice crystal formation at sub-zero temperature storage appear to be minor compared to those occurring during freezing at -20℃ (Einarsson, 1988). It has also been reported that, in order to avoid the influence of low quality in the superchilled food, it is necessary to have the degree of superchilling between 5% and 30% inside the products and that the degree of superchilling larger than 30% inside the product will result in low quality of the food (Stevik and Claussen, 2011). In this study, 20 % has been used. Ronsivalli and Baker (1981) also report that the superchilling process is effective and practical, provided that the temperature does not fall below the point where freezing is discernible (i.e. -2℃). This was the recommendation which derived from research teams from England and later by teams from Canada, The Federal Republic of Germany, and the United states (Ronsivalli and Baker, 1981). However, at this temperature (-2℃), ice crystals will still form since the initial freezing points of most foods are between -0.5℃ and -2.8℃. Therefore, there is a need to analyse the ice crystals during the superchilling process and storage, in order to understand the mechanism of ice crystals in superchilled food because this will give suitable information on the characteristics of ice crystal. Thus, the control of temperature during superchilling, the optimal degree of superchilling, and hence the information on the development of ice crystals, are all essential if the damaging effects during storage are to be avoided.

1.3. Aims of the study

A lot of studies have been done on superchilling. Most of these studies have focused on product quality and shelf life. The main feedback from those studies is, superchilling method extends the shelf life of foods compared to the traditional chilling and maintains high quality foods. Nevertheless, the superchilling temperatures are low enough to significantly suppress microbial activity but other chemical and physical changes may take place and in some cases even accelerate. Therefore, more research is required to study the factors which may influence on these changes such as degree of superchilling and characteristics of ice crystals. The information on the development of ice crystals during the superchilling process and superchilled storage and the optimal degree of superchilling are useful because of their strong influence on the quality of the final superchilled food. To date, there is no quick and better way for defining the degree of

superchilling in food. In addition, there is limited information on the development of ice crystals during the superchilling process and following storage.

Therefore, the main objective of this thesis was the modelling and studying of ice crystallization/recrystallization of food during the superchilling process and storage. In order to fulfil the objective the following research activities have been carried out:

1. A one-dimensional model for predicting partial freezing time necessary to achieve an optimal degree of superchilling in foods (salmon was used in this study) was developed. The model was validated experimentally using salmon.
2. The study of the ice crystallization/recrystallization (characteristics of ice crystals) of food in superchilling technology was studied based on the superchilling rate (small verses high), and the state of food muscle (pre – and post – rigor) which are important parameters for determining the characteristics of ice crystals. The characteristics of ice crystals were further studied by comparing the ice crystals formed in the red and white muscles of salmon.
3. Packaging is also an important parameter to consider during superchilled storage because it defines the shelf life and maintains the quality of the final superchilled food. Therefore, the comparison study between vacuum and air-packed salmon was carried out.
4. The relationship between the development of ice crystals in salmon and quality parameters during the superchilled storage was also studied. Quality changes have been studied with a focus on physical measurements, water holding capacity (WHC) and drip loss.
5. The final activity in this thesis was to study differences in the superchilling storage methods, shell freezing (i.e. initial surface freezing of salmon and the followed storage at superchilling temperature) and non- shell freezing (i.e. storage of salmon at superchilling temperature without initial surface freezing/shell freezing) in order to understand the effect of ice crystal development in salmon muscle during superchilled storage.

Chapter 2

2. The modelling and simulation of a food product in superchilling process

The main task in modelling a food product is to develop a set of simultaneous equations which represent heat conduction within the product, boundary conditions, and the initial condition (Cleland, 1990), while programming is to keep track of the average degree of superchilling (frozen water) during all stages of the product handling.

During the superchilling process a thin frozen layer of about 1 - 3 mm is formed on the surface of food depending on the superchilling rate and thickness of the product. This section focuses on developing a model for predicting/estimating the partial freezing time necessary to achieve an optimal degree of superchilling in food. The partial freezing time can be predicted using numerical solutions of the heat transfer equations. The advantage of numerical methods is that the effects of phase change over a range of temperatures, changing thermal properties and the heterogeneity of food products can be considered (Resende et al. 2007; Zuritz and Singh, 1989). If numerical methods are formulated and implemented correctly to reduce truncation and rounding errors, they are generally considered the most accurate, reliable and versatile superchilling process prediction methods. For realistic and thus more complicated heat transfer problems usually no analytic solution is available, and a numerical solution becomes mandatory (Abbas *et al.* 2004; Kreith *et al.* 2003). These numerical methods are capable of handling any type of boundary condition and product geometry.

2.1. Numerical methods

In the numerical solution of heat conduction problems with phase change by finite differences, enthalpy methods or temperature methods can be used (Pham, 1985). The heat diffusion equation can be expressed in the following two ways (Pham, 1985; Delgado and Sun, 2001; Lind, 1991)

$$C_p T \frac{\partial T}{\partial t} = divkTgrad\ T \qquad 2.1$$

$$\frac{\partial H}{\partial t} = div\ k\ H\ grad\ T\ H \qquad 2.2$$

The equation 2.1 uses temperature as the only dependent variable, while the equation 2.2 represents the enthalpy methods, which have two dependent variables, enthalpy being the primary, and temperature the secondary variable. The enthalpy method requires either an explicit technique, with the consequent problem of convergence, or implicit procedures in which iteration at each time step is used, consuming more computational time. However, to avoid the need for iteration, Pham (1985) proposed the 'temperature-enthalpy correction method', a hybrid temperature-enthalpy method. At each time step, the conduction equation is solved by an implicit method in the usual manner. Moreover, the enthalpy method has more advantage, the change in the relative enthalpy content of the product, ΔH, during thawing and freezing is continuous with temperature (Lind, 1991). On the other hand, the temperature method, the latent heat is represented by a large but finite wide peak of the curve C_p vs. T. The peak in the C(T) curve is narrow (Figure 1), so if the temperature change per time step is too large, a nodal temperature may 'jump' past the freezing temperature range in one step, resulting in the latent heat being ignored [since *Cp(T)* never takes the peak value] (Cleland, 1990; Pham, 1985).

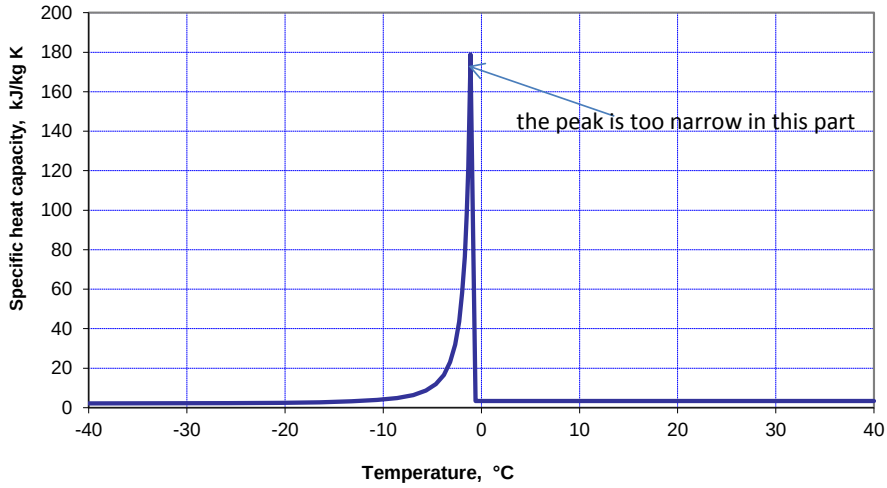

Figure 2.1: Specific heat capacity curve against temperature

This possibility can be checked by on-going heat balance in the calculation, or within a finite difference where the computer program should be regarded as an obligatory checking procedure (Cleland, 1990). Poor heat balances are avoided by the selection of smaller Δt, but this makes

computation times longer. However, several authors have used temperature method and shown good results (Hughes and Charng, 1983; Resende et al., 2007; Wang et al., 2007).

2.1.1. Explicit and implicit scheme

The model used in this study is a simple finite difference method adapted to handle the nonlinear physical properties. The model is implemented in the MATLAB, and has been tried with the built-in ordinary differential equation ODE-solvers with various successes. There are generally two approaches for time integration: explicit and implicit scheme. Our study used the implicit scheme. The explicit scheme is easy to apply but may be computationally demanding due to stability restrictions on the choice of time step. The implicit time integration scheme on the other hand, has no time step restrictions. The success in applying implicit schemes is based on maintaining accuracy in the results, while at the same time avoiding excessive iterations in the solutions of the resulting nonlinear algebraic equations (Swaminathan and Voller, 1992). It seems that in spite of a large time increment, the computational efficiency of the implicit methods is not better than that of the explicit methods (Tavakoli and Davami, 2007). However, the study of (Swaminathan and Voller, 1992) explains well the advantage of using implicit scheme. This article is known as one of the basic and excellent references in the category of phase-change problems. In this article, the authors present a general implicit enthalpy method that has significant efficiency in comparison with other implicit methods. The authors concluded that the results of the explicit and implicit methods are in close agreement.

Implicit methods are generally harder to implement than explicit methods, but they have much better stability properties. In addition, for transient problems with one-dimension, the computational effort per time step for the implicit scheme is not a big problem (Recktenwald, 2011). For transient problems with two or three dimensions, however, the computational effort per time step for an implicit scheme is much greater than the computational effort per time step of an explicit scheme. Nevertheless, the superior stability of the implicit scheme usually provides an overall computational advantage (Clavier et al., 1994; Idelson et al., 1994; Knoll et al., 1999; Muhieddine et al., 2009; Recktenwald, 2011; Tavakoli and Davami, 2007; Trefethen, 1994; Voller, 1987, Swaminathan and Voller, 1992).

Eq. (2.3a to 3e) is a model of the transient heat conduction in a slab of material with thickness L and boundary conditions. The heat transfer equations below were adapted to develop the model in this study.

$$\alpha = \frac{k}{\rho C_p} \tag{2.3a}$$

$$\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} \qquad 0 < x < L \tag{2.3b}$$

$$\frac{\partial T}{\partial t} = \frac{h_a}{\rho C_p}(T_\infty - T) \qquad x = 0\ (surface) \tag{2.3c}$$

$$\frac{\partial T}{\partial t} = 0 \qquad x = L\ (symmetric\ plane) \tag{2.3d}$$

$$T(x) = T_i \qquad 0 < x < L \tag{2.3e}$$

The core element of the model can be visualized as an electrical analogy, with heat storage as the capacitors and conduction as the resistors (Figure 2.1). It assumes that the thermal properties are isotropic within each element. The node temperature is assumed to be in the centre. The model is composed as a row of directly connected elements. Connecting the elements ends in a final electrical analogy for the systems (Figure 2.2), generalized for a specific element. The surface boundary element is similar, but R_i+R_{i-1} includes the surface heat transfer coefficient. For the internal boundary element, $R_{r,N}$ is totally removed.

$$C\ i\ \frac{T'(i) - T(i)}{\Delta t} = \frac{T'(i-1) - T'(i)}{R(i-1)} + \frac{T'(i+1) - T(i)}{R(i+1)} \tag{2.4}$$

Where

$$C = \rho C_p \delta, \quad R = \frac{\delta}{2k}\ and\ \delta = \frac{L}{N}$$

Eq.(2.4) may be expressed as:

$$-\frac{1}{R(i-1)}T'(i-1) + \frac{C(i)}{\Delta t} + \frac{1}{R(i-1)} + \frac{1}{R(i+1)}\ T'(i) - \frac{1}{R(i+1)}T'(i+1) = \frac{C(i)T(i)}{\Delta t} \tag{2.5}$$

The coefficients of $T'\ i-1\ , T'\ i\ and\ T'\ i+1$ designates a(i), b(i), and c(i), respectively form a tridiagonal matrix with diagonal vectors a, b, and c where

$$a(i) = \frac{1}{R(i-1)}$$

$$b(i) = \frac{C(i)}{\Delta t} + \frac{1}{R(i-1)} + \frac{1}{R(i+1)}$$

$$c(i) = \frac{1}{R(i+1)}$$

The right-hand side of Eq.(2.5) the global forcing matrix containing known terms arising from heat generation, and boundary conditions (Pham, 2006) is designated as the vector d, where $d(i) = \frac{C(i)T(i)}{\Delta t}$ (Hughes and Charng, 1983). In conventional thermal food processes the heat generation Q is zero, (Nicolaı¨, et al., 2000).

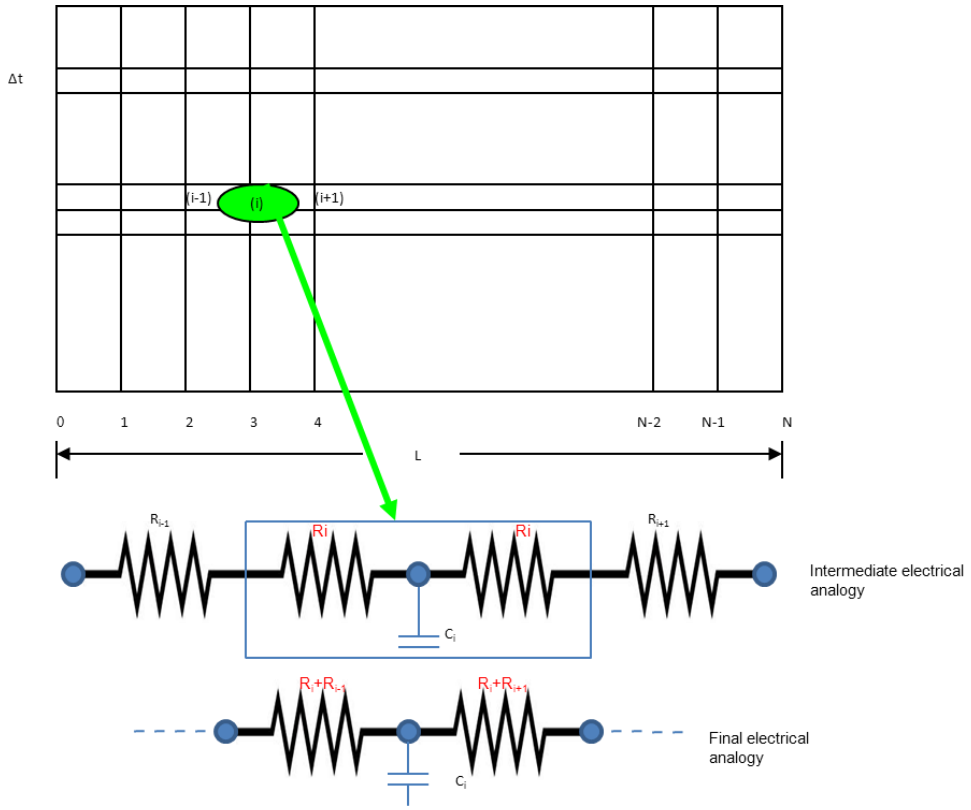

Figure 2.2: The mesh of time and space intervals and thermal resistance connected in series during implicit finite difference solution.

2.1.2. Error estimation of the numerical solutions

Error estimation of the numerical solutions for this kind of empirical nonlinear equations has not been studied in detail. Instead, the model was tried on a specific sample, and a typical superchilling scenario with different resolution in space and time. The simulation model is based on symmetry and models the sample from the centre to the surface. The number of nodes is 50, and all the thermal properties are available in the simulation input. The ice fraction, temperature and the enthalpy are averaged over the total sample and are the most useful output.

Chapter 3

3. Ice crystallization in food at superchilling

In this study, the superchilling technology is divided in two parts, the superchilling process (initial surface freezing) and superchilled storage. The superchilling process consists of two stages; cooling the product to its freezing point (pre-cooling or chilling stage) and removing the latent heat of crystallization (phase transition stage) whereby about 5–30% of the free water is frozen inside the food products. These stages are achieved at the surface of the food products, to a depth of 1-3 mm, depending on the degree of superchilling required, superchilling rate and the thickness of food. The ice crystallization of water occurs during the phase transition part of the superchilling process and is the key step determining the efficiency of the process (Kiani and Sun, 2011; Kiani et al., 2011). During the phase transition stage, water will undergo the stage of ice nucleation, followed by the growth of ice (Liu and Du, 2004), which are important stages for the crystallization process.

Crystallization is a process whereby a crystalline phase is created as a consequence of molecular aggregation in a solution, leading to the formation of nuclei and later, crystal growth (Delgado and Sun, 2012). The quality of superchilled food is mainly related to the properties of the ice crystals, such as size, location (i.e. extracellular and intracellular) and morphology during the superchilling process (Alizadeh et al., 2009; Martino and Zaritzky, 1986; Martino et al., 1998; Petzold, and Aguilera, 2009). The interaction between the nucleation and crystal growth, which are the main processes in crystallization, determines these characteristics of ice crystals (DeMan, 1999; Kiani et al., 2011; Kiani and Sun, 2011).

3.1. Nucleation and crystal growth

The effects of superchilling process conditions on the quality of superchilled food are often related to the characteristics of ice crystals. The primary factor affecting the characteristics of the ice crystals immediately after the superchilling process is the rate of nucleation (Roos, 2012). This is useful information that allows the control of the ice crystal's size and ultimately, in cellular food, the formation of extracellular and intracellular water.

Nucleation is the combining of molecules into ordered particles of a size sufficient to survive and serve as a site for crystal growth (Cubillas and Anderson, 2010; Einarsson, 1988). Nucleation is the start of the crystallization process, and involves the birth of a new crystal (Schwartz and Myerson, 2002). There are two types of nucleation; primary nucleation and

secondary nucleation. Primary nucleation involves the formation of a crystal in a solution containing no existing crystals (Delgado and Sun, 2012; Chow et al., 2005). The classical theory of primary nucleation defines the total work $W = W_s+W_v$, required to create a nucleus as the sum of the work required to form a surface, Ws, and the work required to form the bulk of the particle, W_v (Kiani and Sun, 2011). Secondary nucleation involves the production of new crystals in a solution containing pre-existing crystals, and it can occur either by the crystals acting as templates for a new crystal's nuclei to be formed or by the crystals fragmenting to produce more nucleation sites (Delgado and Sun, 2012; Chow et al., 2005).

Nucleation can be homogeneous, in the absence of foreign particles or crystals in the solution, or heterogeneous, in the presence of foreign particles in the solution (Cubillas and Anderson, 2010). Homogeneous nucleation happens only in pure water, in the absence of any foreign material, at a homogeneous nucleation temperature of about -40 ℃ (Pham, 2012; Roos, 2012). Homogeneous is unlikely in a food system as (1) the cell walls and polymeric components provide surfaces for nucleation; (2) dissolved substances cause depression of the chemical potential of water and the freezing temperature; and (3) foods contain impurities that enhance nucleation (Roos, 2012). Heterogeneous nucleation is therefore the prevailing mechanism in food partial freezing (Pham, 2012; Roos, 2012). Nucleation conditions are of fundamental importance in the control of the superchilling process and storage of superchilled food properties. The main property of the partial frozen materials affected by the superchilling rate is the size of the ice crystals, which can be explained by the effects of supercooling, on the rate of nucleation and crystal growth (DeMan, 1999; Roos, 2012). Supercooling (the difference between the actual temperature of the sample and the expected solid-liquid equilibrium temperature at a given pressure) is the driving force for ice nucleation and is an important parameter that controls the size and number of ice crystals formed (Mittal and Griffiths, 2005). This is because a high level of supercooling increases the rate of nucleation and the number of nuclei can then grow to a large number of small ice crystals. Consequently, the cells maintain their integrity, which in turn minimises the drip loss during thawing (Pham, 2012; Smith, 2011). At low levels of supercooling, the rate of nucleation is low while there is a high rate of crystal growth. This allows a small number of nuclei to grow and form a small number of large ice crystals (DeMan, 1999; Roos, 2012).

Crystal growth, in contrast to nucleation, occurs readily at temperatures close to the freezing point (DeMan, 1999). The two important mechanisms in crystal growth are the diffusion of molecules from bulk to the crystal surface and surface integration, that is, the incorporation of a

growth unit into a lattice (Delgado and Sun, 2012). In superchilled food only 5 to 30 % of the water is in a solid state. The size and location of the ice crystals is vital to the quality of the superchilled food (Einarsson, 1988), particularly for the surface layer which is totally frozen.

3.2. Methods for the evaluation of ice crystals

This section addresses the methods of evaluating the ice crystals during the superchilling process and storage. The evaluation of ice crystals' properties can be done using direct or indirect methods (Nurzahida et al., 2010). Russell et al. (1999) and Evans et al. (1996) have used direct method to view frozen specimens on a microscope cryostage (cryo-scanning electron microscope) and confocal laser scanning miscopy, respectively.

For the indirect method, the evaluation of ice crystals in the partially frozen food products can be carried out after thawing the sample; or freeze drying or using a freeze substitution before observation (Kiani and Sun, 2011). Nurzahida et al. (2010) used the freeze drying method to study the spaces left by the ice crystals. Histological evaluations have also been among the methods employed for the evaluation of ice crystal characteristics and the damages to the tissue (Kiani and Sun, 2011). Freeze substitution (Alizadeh et al., 2007; Feder and Sidman, 1958; Martino and Zaritzky, 1988) and freeze fixation (Miyawaki et al., 1992) has also been reported. Freeze substitution was described as an excellent method of preparing tissue for study in the microscope (Feder and Sidman, 1958). It is a convenient method for generating high quality histological material and with only minimal denaturation (Knight, 2009). It is compatible with the use of wax sectioning; it is also more convenient than cryo-fixation (Knight, 2009). Freeze-substitution is based on solution substitution of ice at temperatures well below 0 ℃ (Feder and Sidman, 1958; Kiani and Sun, 2011).

In this study a freeze-substitution method was implemented. Freeze-substitution is based on the freezing/partial freeze of the tissue and then the ice within the tissue is slowly dissolved in polar solutions capable of substituting for ice, such as ethanol or acetone. The main purpose for doing this is to fix the ice crystal and preserve the structural integrity of a specimen so that it can be viewed microscopically. The entire process is called fixation. There are many methods of sectioning tissues such as the agar, gelatin or wax methods. The most widely used is the paraffin method. For the case of paraffin, which is also used in this study, all samples must pass through different procedures i.e. dehydration, infiltration and embedding in paraffin, sectioning with a microtome, mounting on microscope slides, clearing and staining and preparation of permanent mounts, before microscopical examination. The detailed method is explained in papers III, IV, V, VI and VII.

3.3. Factors affecting quality of the superchilled products during storage

The quality of superchilled food depends on many factors such as the superchilling process, packaging, storage temperature and quality of the raw material. The raw material quality is important, and this quality must be preserved during processing and storage (Blond and Meste, 2004; Margeirsson et al., 2011; 2012). There is no single universal rule governing superchilled food preservation; just as with optimal superchilling rates, which vary from product to product, the storage time depends not only on the temperature but also on the type of product and packaging (Blond and Meste, 2004). Moreover, it is well known that superchilled foods stored at fluctuating temperatures have not the same quality as products stored at constant temperatures. In order to ensure product quality, temperature control is necessary throughout the cold chain, and the required temperature must be maintained during distribution or storage. Magnussen et al. (2008) reported that a typical accuracy of ± 0.5 ℃ gives poor feedback from product to process. While accurate temperature measurements less than ± 0.5 ℃ can be carried out under laboratory conditions, one cannot expect to improve on an accuracy of less than ±0.5 ℃ under industrial conditions. A temperature fluctuation≤0.3 ℃ was proved to have no influence on the growth of ice crystal size during the superchilled storage of salmon fillets (Kaale et al., 2013c; 2014) which also resulted in the good quality of the superchilled fillets, based on the physical measurement done in this study.

Temperature fluctuation during superchilled storage can result in recrystallization, which is an important factor affecting superchilled food quality during storage. Recrystallization is a temperature-dependent process, which is enhanced by temperature fluctuations (Roos, 1995). Small ice crystals are thermodynamically unstable, having a high surface–volume ratio and therefore a high excess of surface free energy (Alizadeh et al., 2007; Russell et al., 1999; Shenouda, 1980; Zaritzky, 2012). Recrystallization basically involves the small crystals disappearing, large crystals growing, and crystals fusing together, and affects the quality of the products because small ice crystals indicate better quality while large crystals often produce damage during partial freezing (Kaale et al 2013b; Pham and Mawson, 1997; Roos, 1995; Zaritzky, 2012). The principal mechanisms of recrystallization in partial frozen foods are the iso-mass, migratory and accretive.

Surface iso-mass recrystallization: refers to a change in the crystal structure, which occurs as a crystal enters a lower energy level maintaining a constant mass of ice with surroundings (Blond and Meste, 2004; Roos, 1995; Zaritzky, 2012). **Migratory recrystallization:** is the result of differences in the surface energies of large and small crystals. Small ice crystals

disappear as large ones grow, as a result of the difference in their melting points, small crystals melt, and the melted water recrystallizes on larger crystals. The number of crystals decreases with time and their mean size increases with time (Blond and Meste, 2004; Karel and Lund, 2005; Roos, 1995; Zaritzky, 2012).

Accretive recrystallization*:* This is the joining together of two ice crystals, increasing the crystal size and decreasing the number of crystals and the surface energy of the crystalline phase. The phenomenon again leads to increased stress and irreversible damage to tissues, and hence increased drip loss and textural changes (Archer and Kennedy, 1998; Blond and Meste, 2004; Zaritzky, 2012).

3.4. Control of recrystallization during storage of superchilled products

Knowledge of the crystallization mechanisms and effects of temperature and time on the physical state can be used to control ice formation and recrystallization in partially frozen foods (Roos, 1995). There are four strategies for the control of ice crystals in foods; inhibition of nucleation, control of nucleation, exploitation of the glassy state and control of ice crystal growth (Roos, 1995). The **nucleation of ice** in food materials can be inhibited by the addition of large amounts of osmotically active materials such as sugars. Then, the unfrozen state together with a low temperature reduces the rates of chemical and physical changes, but the detrimental effects of partial freezing and partial freezing-concentration can be avoided (Roos, 1995). The **control of nucleation** can be achieved by employing a high rate of superchilling to produce a large number of small ice crystals while a low rate of superchilling will produce large ice crystals. The control of the ice nucleation, crystal growth, and ice recrystallization by the **exploitation of the glassy state** is based on the rate-controlling effect of the T_g without added compounds. The control of **ice crystal growth** uses **anti-freezing agents** such as salt or anti-freeze proteins. The possibility of using anti-freeze proteins, a technology which has been studied extensively for nearly 30 years with research focusing on their structures, function and mechanisms of action (Feeney and Yeh, 1998), could be one way of doing superchilling. Anti-freeze proteins have the ability to influence ice growth by interacting directly with the ice surface (Wang and Sun, 2012). Antifreeze proteins lower the freezing point of water, but not the melting point, by a specific non-colligative mechanism termed adsorption inhibition (Feeney and Yeh, 1998; Payne et al. 1994). These antifreeze proteins are able to inhibit ice crystal growth and reduce recrystallization (Mishra et al. 2010; Payne et al. 1994; Wang and Sun, 2012). These proteins are able to inhibit ice crystallisation both at low concentrations and high concentrations; they are able to completely inhibit ice crystal formation over a temperature

range, which is dependent on the AFPs themselves. This protection however is not complete, and ice crystal growth will still increase under severe temperature abuse, though less rapidly than without AFP (Mishra et al. 2010; Payne et al. 1994; Wang and Sun, 2012). Their ability to inhibit ice recrystallization has been shown to reduce the cellular damage in meat and inhibit the appearance of ice crystals in frozen food such as ice cream (Wang and Sun, 2012).

The freezing point of food can also be lowered by using salt. James et al. (2005) reported that the freezing point of cured meat was lowered using salts. The initial freezing points were -1.4, -3.1, -4.1, -5.2 and -6.3 at salt contents of 0.5, 2, 3, 4 and 5 kg salt/100 kg sample respectively (James et al., 2005).

The **control of nucleation** has been done in this study (paper III, IV and VII), and was proved to give small and well distributed ice crystals when a high superchilling rate was applied, compared to a slow superchilling rate where most of the ice crystals were formed in the extracellular space of post-rigor salmon muscle. However, this is possible only during the superchilling process (initial surface freezing). During superchilled storage (inner layer or centre layer) using control of the nucleation is not possible and other methods like using the anti-freezing agents such as anti-freeze proteins have to be tested. Therefore, in the future, it is recommended to test these methods to see if they will help prevent recrystallisation during storage of the superchilled products, particularly during the first day of storage where recrystallization is unavoidable.

Chapter 4

Summary of the papers

4.1 Paper I

Title: Superchilling of food: A review

This paper represents an overview of the recent work within the superchilling area. The review summarises the quality and shelf life of superchilled foods and the numerous benefits of applying superchilling process to food products were found. This article concluded that it is necessary to develop a tool which can define an optimal degree of superchilling immediately after the superchilling process. It is also concluded that information on the development of ice crystals during the superchilling process and storage is required.

4.2 Paper II

Title: Modelling and simulation of food products in superchilling

A new development to determine the degree of superchilling immediately after the superchilling process should be established in order to avoid a negative impact on the quality of the superchilled product. Modelling/simulation is an important tool on predicting degree of superchilling because it is cheaper and easier to implement.

This paper presents the modelling and simulation of salmon in the superchilling process. A one-dimensional model for predicting/estimating the partial freezing time (time spent in the freezers) necessary to achieve an optimal degree of superchilling in food products was developed. A finite differential numerical method under implicit time integration was used. The model was validated experimentally using the calorimetric method, temperature measurement and histology method. Although the results show a good level of agreement between the numerical simulation and the experimental results using salmon, tremendous efforts are needed to further quantify the model using other products and degree of superchilling (i.e. 5, 10 and 30 %).

4.3 Paper III

Title: The effect of cooling rates on the ice crystal growth in air-packed salmon fillets during superchilling and superchilled storage.

Paper III addresses the development of ice crystals in salmon muscle during the superchilling process and storage. It also illustrates the effect of the superchilling rate on the ice crystal formation in salmon muscle. The samples partially frozen at slow superchilling rates contained large and extracellular ice crystals during the superchilling process (partial freezing) and following storage of the superchilled salmon, while the samples that were partially frozen at fast rates had smaller and finely distributed ice crystals.

A significant difference between the size of ice crystals formed during the superchilling process and superchilled storage was observed. The study also discovered different layers with different sizes of ice crystals within the superchilled salmon. This was due to temperature fluctuations and thermal conditions inside the superchilled salmon.

4.4 Paper IV

Title: Ice crystal development in pre-rigor Atlantic salmon fillets during the superchilling process and following storage.

Paper IV reports on the development of ice crystals in pre-rigor salmon muscle. The availability of high quality product to the market relies on early processing. The consumers prefer fresh foods, but the most important issue is a product with a good nutritional value. Therefore, this paper also addresses the effects of both the rapid, and slow partial freezing of pre-rigor salmon fillets on the development of ice crystals during the superchilling process and storage.

Differences in the size of the ice crystals formed at slow and high rates of superchilling were found. It is clearly shown that during rapid superchilling, a large number of smaller ice crystals were formed within the cell. Nevertheless, during the slow superchilling of pre-rigor salmon muscles, the ice crystals were formed inside the cells. However, a small number of large ice crystals were observed compared to those in rapid superchilling. The formation of the ice crystals inside the cells, regardless of the superchilling rate, is still very important for the quality of superchilled products. It was also observed that there was no significant difference between the sizes of the ice crystals formed in pre-rigor compared to those formed in post-rigor. The results revealed that the location, size and distribution of ice crystals in muscle tissue are the functions of the superchilling rate (slow verses high), the state of the muscle tissue (pre - and post - rigor muscle) and the final temperature of the process.

4.5 Paper V

Title: A study of the ice crystals in vacuum-packed salmon fillets (*Salmon salar*) during the superchilling process and following storage.

The results in this paper present the importance of packaging as well as explaining the effect of thermal gradient and temperature fluctuation on the development of ice crystals during the superchilling process and storage. Paper III also addresses the same information on the development of ice crystals, which results in to different layers with different sizes of ice crystals. However, in paper III only two layers (surface and mid centre layers) were analysed, while in this study (paper V), the ice crystals were analysed in three different layers (surface, mid centre and centre).

Three different layers with different sizes of ice crystals were observed. The work showed how the ice crystals progressed from the surface to the centre immediately after the superchilling process and developed different layers within the superchilled salmon muscles. The previous work (paper III) white/brown coloured spots (i.e. freezer burn) were observed in some of the samples during storage. It was also not possible to analyse the samples on day 28 due to the spoiled odour, which may have been caused by oxidation or microbial growth. The samples were stored in the normal plastic bags (air packed) which might be one of the reasons. In the present study, paper V (vacuum packed), the samples did not exhibit any concerning features related to freezer burn or spoilage for the entire storage time.

4.6 Paper VI

Title: A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (Salmo Salar) fillets during superchilling process and storage.

This paper presents the ice crystallization/recrystallization in the post-rigor red salmon muscle during the superchilling process and storage. There was also a significant difference between the sizes of the ice crystals formed during the superchilling process and superchilled storage (in the red muscles) as those observed in the white muscles (papers III – V). The paper also addresses the differences between the ice crystal sizes formed in the red and white muscles. The size of the ice crystals formed in the white muscle was larger than that formed in the red muscle. It was, however, not known why the size of the ice crystals in the red muscle was smaller than in the white muscle.

4.7 Paper VII

Title: A study of the ice crystal sizes of red muscle of pre-rigor Atlantic salmon (Salmo salar) fillets during superchilled storage

The effect of the superchilling rate was studied during the superchilled storage of the pre-rigor red muscle. The size of the ice crystals partially freezing at a higher rate was significantly smaller than at a slower rate. The size of the ice crystals formed in the white muscle was larger than that formed in the red muscle. This was similar to the results observed in paper VI. It was also observed that the size of the ice crystals formed in the pre-rigor red muscle was significantly smaller than the size in the post-rigor red muscle of salmon.

4.8. Paper VIII

Title: Changes in water holding capacity and drip loss of Atlantic salmon (Salmo salar) muscle during superchilled storage.

Changes in water holding capacity and drip loss are useful tools for describing the quality changes in muscle foods. This article is addressing the quality of the superchilled product, referring to water holding capacity and drip loss in relation to ice crystal development during the superchilled storage of salmon.

Due to the significant differences in ice crystal sizes observed in the previous study (paper V), the WHC was analysed separately at the surface (approximately 2-3mm from the surface) and centre of the superchilled samples. The results showed that the liquid loss decreased with storage time (i.e. WHC increased with storage time), both at the surface and in the centre of the superchilled samples. No significant differences were found in WHC between the surface and centre parts of the superchilled samples. In addition, the drip loss was significantly lower in the superchilled samples compared to chilled and frozen samples.

4.9 Paper IX

The influence of superchilling storage methods on the characteristics of ice crystal (distribution/ location) during storage of Atlantic salmon (*Salmo salar*).

The final work of this thesis was to study differences in the superchilling storage methods, shell freezing (initial surface freezing of salmon following storage at a superchilling temperature) and non- shell freezing (storage of salmon at a superchilling temperature without initial surface freezing), in order to understand the effect of ice crystal development in salmon white muscle during superchilled storage. Ice crystals were mainly formed in extracellular spaces in the non-

shell frozen samples. Fine and well distributed ice crystals were formed in both extracellular and extracellular spaces in the shell frozen samples. The water holding capacity and drip loss were also studied in both storage methods. There was no significant difference between the methods with respect to WHC and drip loss.

Chapter 5

5. Discussion

5.1 General overview

There is growing demand for fresh and quality foods worldwide. In recent years, consumers are increasingly concerned with their health, and are demanding foods that are beneficial to their health and help prevent diseases. Due to this, the market for fresh and quality foods is growing rapidly and superchilling seems to be a good technology in maintaining the quality and freshness of foods. Superchilling has recently been established at an industrial scale. In Norway, one superchilling facility is operated at Trøndelag County by Nortura SA for superchilling pork meat. This shows that superchilling technology can be scaled up to industrial scale.

During the last 10-15 years the interest in fresh and high quality foods has increased. The storage temperature of chilled food is normally in the region of between 4 and 8 ℃ (Einarsson, 1988). At these temperatures, the activity of many spoilage microorganisms is still high and the growth of some pathogenic bacteria will occur. A technology, for preserving the nutritional value of food while simultaneously supressing spoilage microorganism is required. Therefore, superchilling has been recommended as a method for maintaining the freshness, preserving the high quality and extending the shelf life of the food.

The NTNU/SINTEF food engineering group has introduced a new employment of superchilling. They are performing superchilling by shell freezing the food (initial surface freezing), followed by the storage of food at 1 – 1.5 ℃ below its freezing point. The purpose of shell freezing (initial surface freezing) is to facilitate temperature equalization, and hence good mechanism of ice crystal within the superchilled food (Kaale et al., 2013b). The ice formed will be used as a cold reservoir during distribution or short-term storage. The main advantage of the superchilling technology is to extend/prolong the shelf life compared to traditional chilling and maintain high quality of foods (Duun and Rustad, 2008; Einarsson, 1988; Kaale et al., 2011; Stevik and Claussen, 2011).

However, more study is required to make the technology more suitable in preserving foods. To date, there is not a quick and better way for defining the degree of superchilling in food. In addition, no study has been done on the development of ice crystals during the superchilling process and following storage. The degree of superchilling and characteristics of ice crystals

(development of ice crystals during superchilling process and storage) are important parameters to study because of their strong influence on the quality of the final superchilled food. The study by Bahuaud et al. (2008) reported on the development of ice crystals during the superchilling process, but the samples were not stored at superchilling temperatures. The study reported that the ice crystals were large and formed in the extracellular spaces which might affect the nutritional value of the superchilled products. Again however, the study did not establish and study the effect of the superchilling rate, which is very important because it has a large influence on the characteristics (location, extracellular vs. intracellular spaces, size and shape) of ice crystals. In reality, one should not speaks about the quality of food before understand the entire process (i.e. freezing, superchilling, drying). The processes should be studied effectively and understand precisely the relationship between the processes and the quality of foods. For example if one is using a very low superchilling/freezing rate, large and extracellular ice crystals are expected and this may influence on the low quality of the final superchilled food such as low water holding capacity, high drip loss and change of texture in the food products.

5.2. The modelling and simulation of salmon.

Paper II presents the modelling and simulation of salmon in the superchilling process. The main idea was to estimate partial freezing time (time spent in the freezers) required to achieve an optimal degree of superchilling in food. There was good agreement between the numerical and experiment results, particularly that from temperature measurements and the histology study; however, the results from calorimetric measurement showed some deviations. There are many factors which could contribute on the deviation such as uncertainties present in the calorimetric measurement and heterogeneity of food.

The results from temperature measurements and microscope analysis coincided well with the numerical results, which makes the model more accurate than relying only on the calorimetric method. The temperature was measured at 4 different locations within the salmon fillet (Figure 5.1) to confirm the temperature gradients which were also observed in the numerical results.


Figure 5.1: Temperature measurement in salmon at different locations during superchilling process at an impingement freezer (paper II)

The histology steps and microscopic analysis on the samples were performed immediately after the superchilling process. It was observed that the ice crystals were formed in the outer layer (to a depth of 1-3 mm from the surface) of the superchilled salmon. No ice crystals were formed at the centre of the salmon. This confirmed the results from temperature measurement (Figure 5.1), as well as the results from numerical that there were no ice crystals formed at the centre of the superchilled salmon.

5.3. Ice crystallization in white salmon muscles during the superchilling process and storage.

The study of ice crystal development during the superchilling process and following storage was also given more attention in this study (papers III - V). During the superchilling process the ice crystals are formed in the outer layer of the superchilled product. It is important that cells in this layer are not damaged during superchilling processing (initial surface freezing) and therefore the superchilling rate should be considered. Paper III established and studied the effect of the superchilling rate on the characteristics of ice crystals. The formation of fine ice crystals during the superchilling process that are evenly distributed both inside and outside the cells, leads to a better preservation of quality of the product due to less damages to the tissue (Chevalier et al. 2001; Dincer, 1997; Ferna´ndez et al. 2008; Kiani and Sun, 2011; Martino and Zaritzky, 1986; Martino et al. 1998; Petzold, and Aguilera, 2009). This can be achieved at a higher superchilling rate (Figure 5.2 and paper III). The results from this study showed that the samples, partially frozen at a slow superchilling rates, contained larger and extracellular ice crystals during the superchilling process (partial freezing) and following storage of the superchilled salmon, compared to the samples that were partially frozen at fast rates (Figure 5.2 and paper III).

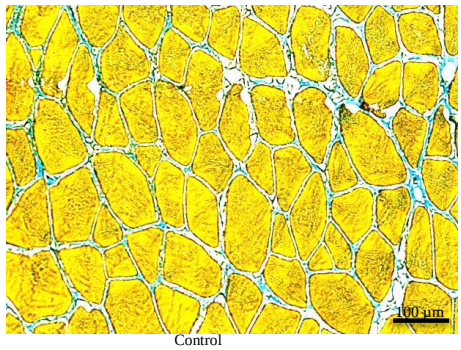

Figure 5.2: Micrographs of salmon at surface layer, post-rigor at fast and slow superchilling rate (paper III).

The large and extracellular ice crystals might destroy the cells in food muscles which reduce the quality of superchilled food like low water holding capacity, high drip loss and change of texture. On the other hand, rapid superchilling of post-rigor muscle also results in the initial formation of extra-cellular ice. However, the extracellular crystals formed during rapid superchilling are much smaller and more finely distributed than those in slow superchilling

(Figure 5.2). The formation of extra-cellular ice dehydrates the cells, but as the temperature decreases rapidly, the cells become supercooled and the remaining intra-cellular water freezes before it has time to diffuse out of the cell.

The state of the muscle food also has an effect on the characteristics of ice crystals. The results in this study showed that partial freezing/shell freezing of muscle food in pre-rigor states results in the formation of ice crystal inside the muscle regardless of the superchilling rate. However, at a slow rate the ice crystals were fewer and larger than at a higher superchilling rate (Figure 5.3 and paper IV). In pre-rigor muscle, the cell fluids are tightly bound to the intracellular proteins, and the diffusion from inside to outside the cell is therefore limited, resulting in the formation of intracellular ice crystals independent of the superchilling/partial freezing rates (Shenouda, 1980).

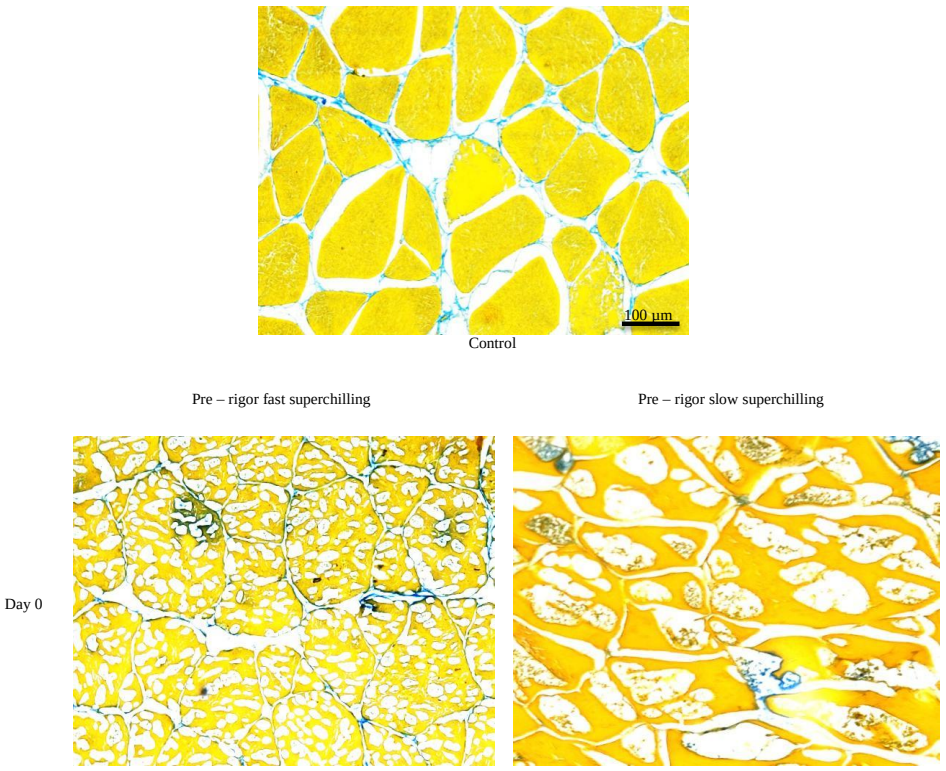

Figure 5.3: Micrographs of salmon, pre-rigor at fast and slow superchilling rate (paper IV).

5.4. Packaging of superchilled salmon.

Packaging is also an important parameter to consider during superchilled storage because it defines the shelf life and maintains the quality of the final superchilled food. Good packaging will prevent dehydration, protect the product from exposure to oxygen and evaporative water loss from the surface of the food, and it can minimize the effects of freezer burn (Pornchai and Chitsiri, 2011). Freeze burn is a surface desiccation defect that can occur when partial frozen tissues are stored without an adequate moisture barrier packaging (Zaritzky, 2006). In this study it was discovered that the type of packaging is very important for the quality of the final superchilled food. The white/brown coloured spots (i.e. freezer burn) were observed in the air-packed samples during storage. This is probably due to excessive hydration loss from the product surface that often limits the quality and shelf life of partially frozen foods (Pornchai and Chitsiri, 2011). It caused by sublimation of ice on the surface region of the tissue where the water pressure of the ice is higher than the vapour pressure in the environment. Freeze burn is prevented if a product is packed in tight-fitting, water- and vapour-proof material, because evaporation cannot take place. We also observed a spoiled odour in air-packed samples after 21 days of storage, which may have been caused by oxidation or microbial growth. The study of Hansen et al. (2009) showed that the salmon fillets packed in air had the highest bacterial growth, unaffected by short-term superchilling prior to packaging. Therefore, product, process and packaging (PPP) are important parameters to consider in superchilling technology.

5.5. Recrystallization of ice in white salmon muscle during superchilled storage.

The results from these studies (Figure 5.4, papers III, IV and V), showed that the size of the ice crystals increased significantly within 24 hours. However, the results showed that after temperature equalization (temperatures at the surfaces equal to temperatures at the centres of the superchilled samples) and with control of the temperature fluctuation during storage, the increase in ice crystal sizes was not significant ($P < 0.05$) at any storage times. This agrees with the study of Bevilacqua and Zaritzky (1982), which reported that when temperature is constant, the recrystallisation occurs at a significant rate only when the specimen contains crystals with diameters less than 24μm. However, temperature fluctuation enhances recrystallisation even if they have larger diameters.

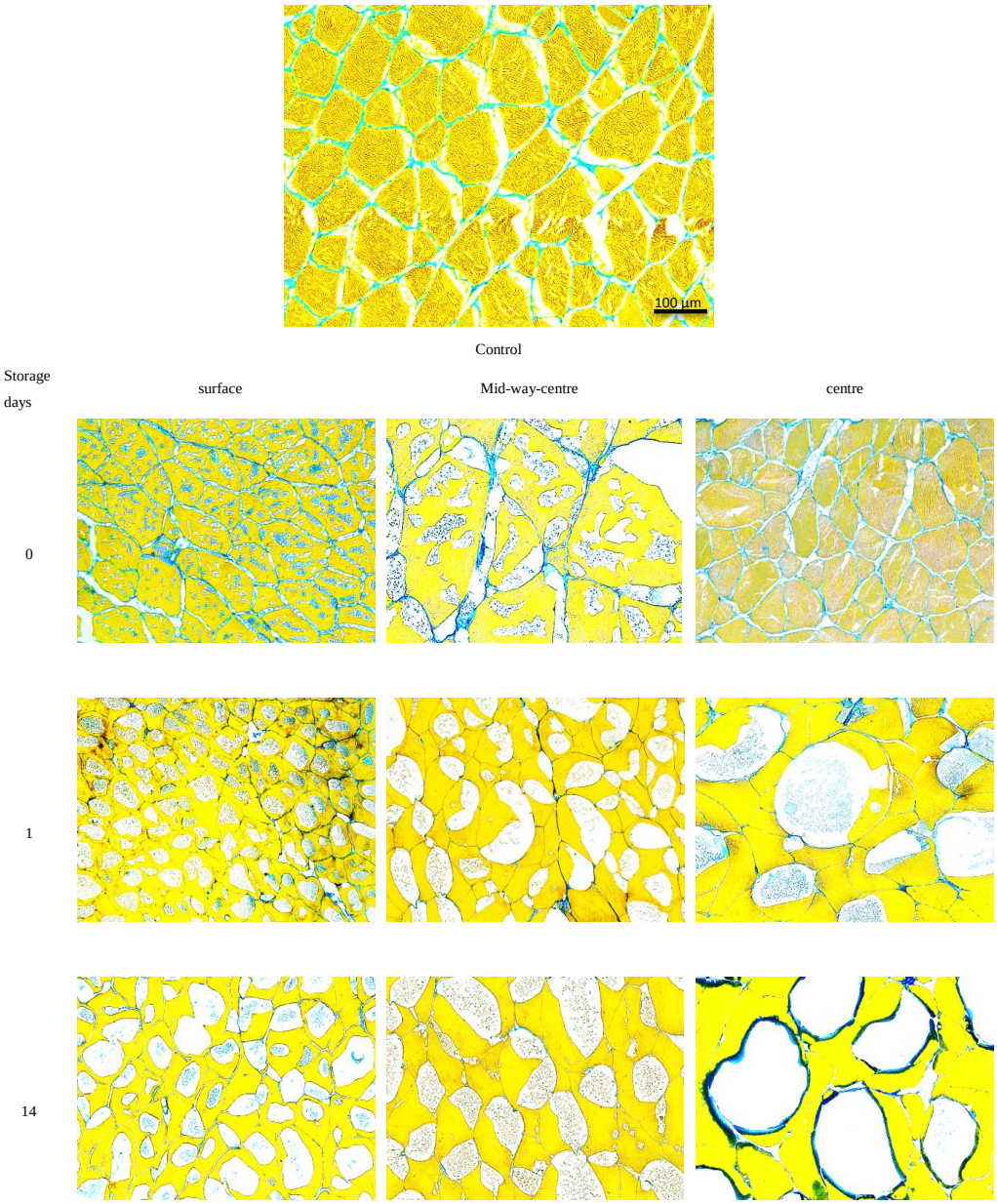

Figure 5.4: Micrographs of salmon, post-rigor shows surface, mid-centre and centre layers within the partial frozen salmon fillet (paper V).

Nevertheless, the physical analysis, water holding capacity and drip loss results (paper VIII) and the results from previous studies Duun (2008); Duun and Rustad (2007); Stevik and Claussen

(2011); Stevik et al. (2010) show that the superchilling method does not seem to change the nutritional value of food compared to fresh product. This might be due to the fact that in fish and meat the destructive effect of ice crystal formation is minimised due to the elasticity of the cellular structure in muscle (Smith, 2011). Furthermore, the loss of quality in fish and meat is largely associated with the loss of the functionality of proteins. When ice is formed, there is an increased concentration of enzymes and build-up of salt concentrations which both cause protein denaturation, and therefore effect the protein functionality (George, 1993; Shenouda, 1980; Smith, 2011). In superchilling the protein denaturation may be minimal because only a small amount of free water is frozen, 5–30%, which results in less enzyme and salt concentration in the remaining water. In addition to that, shell freezing/initial surface freezing of food and the following storage is also important because facilitates temperature equalization and hence suitable mechanism of ice crystals growth. It has also been reported that the structural changes due to ice crystal formation at sub-zero temperature storage appear to be minor compared to those occurring during freezing at -20 ℃ (Einarsson, 1988). Therefore, a combination of high quality raw material, an optimal degree of superchilling, a high superchilling rate, good packaging and the control of temperature during storage will result in high quality superchilled foods.

5.6 Ice crystallization/recrystallization in the red salmon muscles during the superchilling process and storage.

Fish muscle has a unique arrangement of muscle fibres. There are two major types of skeletal fish muscles: red and white (Figure 6.5). The red muscle lies along the side of the body next to the skin, along the lateral lines. The relationship between muscle fibre composition and size, and also the information on fat content in both white and red muscles have long been debated (Ayala et al., 2005; George, 1962; Jiag and Lee, 2007; Nielsen and Nielsen, 2012; Pritchard et al., 1971). Ghaly et al. (2010) also reported that lipid oxidation is a major cause of deterioration and spoilage for species that contain high amounts of red muscles, due to the presence of high content of oil/fat.

However, to date no study has been made of the superchilling process as well as the freezing processes on the ice crystal sizes in the red muscle both pre- and post - rigor. Therefore, this study addresses this lack of information in the superchilling process. The ice crystal size in the white muscle was larger than in the red muscle (Figure 5.5), papers VI and Paper VII). However, based on the literature as well as on this study, no information exists which explains why the size of ice crystals formed in the white muscle is larger than that formed in the red

muscle. More research is required to understand why the ice crystal in the white muscle is larger than those in the red muscle.

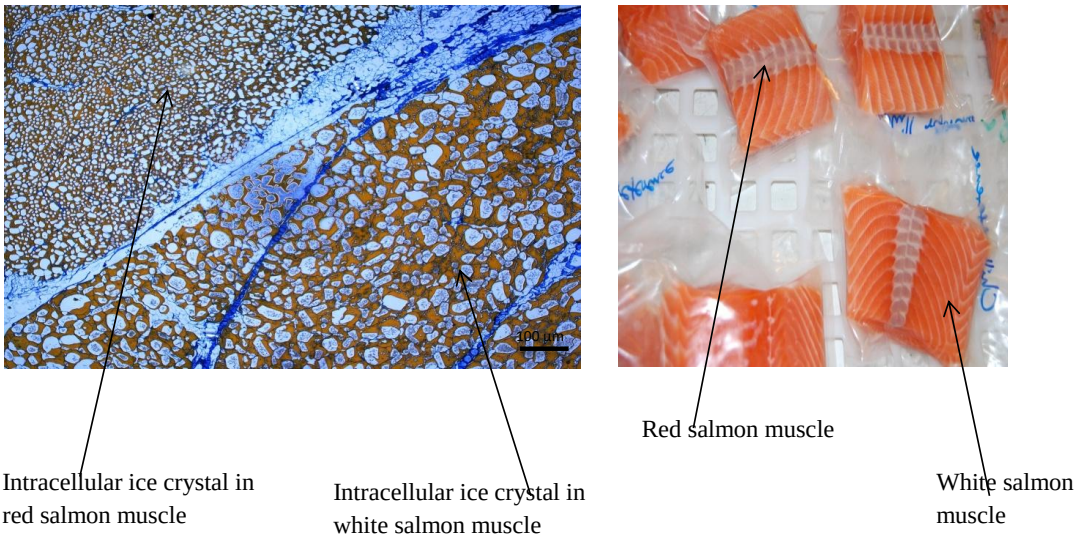

Figure 5.5: Intracellular ice crystals in red and white muscles of pre- rigor salmon (paper VII).

The study also discovered that the size of ice crystals formed in the post-rigor red muscle was significantly larger than in the pre-rigor red muscle of salmon. These findings are of significance for industries because small ice crystals indicate better quality while large crystals often produce damage during partial freezing.

5.7 The influence of superchilling storage methods on the characteristics of ice crystal (distribution/ location) during storage of salmon.

Superchilling has been defined/performed differently. Some researchers have stored food just below 0 ℃ where ice crystals are not formed (Ando et al., 2004). The main idea is that the formation of ice should be avoided within the product in order to retain its original characteristics. Superchilling has also been defined as a technology where food is stored at 1-1.5 ℃ below its initial freezing point. In this study the method has been called non-shell freezing (storage of food at a superchilling temperature without initial surface freezing). The results showed that the ice crystals were formed in the surface and no ice crystals were formed at the centre layer for the entire storage time. Most of the ice crystals were formed in extracellular spaces which might destroy cells at surface layer (Figure 5.6; paper IX).

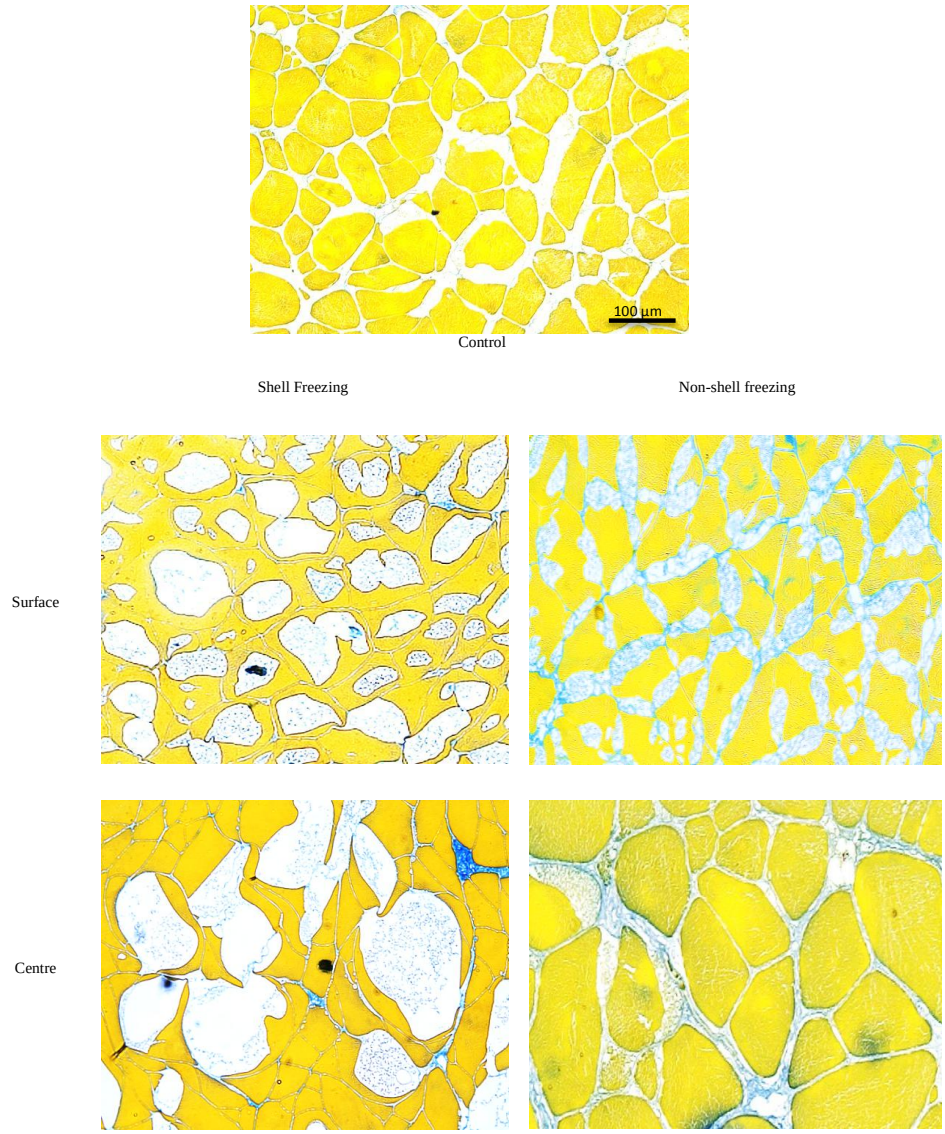

Figure 5.6: Micrographs for shell freezing and non-shell freezing at surface and centre after 1 day of storage (paper IX).

However, it was not possible to detect the destructive effect in this layer because the samples were taken randomly from the surface or the centre of the salmon. In the future, samples should be analysed separately at the surface and centre layers in order to see if the formation of extracellular ice in the surface layer is affecting the cells.

Some researchers Bahuaud et al. (2008); Duun and Rustad (2008); Kaale et al. (2013b); (2013c); (2013c); Stevik et al. (2010) as well as in this thesis have performed superchilling by doing initial surface freezing, and followed by storing food at a superchilling temperature (in this study the method has been called shell freezing). The method allowed good mechanism of ice crystal formation (Figure 5.6) which seems to retain the originality of food properties. However, the method is practicable if the food product is shell freezing fast, with an optimal degree of superchilling, good packaging and strict control of temperature during the superchilled storage. The storage of food at a superchilling temperature range and with control of the temperature during storage will only freeze 5 – 30 % of the free water.

Generally, the initial surface freezing (shell freezing) method is more expensive than the other two superchilling storage methods and needs maximum accuracy with regard to processing parameters in relation to the equipment used. On the other hand, the initial surface freezing (shell freezing) method has many other advantages over the other two methods such as reduced energy and labour costs, reduced transport costs and a reduction of environmental impact. This is because the ice crystals formed at the surface layer will absorb heat form the interior and the temperature will equilibrate within the superchilled product. The small amount of free water (5 – 30 %) which has been converted to ice will be used as an internal cold reservoir so there is no need for external ice around the product during distribution or short term storage.

Chapter 6

6. Conclusions

The main objective of this work was modelling and ice crystallization/recrystallization of foods in superchilling technology (superchilling process and following storage). The relationship between ice crystal development and the quality parameters of superchilled product was also studied. Salmon was chosen as the case study.

The main results and conclusions of this work are:

A one-dimensional model for estimating partial freezing time necessary to achieve an optimal degree of superchilling in food was developed. The model was validated experimentally and there was good agreement between numerical and experiment results. The degree of superchilling is one of the most important parameters because it defines the final quality of the superchilled product. There is no direct/quick way to determine the degree of superchilling, and thus the developed model is of importance in superchilling technology. However, tremendous efforts are needed to further quantify the model using other types and shape of foods.

The superchilling rate has a large effect on the ice crystal characteristics such as location, size and distribution. At a high superchilling rate, smaller and well distributed ice crystals were formed, while at a low superchilling rate larger and extra-cellular ice crystals were formed. The characteristics of ice crystals determine the quality of the superchilled food.

The state of muscle foods (pre - and post - rigor) has an influence on the ice crystal characteristics. In pre-rigor muscle the ice crystals were formed within the cells regardless of the superchilling rate, which can be confirmed by the fact that a large amount of water is inside the muscle cells during superchilling process of pre-rigor muscle, and the water is both inside and outside the cells in the post-rigor muscle. However, at a low superchilling rate the ice crystal size was larger than at a high superchilling rate. The formation of the ice crystals inside the cells, regardless of the superchilling rates, is the most important factor for reducing the damage to food muscles and, hence, maintaining their quality.

There was a significant increase in ice crystal size between the superchilling process (day 0) and superchilled storage (after 1 day of storage). The ice crystals formed in the surface layer were 4 times larger after only 1 day of storage than those formed at day 0. This is due to temperature gradient (-30/20 ℃ to -1.7 ± 0.3 ℃). The recrystallization at this time is unavoidable however,

after temperature equalization (after day 1 of storage) and control of temperature during the superchilled storage there was no significant growth of ice crystals at any storage time.

Different layers with different sizes of ice crystals were observed within the superchilled samples. This is due to the isothermal conditions, and because we have both ice crystals at the surface and water at the centre of the product.

Differences were found between vacuum-packed and air- packed samples. The air packed samples smelled bad (spoilage odour) after 21 days of storage, and it was not even possible to analyse samples at day 28. This may have been caused by oxidation or microbial growth. In addition, freezer burn was detected in some of the air packed samples, which often limits the quality and shelf life of food. In vacuum packed samples, no smell was observed for the entire storage time (after 28 days). In addition, the samples did not exhibit any concerning features related to freezer burn during storage. This indicated that the type of packaging is of high important for the quality and freshness of superchilled food.

The ice crystal size formed in red muscle was smaller than those formed in white muscle. In addition, the ice crystals formed in pre-rigor red muscles were smaller than those formed in post-rigor red muscles. These findings are significant for the superchilling industries because small ice crystals indicate better quality.

Different quality parameters have been studied in superchilling technology by previous researchers, and they were found to have many advantages when compared to those of conventional chilling and freezing technologies. In this study the physical measurement, water holding capacity and drip loss were studied, which are among the most important parameters for determining the quality of foods. Due to the significant differences in ice crystal sizes observed between the surface and centre, the liquid loss (LL) was analysed separately at both the surface and centre of the superchilled samples. No significant differences were found in LL between the surface and centre parts of the superchilled samples. No significant differences were found in the WHC and drip loss between 1 and 14 days of storage in superchilled salmon. It was also observed that the drip loss was lower in superchilled salmon compared to that of conventional chilling and frozen salmon between 1 and 14 days of storage.

The two superchilling storage methods showed differences in the development of ice crystals within the superchilled salmon. In non-shell frozen samples, the ice crystals were mainly formed in the extracellular spaces. Fine and well distributed ice crystals were formed in both the intracellular and extracellular spaces in shell frozen samples. Based on the quality parameters

evaluated in this study, there was no significant difference between shell and non-freezing samples however, it is important to shell-freeze the samples before storage in order to facilitate temperature equalization. This can reduce energy and labour costs, reduce transport costs, easier handling during processing and reduce the environmental impact. In addition, since most of the ice crystals were formed in extracellular spaces the cells in the surface layer might have been destroyed.

Superchilling technology preserves the freshness and maintains the high quality of food, and gives the product nearly same quality as the original product based on the physical measurements found in this study. In order to achieve this, a combination of high-quality raw materials, good superchilling process, stable temperature during storage of the superchilled products and good packaging are most important.

Generally, the results found in this study have given more information in the superchilling area. The developed model which can be scaled-up to the industrial level, together with information on the development of ice crystals, which has a large influence on the quality of the final superchilled food are useful for the industry in estimating the refrigeration requirements for a superchilling system and designing the necessary equipment. In addition, the quality study revealed that the superchilling is practicable if the product is partially freezing fast, with an optimal degree of superchilling (5 - 30 %), good packaging and strict control of the temperature during superchilled storage.

Chapter 7

7. Suggestions for future research

A-One dimensional model for predicting/estimating partial freezing time (time spend at freezers) necessary to achieve an optimal degree of superchilling in food was successfully developed. A study on the development of ice crystals during the superchilling process and following storage was carried out in this thesis, and useful information in the superchilling area was obtained.

However, more research in superchilling technology is required to further quantify the model, using different degrees of superchilling (i.e. 5, 15 and 30 %) and other food products. Foods are heterogeneous and have different thermal-physical properties.

There is also a need to establish a recrystallization model. To develop a mathematical model for interpreting the recrystallization of ice, to determine the kinetics of the recrystallization process in superchilled foods and to discuss the mechanisms involved in this phenomenon are of great importance in the future. Understanding the mechanisms of recrystallization of ice in the superchilled food can help for improving superchilling process.

It has been reported that the formation of ice in extracellular space is due to slow superchilling rate and this may reduce the quality of superchilled food (Ferna´ndez et al., 2008; Kiani and Sun, 2011) such as low water holding capacity, high drip loss and change of texture. The two superchilling storage methods studied in this study show differences in the development of ice crystals within the superchilled salmon. In non-shell frozen samples, the ice crystals were mainly formed in the extracellular spaces while in shell frozen samples the ice crystals were finely distributed in the intra- and extracellular spaces. However, there was no significant difference between shell and non-freezing samples based on the physical measurements done in this study. Nevertheless, since most of the ice crystals were formed in extracellular spaces in non-shell frozen samples, the cells in the surface layer might have been destroyed. The samples in this study were taken randomly from the surface to the centre, and therefore, it was not possible to detect the destructive effect in this layer. In the future it is necessary to analyse the samples differently at both the surface and centre in order to see if the formation of extracellular ice has an effect on the quality of superchilled food. It is also necessary to perform biochemical and microbiological analyses in both methods in order to compare their capabilities of storing foods in relation to shelf life and final quality. If non-shell freezing method will give positive

impact on the quality of the final superchilled food, this might be a good alternative of doing/performing superchilling because is cheaper than shell freezing method. Non-shell freezing does not need initial surface freezing (i.e. no need of freezer), will only need a flexible and effective storage facility. In addition to this, Duun and Rustad (2008) as well as in this study found a development of white spots on the surface of fillets during superchilled storage. The white spots were found in shell frozen samples and not found in non-shell frozen samples. This is a challenge in this technology since the product looks undesirable to the customers.

Superchilling is a promising technology for preserving the freshness and high quality food during distribution and storage. In order to achieve the best in superchilling technology, control of the superchilling process, storage temperature and degree of superchilling should be kept within a narrow margin. In the future it is recommended to implement the following tasks in order to make the technology more suitable in preserving the freshness and high quality, and extend the shelf life of the superchilled product;

(a) Develop a dynamic process control: i.e. the input and output of the process should be documented online. Introduction of on-line measurement techniques to understand and control the degree of superchilling and temperature distribution in foods during superchilling process and superchilled storage is also important in this technique. This will give more information on the quality of the final superchilled food.

(b) In order to fulfil with the demand for short processing times, a technique for quick/fast shell freezing food products which result in better production yields, improved product quality and a longer shelf life is required. Today most of the equipment are not suitable to carry out the superchilling process. The equipment producers, researchers and processing companies have to work together in order to produce appropriate equipment for superchilling processes. The impingement freezer which has been identified as an alternative to conventional freezing methods, because of its high turbulence characteristics, which enhance heat transfer and therefore quality product is very expensive and alternative equipment with the same characteristics but to be commercially interesting is require.

(c) On the other hand, developing a flexible and effective storage facility is important. More research on how to control and keep the required storage temperature after initial surface freezing is needed. The temperature should be stable enough to avoid significant levels of ice crystal growth that can cause structural damage during the storage of the

superchilled product as well as to keep the degree of superchilling within a narrow margin.

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Review

Superchilling of food: A review

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ABSTRACT

Food preservation is very important for the safety and the reliability of the product. Superchilling as used for preserving foods, has been defined as a process by which the temperature of a food product is lowered to 1–2 °C below the initial freezing point. Fresh and high quality food products are in great demand worldwide. Temperature is a major factor determining the shelf life and quality of food products. Fish and meat are perishable food commodities, where better and more advanced preservation technology is needed. Deterioration of these foods mainly occurs as a result of chemical, enzymatic and bacteriological activities leading to loss of quality and subsequent spoilage. Storing food at superchilling temperature has three distinct advantages: maintaining food freshness, retaining high food quality and suppressing growth of harmful microbes. It can reduce the use of freezing/thawing for production and thereby increase yield, reduce energy, labour and transport costs. The study on the growth mechanism of ice crystals, modelling and computer simulation of foods during superchilling and superchilling storage is needed.

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1. Introduction

With increasing world population and the need to supply people with fresh and healthy food, food preservation becomes increasingly necessary in order to increase the shelf life and maintain the nutritional value, texture and flavour of food. A main challenge in this respect is to maintain a stable and sufficiently low temperature which is often more difficult in fresh foods than in frozen foods. Studies on the chill chain have shown that it is a challenge to maintain an acceptable temperature during distribution and storage of food products (Aune, 2003). Superchilling is one method that can be used to maintain food products at a low temperature. Superchilling implies temperatures in the borderline between chilling and freezing. Superchilling is a process by which the temperature of a food product is lowered to 1–2 °C below the initial freezing point of the product (Duun and Rustad, 2007). At superchilling temperatures, microbial activity is reduced and most bacteria are unable to grow. Microbial growth is the most important factor limiting the shelf life and quality of fresh food products. Ghaly et al. (2010) stated that about 30% of the landed fish are lost through microbial activity alone, while chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year. In addition, food systems and quality deterioration mechanisms are complex and consumers are a heterogeneous group. Fresh food, especially meat and fish, are highly perishable products due to their biological composition. The shelf life of refrigerated meat and fish is limited, primarily due to microbial activities (Duun, 2008; Fernández et al., 2010; Lambert et al., 1991). Controlling microbial activities is the key to extension of shelf-life during processing, distribution and storage of food. Temperature is one of the most important parameters affecting the growth of microorganisms (Borch et al., 1996; Bréand et al., 1997, 1999; Constantin, 1985; Doyle, 1989). The rate of food spoilage processes depends on temperature. To reduce spoilage and biochemical degradation, different preservative methods, mainly based on low temperature, have been employed for storage and distribution of food products. The most used methods include refrigerated ice storage between 0 °C and 4 °C, superchilled storage in the range of −1 to −4 °C, by means of slurry ice or in superchilled chambers without ice, and frozen storage at −18 to −40 °C (Gallart-Jornet et al., 2007).

In addition to microbial growth, enzymatic activities are important for the determination of shelf life and quality of food. The initial quality loss in most of food products such as fish is primarily caused by autolytic changes and is unrelated to microbiological activity (Gram and Huss, 1996). Of particular importance in this respect is the degradation of nucleotides (ATP-related compounds) which is caused by autolytic enzymes. Lipid oxidation (both chemical and enzymatic) leading to rancidity can also take place at low storage temperature (Duun, 2008). Due to the presence of highly

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unsaturated fatty acids, fish is more susceptible to lipid oxidation than other muscle foods such as poultry, pork, beef, and lamb (Lee et al., 2006). Lipid oxidation products are known to react with nitrogenous materials in biological systems including amino acids, proteins, phospholipids, and DNA to form brown pigments and fluorescent compounds which may have negative health effects (Kubow, 1992).

The volume and value of fresh, refrigerated foods, and also the flow of these products between countries is increasing. The level of education among consumers has increased in recent years as well as has the knowledge about nutrition and the amount of money available to spend for food products. In addition to purity and safety, product freshness is a major part of product quality and will help to prevent health problems (Matthias, 2005). Frozen foods have a shelf life of months or years while fresh products have a shelf life of days or weeks. The shelf life of superchilled food is far shorter than that of frozen food but longer than that of chilled food (Duun, 2008) and with an increased focus on fresh products, the profitability of the superchilling process from harvest (catch) transport-consumer is of great importance. The aim of this paper is to review the superchilling technology for the preservation of fresh food products.

2. Superchilling

The process of superchilling was described as early as 1920 by Le Danois, even though the terms 'superchilling', 'deep-chilling' or 'partial ice formation' were not used. The superchilling technology combines the favourable effect of low temperatures with the conversion of some water into ice, which makes it less available for deteriorative processes (Aune, 2003). The ice formed on the surface will absorb heat from the interior and will eventually reach equilibrium. Superchilling gives the food product an internal ice reservoir so that there is no need for external ice around the product during transportation or storage for shorter periods, however, for long term superchilled storage, refrigerated storage at superchilled temperatures will be needed. Generally, superchilling is positioned between freezing and refrigeration (conventional chilling), where the surrounding temperature is set below the initial freezing point. The initial freezing points of most foods are between −0.5 °C and −2.8 °C (Duun and Rustad, 2007). Ando et al. (2004) defined superchilling as the temperature zone below 0 °C but where ice crystals are not generated. Beaufort et al. (2009) defined superchilling as a technology where food is stored just below the initial freezing temperature.

2.1. Shelf-life aspects in relation to superchilling technology

There is no generally accepted definition for the term shelf life in the literature. A universal definition of shelf-life is virtually impossible to establish since it is impossible to satisfy all consumers at all times (Bin and Theodore, 1993). Shelf life can be defined as the time period for the product to become unacceptable from sensory, nutritional or safety perspectives. Shelf life is the time during which the product will remain safe, to retain the sensory, chemical, physical and microbiological characteristics, and comply with any label declaration of nutritional data (Kilcast and Subramaniam, 2000). The shelf life of foods is a function of composition, processing, packaging and environmental factors, including humidity, and temperature (Bili and Taoukis, 2007).

Studies on superchilling have shown extended shelf life of food products compared to conventional chilling. According to Einarsson (1988), superchilling results in better quality, and extends shelf life of stored food 1.5–4 times compared to conventional chilling. Carlson (1969) reviewed superchilling of fish and found that as temperature was reduced from −1 °C to −3 °C, the shelf-lives increased from 21 to 35 days. Sivertsvik et al. (2003) reported a sensory shelf life of 21 days for superchilled salmon in air, whereas modified atmosphere and air stored fillets at chilled conditions were spoiled after 10 and 7 days, respectively. The superchilled MA packaged salmon had a negligible microbial growth (<1000 colony-forming units (CFU)/g) for more than 24 days (aerobic plate count, H_2S-producing, and psychotropic bacteria) (Sivertsvik et al., 2003). This is in accordance with the results of Duun and Rustad (2008) who found a doubling of shelf life of superchilled salmon stored at −1.4 and −3.6 °C compared to ice chilled storage with respect to microbial and chemical analyses, and also with the results of Stevik et al. (2010). Fernández et al. (2009) who reported the shelf life of 22 days in superchilling in combination with modified atmosphere packaging (MAP) based on sensory, chemical, and microbiological analyses compared to 11 days control sample. In a study of MAP and superchilled storage to extend the shelf-life of fresh cod (*Gadus morhua*) loins, Wang et al. (2008) found that superchilled-MAP storage had a shelf life of 21 days compared to 14 days for chilled-MAP storage. Olafsdottir et al. (2006) found shelf-life of superchilled cod fillets based on microbial, Chemical Quality Indicators (Torry – score) and total volatile basic nitrogen (TVB-N) to be 15 days at −1.5 °C compared to 12.5–14 days at 0 °C for iced chilled cod fillets, while Duun and Rustad (2007) found that the microbial shelf life (with respect to reduced growth of sulphide producing bacteria) in superchilled vacuum-packed cod fillets stored at −2.2 °C was extended by several weeks compared to chilled cod. When comparing effect of brining and MAP on the shelf life and quality of cod loins, a shelf life of 21 days for the superchilled samples was found while for chilling the shelf life was about 14–15 days (Lauzon et al., 2009), the experiments were evaluated based on sensory, microbial, and chemical analyses. Zeng et al. (2005) showed that the total viable counts (TVC) of bacteria increased most rapidly in shrimp stored in flake ice and in brine mixed with flake ice, followed by those in liquid ice at +1.5 °C, while the lowest counts were observed in shrimp stored in liquid ice at −1.5 °C. For meat a longer extension of shelf life with respect to quality parameters (sensory, physical, biochemical and microbiological) has been found, superchilled pork roasts had a shelf life of at least 16 weeks compared to 2 weeks for the chilled references (Duun et al., 2008).

2.2. Quality aspects in relation to superchilling method

Quality is an arbitrary term and one which causes confusion among consumers, processors and researchers. Product quality is a very complex concept (Gao, 2007) which includes nutritional, microbiological, biochemical and physiochemical attributes. Microbial growth, colour, texture, off-flavour and oxidation are important factors for the safety and quality of food products. Due to the fact that colour, texture, and flavour characteristics are the main quality parameters in food products, conventional technologies such as freezing are often not preferred. Freezing may induce undesirable changes such as protein denaturation, reduced water holding capacity and increased drip loss on thawing. Methods reducing these problems are therefore wanted.

Superchilling processing technology has shown several advantages on the quality of food products, for example had superchilled salmon fillets lower bacterial counts compared to the corresponding chilled fillets (Hansen et al., 2009). Superchilling was found to be a promising method for storing raw material before salting, slowing down biochemical quality degradation while at the same time the degree of protein denaturation was low and the degree of structural damage was less than in frozen storage (Gallart-Jornet et al., 2007). On the evolution of *Listeria monocytogenes* and organoleptic characteristics of cold-smoked salmon samples, Beaufort

et al. (2009) reported that storage at −2 °C (superchilling process) for 14 days did not have any serious consequences on the quality of cold-smoked salmon compared to controls (absence of superchilling). However, the prevalence of *L. monocytogenes* and organoleptic properties were higher after 28 days at superchilling, followed by 28 days in chilling, compared to samples stored at superchilling/chilling for 14 days before chilling for 28 days. During superchilled storage of kuruma prawn, the brightness of the tail colour could be retained compared to traditional refrigeration where unfavorable changes in quality such as discoloration, deterioration of texture and a rapid rise in the amount of inosine and hypoxanthine in relation to the total amount of ATP and substances derived from ATP (*K*-value) were found (Ando et al., 2004). On evaluating the impact of superchilling on the quality of pre-rigour Atlantic salmon (*Salmo salar*) fillets, Bahuaud et al. (2008) found that superchilling prevented the fillets from rigour contraction. The study of Sivertsvik et al. (2003) found no negative texture changes in the superchilled salmon and insignificant increase in drip loss, this is in accordance with the result of Duy et al. (2007) who found no negative effects on the quality in superchilled Arctic Charr fillets. These differ from the results of Duun (2008) and Duun and Rustad (2007) who found drip loss to be lower in superchilled samples than in chilled samples both in cod and salmon fillets as well as in pork roasts. Temperature fluctuations during superchilled processing and storage should be avoided (Duun, 2008; Duun and Rustad, 2007). 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 lot of interest in the use of superchilling technology in food processing. In the specific case of chilling of food products, superchilling may become an alternative method over conventional chilling. Duun and others (2008) reported extended shelf life and improved quality for superchilled food products. This may be a direct result of the reduction of tissue or external damage and an indirect result of the reduction of lipid deterioration and protein oxidation in the final food product.

However, some negative effects on quality have also been found in superchilled foods. Bahuaud et al. (2008) reported on freeze damage during superchilling, the upper layer of the super-chilled fillets showed freeze damage characterized by the formation of large intra- and extracellular ice crystals during superchilling. Freeze damage due to superchilling accelerated the amount of detachments between myofibres and increased the amount of myofibre breakages during storage time. Super-chilling accelerated the release of the proteolytic enzymes cathepsin B and L from the lysosomes, causing an acceleration of fish muscle degradation (Bahuaud et al., 2008). However, Duun and Rustad (2008) concluded that super-chilling did not influence the total cathepsin B and L activity in salmon muscle stored at −1.4 °C and −3.6 °C. Duun (2008) found that myobrillar proteins denatured more easily during superchilled than during chilled storage both in salmon and cod fillets and the amount of free amino acids increased more rapidly due to exoproteolytic activity. Duun and Rustad (2007) also found a higher liquid loss (LL) in superchilled samples compared to ice chilled cod fillets. The high LL was correlated to a reduction in amount of salt soluble proteins which was significantly lower in the superchilled samples than in the ice chilled samples.

Texture is an important quality parameter, and may vary depending on species, part of muscle, storage, and processing (Hansen et al., 2009). Superchilling in combination with MAP had a negative effect on the texture of salmon fillets. This is in accordance with the results of (Gallart-Jornet et al., 2007; Wang et al., 2008), but in disagreement with the results of Bahuaud et al. (2008) who found that MAP did not influence the effects of super-chilling. Measurements of texture, liquid loss and protein denaturation during superchilling in the reviewed studies indicate that the superchilling process still needs to be optimized before further commercial implementation.

3. Superchilling technologies

The superchilling process can be carried out in special cold-producing machines called freezers; mechanical freezers, cryogenic freezers, or impingement freezers. The three technologies, and all freezers, have different advantages, drawbacks and limitations. In this paper, we will compare mechanical, cryogenic and impingement freezing technologies rather than specific systems. The selection of suitable freezing equipment helps to maximize product quality, operating flexibility and return on investment (ROI) while minimizing waste, costs and downtime.

3.1. Mechanical freezers

Use a circulating refrigerant to achieve temperature reduction by heat exchange against air to the food product. Mechanical freezers are commonly used to freeze foods. Mechanical freezers, especially in continuous belt freezers, have lower operating costs than cryogenic freezers. However, mechanical freezers require higher processing times due to low heat transfer coefficients ($h \ll 50\ W/m^2\ K$) which, in turn, lead to a lower quality product (Salvadori and Mascheroni, 2002).

3.2. Cryogenic freezers

Generally, the term cryogenics is applied to temperature below −150 °C, but in food processing, the term cryogenic freezing is widely used to identify freezers using either nitrogen liquid (−196 °C) or carbon dioxide (−78 °C as a solid) which are applied directly to the food product to achieve temperature reduction. Cryogenic freezing offers shorter freezing times compared to conventional air freezing because of the large temperature differences between the cryogen and the product surface and the high rate of surface heat transfer resulting from the boiling of the cryogen (Zhou et al., 2010). Cryogenic freezing requires no mechanical refrigeration equipment; simply a cryogen tank and suitable spray equipment. However, there may be some distortion of the shape of the product caused by the cryogenic process that might impact on the commercial application (Zhou et al., 2010). Furthermore cryogenic freezing has a high refrigerant consumption (>1 kg of N_2 per kg of processed product) and has very high operating costs (Salvadori and Mascheroni, 2002; Soto and Bórquez, 2001). This makes cryogenic freezing a valid alternative only for expensive products such as seafood or fine fruits.

3.3. Impingement freezers

Is equipment which has a freezing chamber divided into zones where the temperature of each zone is independently controllable so that the temperature profile within the impingement freezer is coldest at a zone adjacent the outlet and warmest at a zone adjacent to the inlet for maximum thermodynamic usage of the refrigerant (Lee and Sahm, 1998). Additionally, the velocity of each of the impingement jets is independently adjustable from zone to zone so that in the zone adjacent to the entrance of the freezing chamber, the impingement jets can be adjusted to have maximum velocity air to produce maximum heat transfer coefficients and thereby an acceptable rate of cooling within the impingement freezer (Lee and Sahm, 1998). Products are placed on conveyor belts, and the high velocity air passes through the conveyor upwards and downwards. Impingement freezer increases heat transfer rates than that seen with traditional mechanical freezers because it

breaks up the boundary layer at the surface and hence reduces processing time (Anderson and Singh, 2006; Erdogdu et al., 2005, 2007; Salvadori and Mascheroni, 2002; Sarkar et al., 2004). Impingement jet systems have been identified as an alternative to conventional freezing methods, given their high turbulence characteristics, which enhance heat transfer and therefore quality product (Dirita et al., 2007; Garimella and Schroeder, 2002; Soto and Bórquez, 2001).

The study of Salvadori and Mascheroni (2002) concluded that the processing times in an impingement freezer are markedly lower than the times required in conventional belt tunnel freezers, thus the use of this equipment increases the production capacity without increasing the size of the facilities. In addition the freezing times and weight losses for impingement freezing are similar to those of cryogenic freezing at a noticeably lower operating cost. The impingement freezer has a good impact on the product, as it reduces processing time by enhancing heat transfer, and therefore good quality of the product. Impingement technology advantages could give the food technologists the best way of preserving food products, for extending shelf life and improve product quality.

4. Ice crystal formation

Ice content, the percentage of the water in a product that is in solid form, is one of the most important parameters of food when freezing is involved. It is also one of the parameters that are elusive and difficult to measure (Aparicio et al., 2008). However, this depends on controlling the temperature (temperature stability) pre and during storage treatment. The process temperature should be stable enough to avoid significant levels of ice crystal growth that can cause structural damage. Temperature measurement during transient chilling and the freezing process is extremely challenging due to temperature variations both in time and space (Magnussen et al., 2008). In practice, controlling and measuring temperature must therefore be performed after chilling and temperature equalization are complete. Accurate temperature measurements can be carried out under laboratory conditions, but under industrial conditions one cannot expect to improve on an accuracy of ±0.5 °C. However, even with this accuracy, calculating the amount of ice in the product is highly uncertain due to the strong dependency of ice content on temperature in the region of interest (Magnussen et al., 2008). Fig. 1: ice content and specific enthalpy in salmon filets with varying temperature. The red columns represent variations in temperature due to error of measurement in the industry. Dotted arrows show how these errors affect the ice content and specific enthalpy (Magnussen et al., 2008).

Bahuaud et al. (2008) reported that the ice crystals formed during superchilling were large enough to damage the integrity of the fish muscle during superchilling. The large intra- and extracellular ice crystals formed during superchilling/freezing has a large effect on morphological changes, cell destruction as well as the denaturation of cell components (Bahuaud et al., 2008; Gab-Soo et al., 2004). These may result in textural changes and increased drip loss during thawing (Bahuaud et al., 2008). Fluctuating temperatures, even if the freezing temperature is quite low, accelerates the growth in size of the ice crystals formed. With a slight rise in storage temperature, the small ice crystals will presumably melt faster than the larger ones, and when the temperature drops down again, forming larger and larger crystals (Shenouda, 1980).

Studies of the characteristics of the ice crystals formed during superchilling and superchilled storage should contribute to a better scientific basis for evaluation of methods for chilling and comparison between technologies (Magnussen et al., 2008). There is, however, few published studies explaining the growth mechanism of ice crystals during superchilling and superchilled storage. For example the growth mechanism of ice crystals during superchilling storage may be related to shelf life of foods and some of the quality parameters (water holding capacity, lipid oxidation, protein denaturation and other quality parameters). Therefore, the information about the growth of ice crystals should be investigated because it could offer another way of improving the quality of superchilled foods and chilling-related technology.

5. Modelling and simulation

Studies show that, the main problem in superchilling technology is to define the degree of superchilling and control the temperature in the process that will improve the shelf life sufficiently and fulfill the demands regarding processability and quality attributes. Modelling and computer simulation of the superchilling process is a tool which could implement this task. Computer simulation and laboratory experiments of superchilled salmon fillets were reported by Aune (2003). Simulation was carried out to find the amount of the frozen water at different mean temperature in the fillets and different chilling times. The computer simulation gave satisfactory results compared to experimental results. As far as we know, there is no published papers concerning computer simulation and modelling in superchilling since 2003.

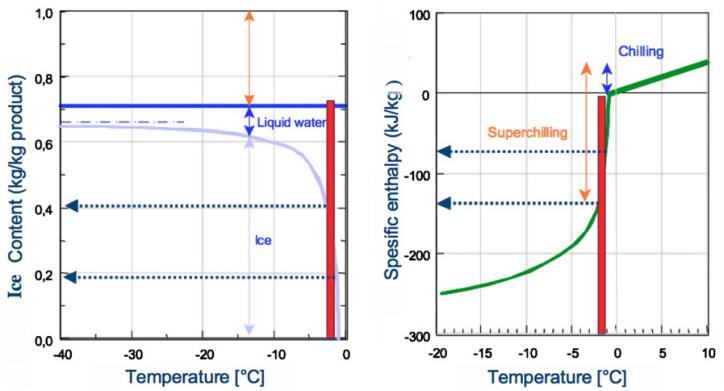

Fig. 1. Ice content and specific enthalpy in salmon filets (Magnussen et al., 2008).

However, different models have been applied in food industries and presented in the literature for simulation in one, two and three dimensions. Moureh and Derens (2000) developed a three-dimensional heat transfer model to predict the food temperature as a function of time and location within the pallet. They concluded that to ensure a better control of the cold chain, the model can help evaluate the benefits of investments such as cold-storage facilities, choice of packaging or the control of ambient temperature. Mannapperuma and Singh (1989) developed a numerical method based on enthalpy formulation of heat conduction with gradual phase change which was then used to develop a mathematical model to simulate freezing and thawing processes in foods, the results agreed reasonably well with published experimental results and with predictions by other published methods. Dolan et al. (1987) developed a one-dimensional heat transfer model in order to calculate temperature profiles and histories within a pallet of frozen food exposed to different environmental conditions. Various simulations were carried out by varying thermal properties, external heat transfer coefficient, and ambient temperature and radiation surface properties of the carton box. Among these parameters, the thermal properties exert the greatest effect on temperature distribution within the pallet.

Mallikarjunan and Mittal (1995) used a validated heat and mass transfer model and a pattern search algorithm to evaluate the effect of the optimum freezing conditions on beef quality after ageing. A computer programme was written using FORTAN 77 and they found that optimum chilling conditions provided a better quality compared to other chilling systems. Lijun and Da-Wen (2002) used a finite element analysis to model the three-dimensional transient heat transfer of roasted meat during air blast cooling process. A user-friendly computer programme developed in visual C++ by the authors was used to solve the model. The temperature predictions were in agreement with experimental values.

The typical shape factor of the food product usually makes a one-dimensional model sufficient to study the thermal behaviour of the product. Such a model has the advantage that it is simple, very fast, and yet detailed enough to estimate the real behaviour of the food product. The physical properties of food have a strongly nonlinear behaviour in the temperature region of freezing. This is especially true for the specific heat capacity, since it represents the latent heat in addition to the sensible heat.

5.1. Computer programming

A finite difference method adapted to handle the nonlinear physical properties of food is adopted to model the freezing/partial freezing processes. Solving these kind of equations need a fine resolution in space and time, and can be implemented using different computer languages such as FORTAN 77, C++, MATLAB, etc. MATLAB is well suited because this tool provides a rich set of built-in facilities for equation solving and visualization. The main task of the programme is to keep track of the average ice fraction during all stages of the product handling. Good knowledge and accurate prediction of the ice fraction-temperature dependence has significant importance for reliable determination of the thermophysical characteristics and enthalpy variation during freezing of foodstuffs as well as for proper selection of the temperature regimes during refrigerated processing and storage (Fikiin, 1998).

Based on this information, optimal time in the freezing facilities such as impingement can be found by repeated simulations. The practical methodology will include simulation and verification of the developed models using experimental data. A major advantage of such a simulation model is a better control of food quality because the process of evaluation can be followed up frequently at a cheap cost. These models, when validated, can be used to design and operate controls of temperature in superchilling.

6. Challenges in superchilling

The main challenges are: selecting optimal process conditions, such as temperature, velocity and control holding time in the superchilling unit. More knowledge is needed on the right degree of superchilling which will increase shelf life and maintain quality of food products. To control temperature during superchilling and superchilled storage is a challenging task. It is also difficult to define the degree of superchilling required to sufficiently improve shelf life and fulfill the demands of the process to achieve the desired quality attributes (Magnussen et al., 2008). The changes in the microstructure of foods during superchilling and superchilled storage, introduction of on-line measurement techniques to understand and control the ice fraction are also main challenges with this technique. Growth of microorganisms, protein denaturation and lipid oxidation at temperature below 0 °C also requires more attention. Duun and Rustad (2007) found development of white spots on the surface of fillets during the superchilled storage. This is a challenge in superchilling process since the product looks undesirable to the customers. Lastly, the superchilling technology is more expensive than conventional chilling and needs maximum accuracy with regard to processing parameters in relation to equipment used. On the other hand superchilling offers advantages with regard to shelf life compared to chilling technology.

7. Conclusions

Storing food at superchilling temperature has three distinct advantages: maintaining food freshness, retaining high food quality and suppressing growth of harmful microbes. Superchilling, as a commercial practice, can reduce the use of freezing/thawing for production and thereby increase yield, reduce energy and labour costs. Superchilling may also lead to reduced transport costs, easier handling during processing and reduction of environmental impact.

Study on the mechanism of the ice-crystals growth during superchilling storage is highly required. Modelling and computer simulation of the superchilling process is an area that needs more attention. Calculating the required superchilling times which will define the degree of superchilling required to sufficiently improve shelf life while maintain the desired quality attributes is highly important as well as maintaining a stable temperature during superchilled storage.

There is a need for improved methods to control the superchilling process. Temperature is not a sufficient parameter and ice-fraction has been introduced as a parameter to describe the degree of superchilling. However, if the positive effects of superchilling shown in the reviewed articles could be implemented at the industrial level, superchilled storage might be used to provide additional value to commercial foods.

Future aspects/trends

In the field of superchilling, progress is expected in the area of:

(i) Modeling: Computer simulation of the superchilling process, including interaction between superchilling method and raw material properties. This is important since computer simulations are cheaper and easier to use to study effect of process parameters and product properties.
(ii) The study on growth of ice crystals during superchilling storage which has effect on changes in proteins, lipids, and changes in microstructure which resulting in changes in appearance and texture of food products is an important parameter for future studies.

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Modeling and simulation of food products in superchilling technology

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Modeling and simulation of food products in superchilling technology

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Abstract

Food superchilling process is of increasing importance because of its benefit in achieving food quality and extending shelf life of food products. The rate of the superchilling process is critical to the products' quality and to the productivity of the process and therefore the superchilling dynamics is of extreme importance. The objective of this work was to develop a one-dimensional implicit finite difference numerical model for predicting partial freezing time necessary to achieve an optimal degree of superchilling in foods and to validate the model experimentally. The evaluation of degree of superchilling was determined using finite slab and measured by using a calorimetry method. There is a good level of agreement between numerical simulation and laboratory experimental results.

1. Introduction

Superchilling is the process of partial ice - crystallization from supercooled water in food products. The superchilling/partial-freezing process has two stages: 1) cooling the product to initial freezing point and 2) removing the latent heat of crystallization (phase transition stage), whereby 5-30 % of the water is frozen (degree of superchilling) and stored within the product. Degree of superchilling (ice fraction) is amount of water (5 – 30 %) which is frozen inside the food product, is one of the most important parameters which define the quality of the superchilled food product (Magnussen et al., 2008; Stevik et al., 2010). Superchilled storage is the storage of food product at 1-1.5℃ below its initial freezing point. During this time, the ice formed will absorb heat from interior and will eventually reach equilibrium. Superchilling provides the food product an internal ice reservoir so that no external ice is required during transportation or short term storage (Kaale et al., 2011).

Superchilling technology has recognized advantages; it results on maintaining food freshness, retaining high food quality and suppressing growth of harmful microbes. Despite the benefits of this technology, one of its main drawbacks is the need to define the degree of superchilling that will sufficiently improve the shelf life and fulfil the demands regarding processability and quality attributes (Kaale et al., 2011). The degree of superchilling can be predicted using either analytical or numerical

methods (finite difference, FDM, finite elements, FEM, or finite volume method, FVM) of the heat transfer equations. Analytical methods offer an exact solution and are mathematically elegant, however, due to their limitations, analytical solutions are mainly for one-dimensional cases with simple initial and boundary conditions and constant thermal properties (Abbas et al., 2004; Bonacina et al., 1973b: Pham, 2008; Tavakoli and Davami, 2007). The advantage of numerical methods over analytical is that effects of the phase change over a range of temperature, changing thermal properties, the step change in thermal conductivity over the same range and heterogeneity of food products can be analyzed (Delgado and Sun, 2001; Pham, 2006; Resende et al., 2007; Zuritz and Singh, 1989; Tavakoli and Davami, 2007; Wang et al., 2007).

Studies on phase change problems have been carried out using finite difference method (Clavier et al., 1994; Pham, 2006; 2008; Tavakoli and Davami, 2007; Idelson et al., 1994; Voller, 1984; 1987; Kim and Kaviany, 1990; Muhieddine et al., 2009; Wang et al., 2007; Wilson and Singh, 1987; Mannapperuma and Singh, 1989; Abbas et al., 2004). These studies have applied the finite difference method by using enthalpy or temperature formulation of heat conduction and have concluded that the method has been succeed to study the phase change problems. Moreover, the finite difference is a simpler method, which leads to satisfactory results for all simple and regular shapes. Finite differences are more difficult to implement for irregular shapes, whereas the complexity of a finite element scheme is not affected by product shape (Cleland, 1990). However, by applying numerical grid generation approach, the finite difference method can be used for irregular geometry as effectively as the more complicated finite element method without sacrificing its simplicity (Delgado and Sun, 2001).

In this study, a one dimensional implicit finite difference numerical model was chosen to predict partial freezing time (time spent at freezers) necessary to achieve an optimal degree of superchilling in food products during superchilling process. Salmon fillet was chosen as a case study. Data of salmon fish compositions as well as the suitable composition based correlations were taken from ASHRAE handbook (2006). Thus, the one-dimensional case allows use of the finite difference method to obtain the numerical solution by implementing the heat transfer partial differential equations in the implicit finite difference form and to solve it via a MATLAB computer program. The model was validated experimentally.

2. Methods and materials

2.1. Numerical methods

The superchilling process is a highly transient process that develops steep thermal gradients in the product near the surface. It is therefore necessary to use a high resolution in the grid and, hence, small time steps. It is well- known that a one-dimensional finite difference model has been used for many

years to study the freezing process (Cleland, 1990; Bonacina et al., 1973a; 1973b; Hughes and Charng, 1983; Pham, 2008; Resende et al., 2007). In this study, the one-dimensional finite difference model for general heat transfer calculations has been used to study the superchilling process. The thermal physical property correlations of food products have been taken from ASHRAE handbook (2006). The temperature method with apparent heat capacity combined with an implicit integration scheme proved to give desirable results on specific test cases. Desirable accuracy was achieved by manually varying the time step and grid resolution. The results of the implicit and explicit simulations were compared for sufficiently fine resolution in space and time and gave no significant differences. The results from the simulation can be visualized in many ways, each showing the consequences of rapid thermal treatment of the product. This paper focused on visualizing the temperature and the ice fraction of the product.

2.2. Laboratory experiments for validation of the model

2.2.1. Materials and superchilling process

Salmon fillets (0.9–1.2 kg) of thickness ranging from 0.025 to 0.027 m were provided by Lerøy Midnor (Hitra, Norway). Approximately 210 g of sample (salmon fillet) was weighed and stored at 4 ℃ for 24 h before the superchilling process to ensure a constant temperature in all samples. The initial temperature of the salmon fillets was approximately +4.1℃ before starting superchilling process. The superchilling process was performed in an Impingement Advantec Lab Freezer (JBT Food - tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. A 2^4-two-level full-factorial design was used with four variables: superchilling medium temperature, surface heat transfer coefficient, superchilling process time, and product thickness. Immediately after superchilling, the ice fraction was measured by a calorimetry method.

2.2.2. Measurement of the temperature during the superchilling process

The temperature was measured at four different locations on the samples during the superchilling process: the surface, near surface, midway to the centre and the centre. Three thermocouples were used at each location (3 - surface, 3-near surface, 3 - midway centre and 3 - centre). The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the sample were recorded every 4 seconds. The thickness of sample was approximately 0.027 m. The temperature was measured by inserting thermocouples approximately 0.001 m from the surface, 0.002 m from the surface (near surface), 0.007 m from the surface (midway to the centre) and 0.014 m from the surface (centre).

2.2.3. Measurements of ice fraction by calorimetry

The ice fraction in the superchilled salmon fillets was measured using a calorimetric method. Steel thermoses (Finemech Inc., Cylindrical Dewar Container, Portola Valley, USA) were used. The steel thermoses were filled with 2 l of water (~24 ℃). Two l was minimum amount of water that could ensure that all of the six thermocouples for each thermos were inside the water. The samples were weighed directly after superchilling. The thermoses were tightly sealed, and the systems were left for temperature equalization under continuous logging (Agilent Technologies Inc., Agilent 34970A, Santa Clara, USA) for approximately 24 h. Analysis was performed on three parallels for each experiment. Based on temperature data from the equalization process, the ice fraction after superchilling was calculated by method of enthalpy balances explained elsewhere (Stevik et al., 2010). The conductive heat loss from the thermoses and the specific heat capacity of the steel thermoses linings were considered.

2.2.4. Surface heat transfer coefficient measurement

For modeling of food superchilling, apart from thermal properties of the food it is necessary to measure the surface heat transfer coefficient (HTC). This is very important in heat transfer calculations. In this study, the experiments for measuring surface heat transfer coefficient (HTC) were performed in an Impingement Advantec Lab Freezer. Aluminum plate (Al- plate) (20 x 10 x 2.5 cm) was used as metal transducer. A hole under the surface of the plate was made in order to insert five thermocouples (T-1, T-2, T-3, T-4 and T-5). The five thermocouples were connected to a temperature recorder while the plate was cooled in the impingement freezer. The initial temperature of the whole plate was controlled before placing it in the impingement freezer at -30℃. The temperature of the cool air and the plate were recorded every 2 seconds. The heat transfer coefficient was measured at 1.5, 2, and 2.5 kPa pressure difference of the fan, and experiments were conducted in three parallel run. Calculations of the heat transfer coefficient were made after the attainment of the cooling curves of the aluminium transducer (Resende et al., 2003).

$$h = \frac{\rho_{al} V_{al} C_{pal}}{A_{al}} G \qquad (1)$$

The coefficients (G) of the linear regression of $ln\ \frac{T_{al}-T_{\infty}}{T_{ial}-T_{\infty}}$ versus time were obtained and used in the Eq. (1) to find surface heat transfer coefficient.

where T_{ial} = initial temperature of aluminum plate, ℃

T_{al} = surface temperature of aluminum plate at any time t, ℃

T_{∞} = cooling air temperature, ℃

h = surface heat transfer coefficient, $W/m^2.K$

ρ_{al} = density of aluminum plate, kg/m^3

t =	time,	second
Cp_{al} =	specific heat capacity of aluminum,	J/kg.K
A_{al} =	surface area of aluminum plate,	m^2
V_{al} =	volume of aluminum plate,	m^3

T_{al} is obtained by the average values from the readings of the five thermocouples located inside the aluminum transducer. The average values (from three parallel runs) of the surface heat transfer coefficient were obtained for each pressure difference of the fan.

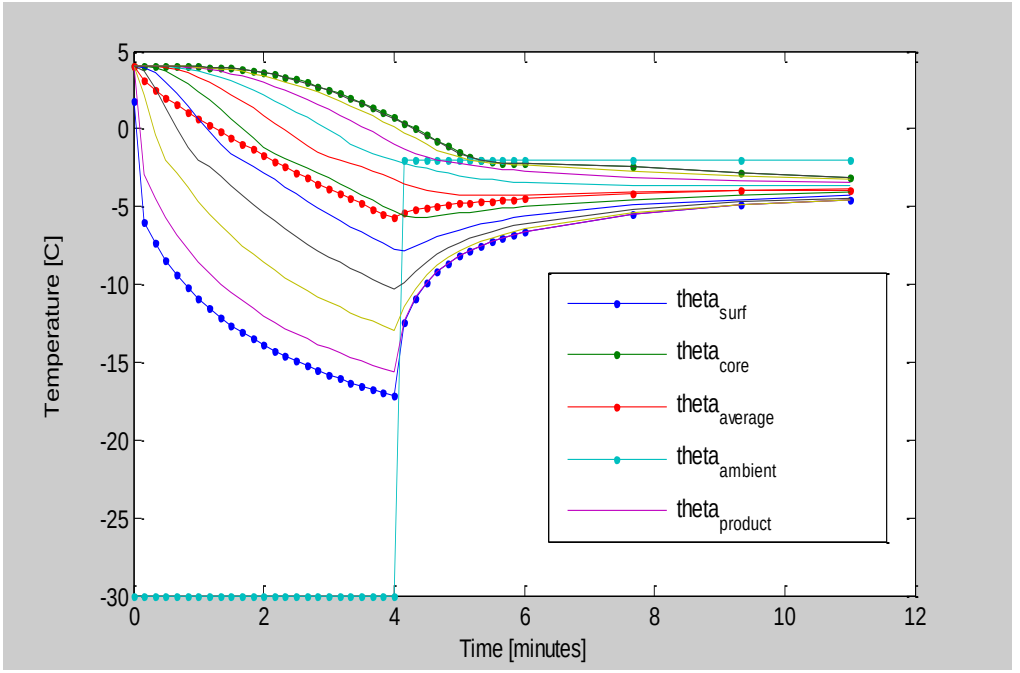
2.2.5. Design of experiment and Statistical data analysis

Minitab 16 software was used in design of experiment and statistical data analysis. A general linear model, (post-hoc test) under Tukey's simultaneous test was applied whenever the analysis of variance (ANOVA) results were significant. The reason for performing the post-hoc test is to compare pairs of numerical results with laboratory experiment results simultaneously to understand why the significant results were obtained for the overall ANOVA. The statistical significance of each run (comparison runs: laboratory experimental and numerical results) was $p < 0.01$.

3. Results and discussions

3.1. Numerical simulation of the salmon fish: Superchilling process

Figure 1 shows the time–temperature profile for the numerical simulation during superchilling process of food products (-30℃, 227 W/m^2.K). The surface heat transfer coefficient value, 227 W/m^2.K is within the range (250 – 300 W/m^2.K) which has been reported by Goransson and Londahl (2005). The initial temperature of the food products was approximately +4.1℃, from 4 minutes. Figure 1 shows the superchilling storage time of the food products, as defined in the previous section, the superchilling storage temperatures is between -1 and -1.5℃. The red line in Figure 1 shows the average temperature during all stages of the product handling, the remaining lines show the temperature at the different points within the food product.



Figur 1: Time - temperature profile of food product by impingement freezing (-30°C, 227 W/m^2.K), numerical results

Figure 2 shows time - temperature profile (-30°C, 227 W/m^2.K) for the laboratory experiment results. These results (numerical and laboratory results) show that the surface of the food experiences a rapid change in temperature compared to the core part of the product. This behaviour is due to both the formation of ice crystals just a few millimeters from the surface during the superchilling process and the lack of ice crystals formed at the centre of the food products. In addition, the temperature dropped slowly because the water-to-ice transition substantially changes the thermo-physical properties of food materials (Alizadeh et al., 2007; Chin and Spotar, 2006).

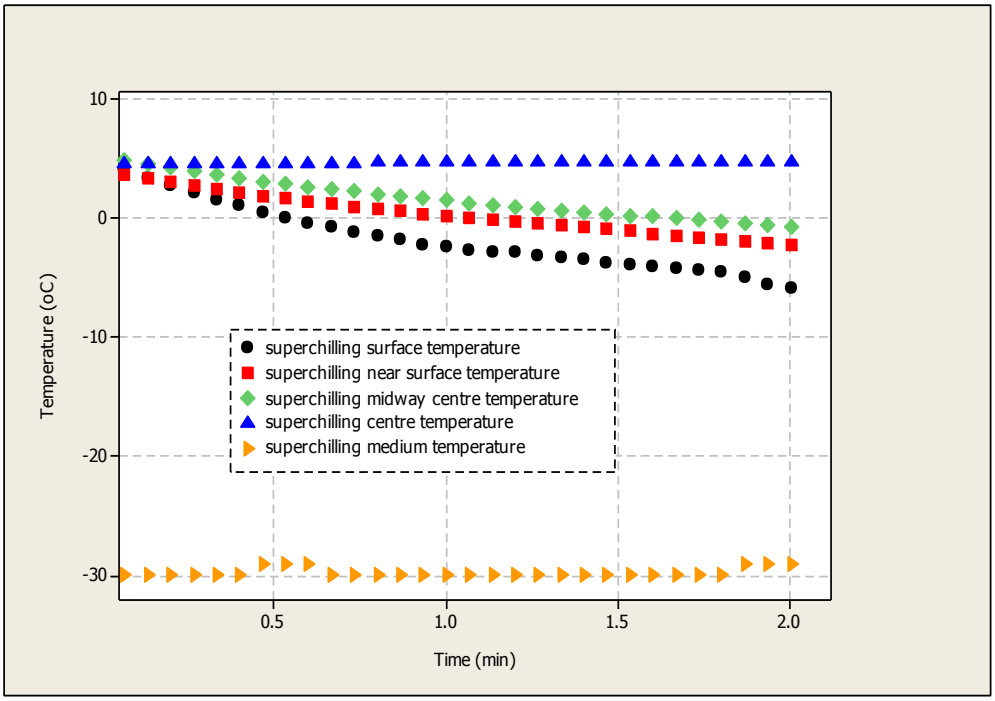
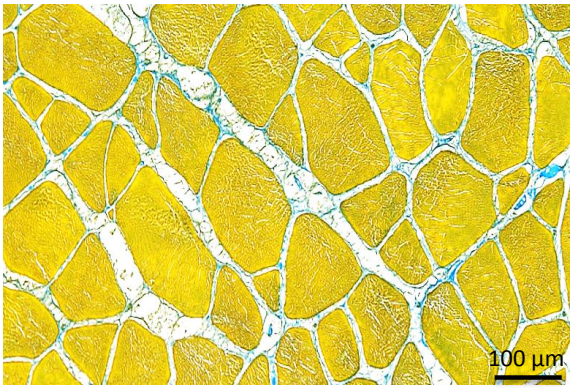


Figure 2: Temperature-time profile at different locations during superchilling process (-30 ^{o}C, 227 W/m^2.K), laboratory experiment

The results were further proved by performing histology steps plus microscopic analysis on the samples immediately after superchilling process. Figure 3, shows the microstructure size of the salmon fillets at -30℃, 227 W/m^2.K for 2 min. Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. It was observed that at the centre of the superchilled samples, there was no ice crystals formed; this agrees well with the finding that the temperature at the centre is higher than the initial freezing temperature of Atlantic salmon, -1.1℃. The initial freezing point was indicated by the beginning of the freezing plateau at the centre of the sample. The initial freezing point of the salmon was determined using separate samples that were totally frozen in the impingement freezer for approximately 30 minutes, and these samples were not used for any other analysis.



Control

surface centre

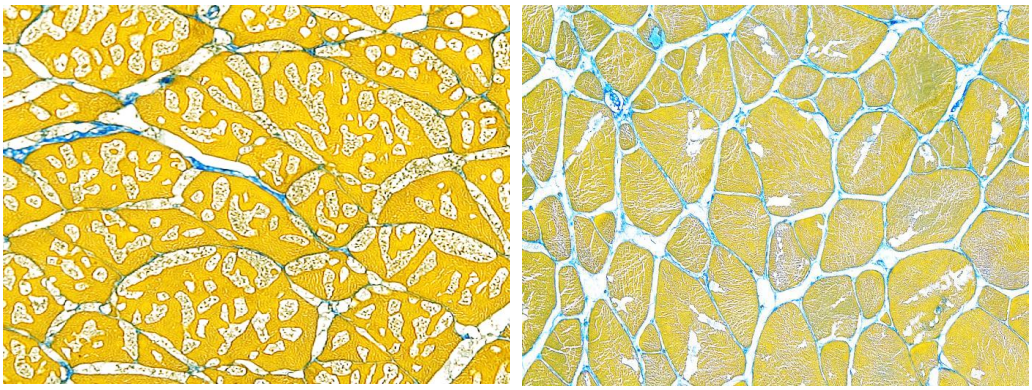


Figure 3: Micrographs of unsuperchilled and superchilled salmon tissues: surface and centre layers

Figure 4 shows the degree of superchilling formed after 4 minutes of superchilling process, -30°C, 227 $W/m^2.K$ and 0.014 m half of the product thickness. The degree of superchilling at the surface was about 50 % and 0.001 % at the center. The red line, is the average degree of superchilling at the food product, which was 32.5 %, and the remaining lines show the ice level in the different points within the food products.

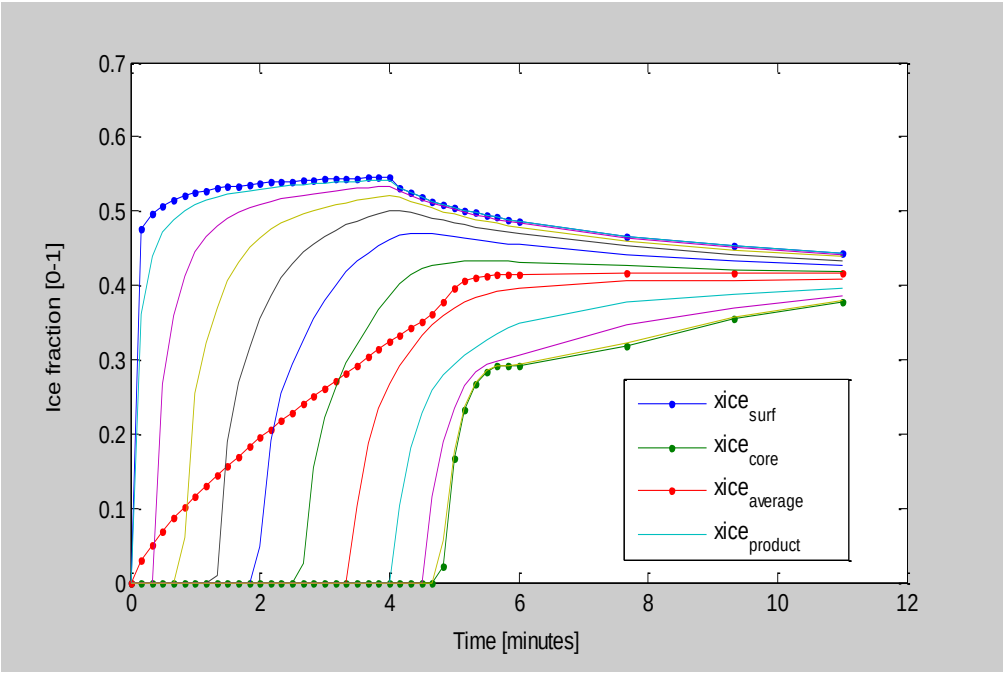


Figure 4: Time- degree of superchilling profile of food product (-30oC, 227.14 W/m2.K). Shows ice-levels at the surface, centre, average and at different parts of the product.

3.2. Calorimetry measurement: Laboratory experiment results compared to numerical results

The formation of ice crystals inside the food products during the superchilling process can be influenced by a number of factors such as, product thickness, density, surface area of the food, temperature difference between product and superchilling medium, surface heat transfer coefficient, superchilling process time, and, of course physical, chemical composition of fish and thermal properties of product. In order to validate the model, design of experiment was performed to study the effect of superchilling medium temperature, surface heat transfer coefficient, superchilling process time, and product thickness during calorimetry measurement of ice crystals formed in the salmon fillets and the results were compared to that of numerical simulation.

3.2.1. Plots of Interaction between Factors

Figures 5 and 6 show the interaction results for laboratory experiment and numerical simulation, respectively. The plots show the interactions (2-way, 3-way and 4-way effects) of all four factors. The interaction plot graphs, the means of the triplicates (degree of superchilling) are organized based on the high and low values of the factors. When lines are parallel, interactions effects is small or no interaction. The more different the slopes, the more influence the interaction effect has on the results.

To visualize these effects, the degree of superchilling was set on the Y- axis for each combination of factors.

Effect of product thickness: It is well- known that smaller, thin product will cool and release heat more quickly than large, thicker products. The product thickness was varied from 0.025 to 0.027 m in order to observe the effect of the ice crystal formation inside the food product. It was found that, the product thickness was inversely proportional to the amount of ice crystals formation during numerical simulation and calorimetry measurement results. However, due to very small difference between the two values of the thickness (only 0.002 m), the influence of product thickness on the results was small. Figures 5 and 6 show lines that are nearly parallel in the column of the product thickness, which indicates that, there is little interaction between thickness product and other factors.

Effect of superchilling medium temperature: The effect of superchilling medium temperature shows influence both in numerical and experimental results. The samples had a higher ice level when superchilled at -30℃ than those superchilled at-20℃. This is because the temperature difference between superchilling medium temperature and food is the driving force for removal of heat (Sun and Zheng, 2006). If the superchilling temperature is lowered, the superchilling rate will always increase. Therefore, lowering the superchilling temperature, from -20 to -30℃ is one method to accelerate the superchilling process. It was also noticed that, when temperature interacted with other factors at the higher superchilling medium temperature, the level of degree of superchilling was higher than in lower superchilling medium temperature

Effect of surface heat transfer coefficient: During superchilling small amount of water is converted to ice crystals, (5 % to 30 %). At this point, the surface heat transfer coefficient is very important because the difference between surface temperature of product and the superchilling medium temperature is high. Statistical results also indicated that, the high level of ice crystals was achieved at higher value of surface heat transfer coefficient (227 $W/m^2.K$) than at lower value (153 $W/m^2.K$).

Effect of superchilling process time: is one of the most important parameters in the superchilling process. It is well- known that the more time the product spends in the freezer, the more ice crystals will be formed within the products. It was observed that the degree of superchilling is directly proportional to the superchilling process time. The interactions between time and other factors were also observed to influence on the results.

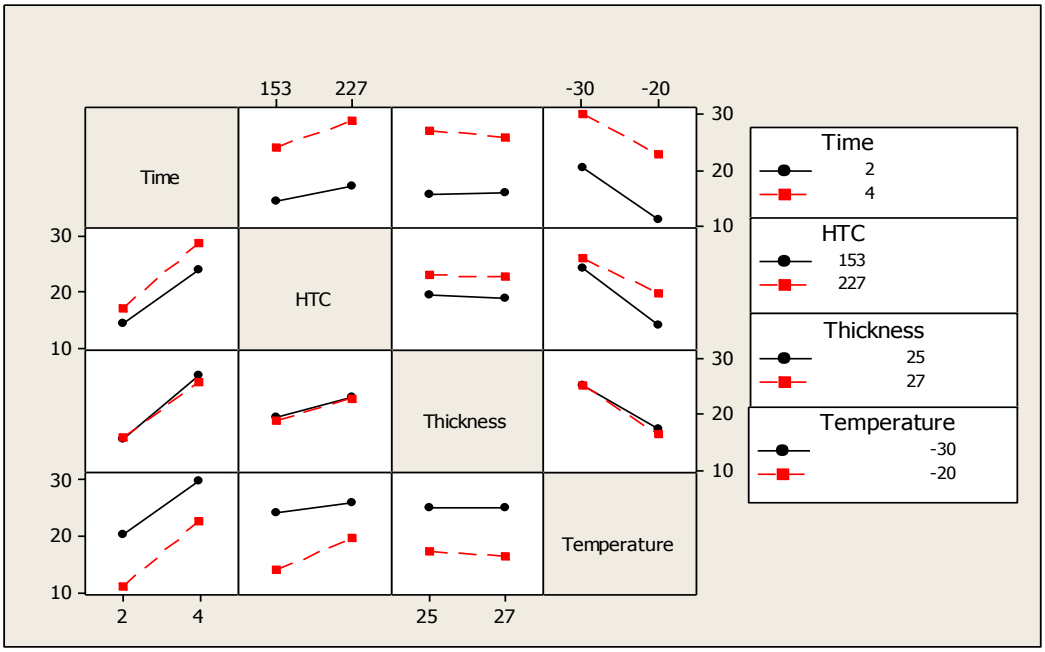


Figure 5: Interaction effect on degree of superchilling laboratory experiment results

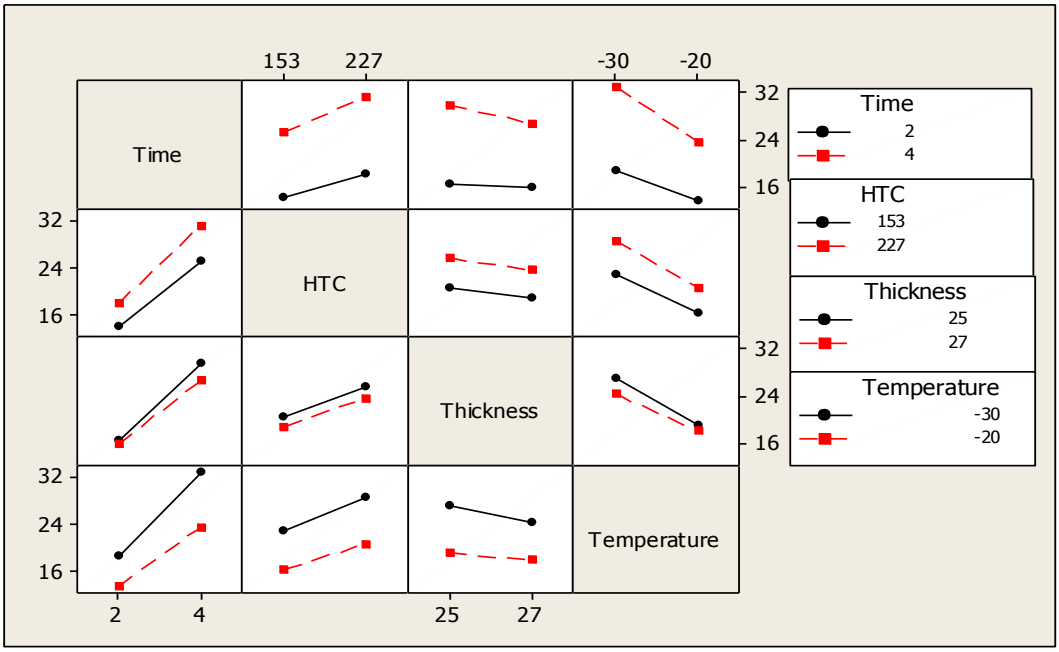


Figure 6: Interaction effect on degree of superchilling numerical simulation results

3.2.2. Residual Plot

The final and necessary step is to check the error in the results using residual diagnostic tools. Figures 7 and 8 show residual plots for laboratory and numerical results, respectively. It can be seen that in the residual plots for laboratory experiment and numerical results, most of the data points are fitted in the line (normal probability plots). This suggests that, the normal distribution is a good model for these data sets. The histogram plots for both experiment and numerical results are more-or-less bell-shaped, which confirms the conclusions from the normal probability plots. Nevertheless, both numerical and laboratory results were correct; the residuals versus fits values spread well in the points for the highest fitted values, which suggest that the models (experimental and numerical) fit the data well. Additionally, individuals control charts (I-charts) of residuals versus observed order, which assess the independence assumptions, do not exhibit any concerning features, both in numerical and laboratory results.

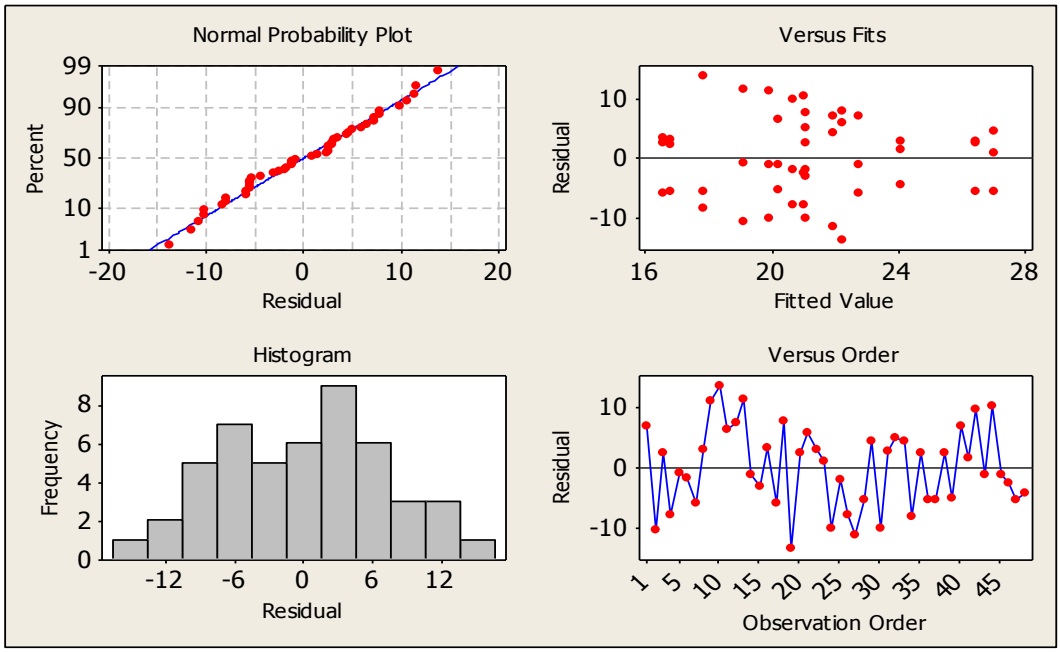


Figure 7: Residual plots for degree of superchilling laboratory experiment results

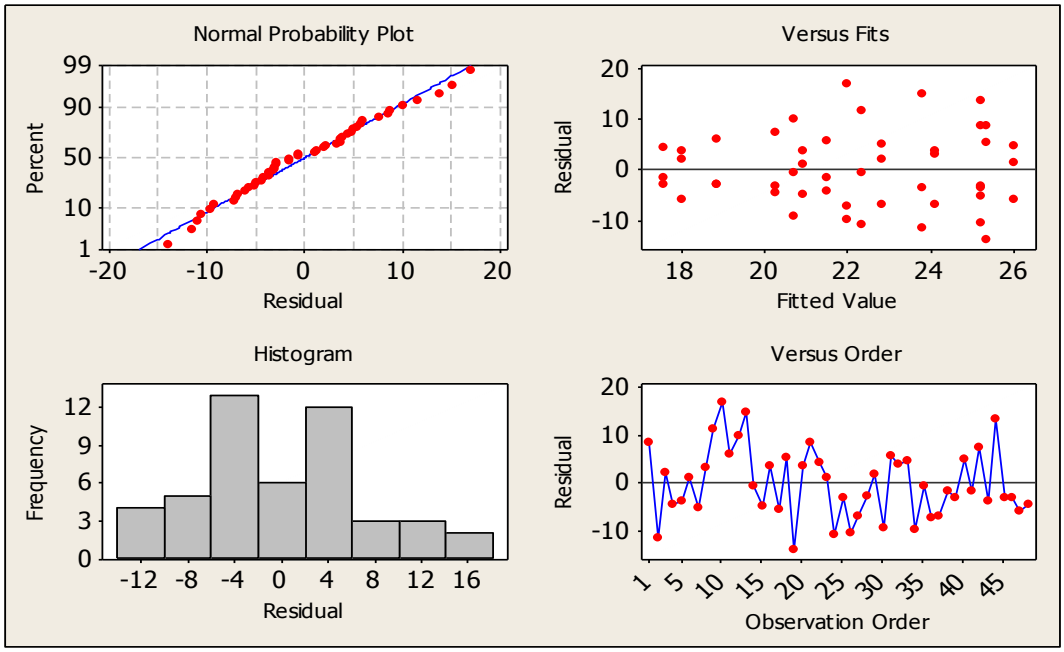


Figure 8: Residual plots for degree of superchilling numerical simulation results

Figure 9 shows the degree of superchilling numerical results versa laboratory experiment results. The relationship is fairly perfect; the effect of experimental results suffice for an entirely accurate prediction of the numerical results, except for runs 4, 7, 20 and 36. ANOVA with Tukey simultaneous test was also carried out to prove the results in the Figure 9. The ANOVA did not show significant differences at $P < 0.01$ between experimental and numerical results, except for runs 4, 7, 20 and 36. The significant differences observed in these runs may have been caused by uncertainties present in the calorimetric measurement such as fluctuation of temperature (room temperature) and reading of the equalization temperature. Thorough testing of the developed model was implemented by carrying out different simulation under different scenarios using MATLAB software and by intensive laboratory experiments. It is worth mentioning that there is a good level of agreement between numerical simulation and experimental results.

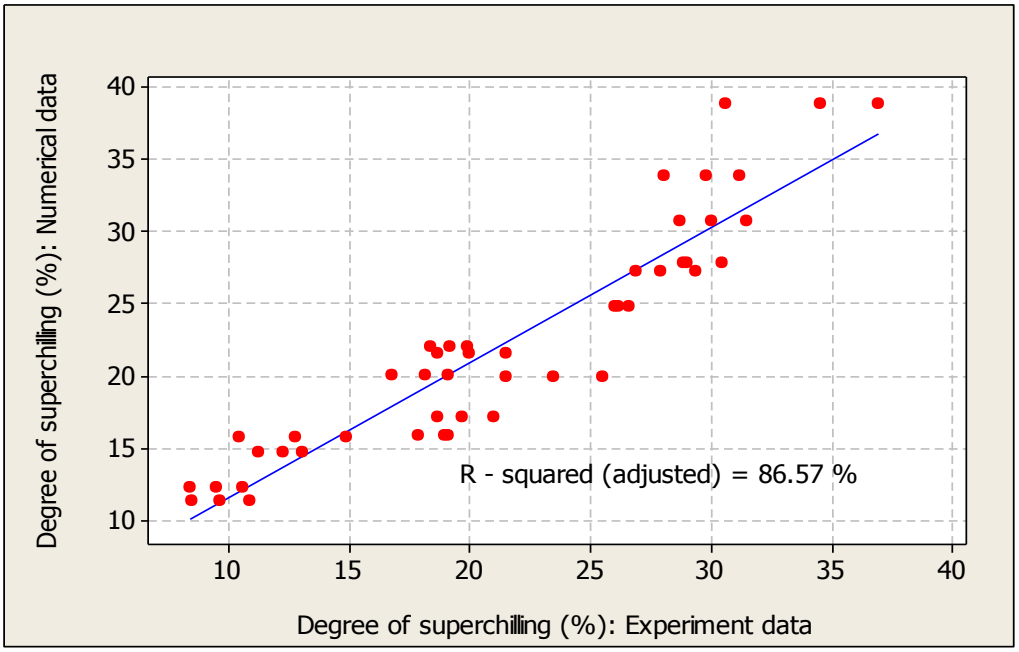


Figure 9: Degree of superchilling, numerical results versa degree of superchilling laboratory experimental results

4. Conclusion

In the present study, a one-dimensional implicit finite difference numerical model for predicting partial freezing time necessary to achieve an optimal degree of superchilling was developed. There is good agreement between numerical simulation and experimental results except 4 runs which show deviation. The significant differences observed in these 4 runs may have been caused by uncertainties present in the calorimetric measurement. The superchilling process time, surface heat transfer coefficient, product thickness and superchilling medium temperature gave satisfactory agreement between simulation and laboratory results.

It should, however, be mentioned that this study is only the first step in enabling superchilling dynamics as an analysis tool for improving the process. Tremendous efforts are needed in forthcoming study to further quantify the model. Determine the optimum superchilling process conditions for extending the shelf life and maintaining the quality of foods is the next step.

Acknowledgements

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Paper III

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The effect of cooling rates on the ice crystal growth in air-packed salmon fillets during superchilling and superchilled storage

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ABSTRACT

Salmon fillets were superchilled in an impingement freezer using four different treatments, A (heat transfer coefficient 153 W m^{-2} K^{-1}, air temperature −20 °C), B (227 W m^{-2} K^{-1}, −20 °C), C (153 W m^{-2} K^{-1}, −30 °C) and D (227 W m^{-2} K^{-1}, −30 °C), and stored at −1.7 °C for 28 days. The influence of these treatments on the microstructure of salmon fillets was studied. The equivalent diameter of the intracellular ice crystals formed were 124 ± 14, 110 ± 4, 114 ± 16, and 95 ± 5 μm for the samples subjected to treatments A, B, C and D, respectively, after one day of storage. Smaller size of the ice crystals were observed in fillets superchilled using treatment D compared with other treatments. Significant differences were observed between the size of ice crystals formed during the superchilling process and superchilled storage. The results further indicated that, the growth of the intracellular ice crystal was significant at ($P < 0.05$) for the entire storage time. However, after temperature equalization (1 day of storage), there was gradually growth of the intracellular ice crystals compared to that observed before temperature equalization (i.e. day 0 and 1). Conversely, spurt growth of the size of ice crystals were observed between 14 and 21 days of storage.

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L'effet des vitesses de refroidissement sur la formation de cristaux de glace dans des filets de saumon emballés sous air lors du surrefroidissement et de l'entreposage surrefroidi

Mots clés : Refroidissement ; Surrefroidissement ; Produit alimentaire réfrigéré ; Taille des cristaux de glace ; Entreposage

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1. Introduction

Superchilling is the process of partial crystallization of ice from supercooled water in food products. The superchilling/partial-freezing process has two stages: 1) cooling the product to initial freezing point and 2) removing the latent heat of crystallization, whereby 5–30% of the water is frozen (degree of superchilling). The phase transition stage of the superchilling/partial-freezing process involves the conversion of water to ice through the crystallization process and is the key step in determining the efficiency of the process and the quality of the resulting superchilled product. The degree of superchilling (ice fraction) is amount of water (5–30%) which is frozen inside the food product, and it is one of the most important parameters which define the quality of the superchilled food product. Superchilled storage is the storage of superchilled food product at 1–1.5 °C below its initial freezing point. During the storage time, the ice formed will absorb heat from the interior and eventually reach equilibrium. Superchilling provides the food product with an internal ice reservoir so that no external ice is required during transportation or storage for short periods (Kaale et al., 2011).

Food products are multicomponent systems of uneven quality containing many substances, with water being the most abundant component (50–95%), which exists in different forms in the tissue (Dincer, 1997; Do et al., 2004; Kiani and Sun, 2011). Superchilling of food products entails the conversion of a minor part of this water (5–30%) into ice crystals. It is the formation of these ice crystals that causes major problems in superchilled food products. The formation of fine crystals during the superchilling process that are evenly distributed both inside and outside the cells leads to better product quality preservation due to reduced damage to the tissue (Clevalier et al., 2001; Dincer, 1997; Fernandez et al., 2008; Kiani and Sun, 2011; Martino and Zaritzky, 1986; Martino et al., 1998; Petzold and Aguilera, 2009). This even distribution usually occurs at high superchilling rates. Slow superchilling rates usually cause texture damage due to the formation of large ice crystals.

The volume and value of fresh, refrigerated foods is increasing along with the flow of these products between countries, and superchilling appears to be a better mode for their preservation (Kaale et al., 2011). This volume increase is due to a number of factors that are driven by changes in technology and lifestyle. However, the sensory and nutritional loss that occurs in superchilled food products is a major concern for consumers. Bahuaud et al. (2008) reported that the ice crystals formed during superchilling were large enough to damage the integrity of the fish muscle during the superchilling process. The large intra- and extra-cellular ice crystals formed during superchilling has a significant effect on the morphological changes, cell destruction and the concentration of the unfrozen matrix, which may result in a change in pH, osmotic pressure, and ionic strength. These changes can affect biochemical and physicochemical reactions, such as protein denaturation, lipid oxidation and enzymatic degradation, in superchilled food products. However, there are few published studies describing ice crystal growth during the superchilling process and during superchilled storage. Studies of the characteristics of the ice crystals formed during the superchilling process and during superchilled storage provide a better scientific basis for the evaluation of the methods for chilling and for a comparison of the different technologies used. Therefore, the objectives of this work were to analyse the ice crystal microstructure in salmon fillets after the superchilling process and to assess the change of ice crystal sizes during superchilled storage under various operating conditions. Salmon fillet was chosen as a case study.

2. Materials and methods

2.1. Materials

Salmon fillets (0.9–1.2 kg) of thickness ranging from 26 to 28 mm were provided by Lerøy Midnor (Hitra, Norway). Skins were removed from the fillets to enhance heat transfer during the superchilling process. This enhanced heat transfer is due to the removal of lipids in the skin of fish, which are good insulators for heat transfer (McClements and Decker, 2008). The salmon fillets were prepared and stored at 4 °C before the superchilling process to ensure a constant temperature in all samples. Superchilling was performed in an Impingement Advantec Lab Freezer (JBT FoodTech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. Minitab 16 software was used in the design of experiment. A 2^2-two-level full-factorial design was used with two variables: surface heat transfer coefficient (SHTC) and impingement superchilling temperature. Four different superchilling treatments were performed and were labelled A (heat transfer coefficient 153 W m^{-2} K^{-1}, air temperature −20 °C), B (227 W m^{-2} K^{-1}, −20 °C), C (153 W m^{-2} K^{-1}, −30 °C) and D (227 W m^{-2} K^{-1}, −30 °C). The experiments for measuring SHTC values were performed in an Impingement Advantec Lab Freezer. Details on experiments set up and equation used to calculate SHTC explained elsewhere (Kaale et al., 2012). Five fillets were used per treatment. Once superchilled, the salmon samples were sliced into seven pieces each, i.e., equal to the number of sampling days, and stored in tightly sealed plastic bags in a cold room at -1.7 ± 0.3 °C for 28 days.

2.2. Impingement freezers

The impingement freezer is a tunnel freezing system which employs high velocity refrigerated air impingement jets to quick freeze food products. During the freezing/partial freezing process, the products are placed on a conveyor belt, and the high velocity air passes through the conveyor upwards and downwards Fig. 1. The range of pressure difference across the fan is 1.5–2.5 kPa and the working temperature range is +20 to −46 °C. Maximum product height is 180 mm and maximum surface area is 500 × 459 mm. Other dimensions such as size/spacing of holes are not available from the manufacturer. Fig. 1.

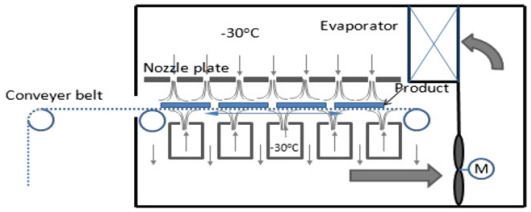


Fig. 1 – Showing general principles of the impingement freezer.

2.3. *Measurement of the temperature during the superchilling process and superchilled storage*

The temperature was measured at three different locations on the samples during the superchilling process: the surface, midway to the centre and the centre. Three thermocouples were used at each location (3 – surface, 3 – midway centre and 3 – centre). The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the sample were recorded every 4 s. The thickness of each sample was approximately 28 mm. The temperature was measured by inserting thermocouples approximately 2 mm from the surface, 7 mm from the surface (midway to the centre) and 14 mm from the surface (centre).

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted in the storage box: one was used to measure the air temperature, and the other two were used to measure the surface and centre temperatures of the superchilled salmon fillets. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately −5 °C (temperature outside the storage box).

2.4. *Microscopic analysis*

Two pieces were cut from the top surface to the bottom surface of each superchilled sample (−1.7 ± 0.3 °C) transversally to the muscle fibre using a standard knife blade that was previously stored at −1.7 ± 0.3 °C. This procedure was conducted in a walk-in freezer to ensure a perfect cold chain. In this study, a fixation method similar to those proposed by Alizadeh et al. (2007), Martino and Zaritzky, (1988) was used to observe the spaces left by the formation of ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The control (unprocessed) samples were fixed using the same solution but at 4 °C. The fixed samples were then heated to room temperature and were subsequently dehydrated using absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fibre using a microtome (Autocut 2055, Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Tissue Clear was used for rehydration, the samples were immersed in 1% blue aniline for 1 min, and xylene was used before mounting.

All the prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan). The images of the slides were recorded and treated using the stereological analysis program CAST2 (Olympus Inc., Denmark). Two parameters, namely the cross-sectional area and the equivalent diameter, were used in the evaluation. The cross-sectional area refers to the surface area of the cross-section of an object (ice crystal or fibre muscle). The equivalent diameter for each ice crystal is defined as the diameter of a circle having the equivalent area, S_p. From the data set of each equivalent diameter, the mean crystal diameter, D_{eq}, was calculated. All analyses were performed for the six different specimens (three – surface and three – midway to the centre) per fillet. For each case considered, more than 100 incidences of ice crystals were evaluated.

2.5. *Statistical analysis*

The observations of the microstructure sizes for different superchilling treatments and the locations with respect to the number of storage days were determined by one- and two-way analyses of variance (ANOVA) using Minitab 16 software. A general linear model (post-hoc test) under Tukey's simultaneous test, was applied whenever the ANOVA results were significant. The reason for performing the post-hoc test is to compare pairs of locations/treatments with storage days simultaneously to understand why the significant results were obtained for the overall ANOVA. Data were expressed as the mean ± standard deviation, and the statistical significance of each experiment was $p < 0.05$.

3. Results

3.1. *Superchilling simulation processes*

The developed model (Kaale et al., 2012) was used to predict the superchilling process times in the present study. Fig. 2 shows the time-degree of superchilling for treatments A, B, C and D. A product thickness of 14 mm, i.e., half the thickness of the sample, was used for each treatment. The initial freezing point was −1.1 °C, which was indicated by the beginning of the freezing plateau at the centre of the sample. The initial freezing point of the salmon was determined using separate samples that were totally frozen in the impingement freezer for approximately 30 min, and these samples were not used for any other analysis. Fig. 2.

3.2. *Time–temperature profiles for the superchilling process and superchilled storage*

Figs. 3 and 4 show the superchilling temperature–time profiles during the superchilling process and during storage,

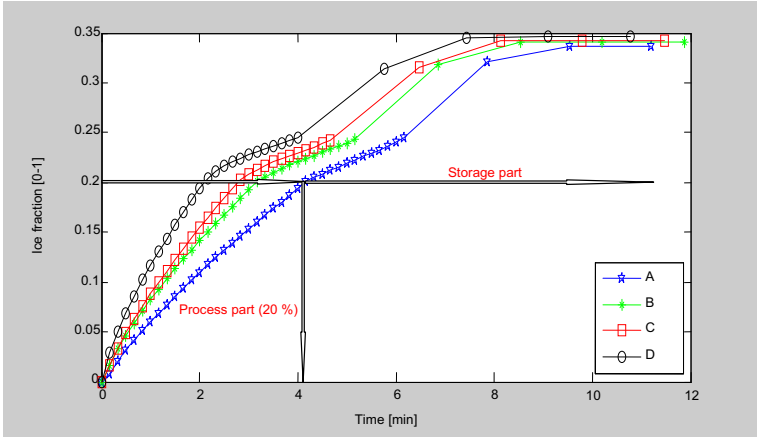


Fig. 2 – Ice fraction vs. time.

respectively. Fig. 3 shows that during the superchilling process, the surface of the food experiences a rapid change in temperature: −6 °C at the surface compared to +4 °C at the core part of the product. The temperature dropped slowly because the water-to-ice transition releases latent heat. Fig. 4 shows the evolution of the air temperature in the storage box and the surface and centre temperatures during superchilled storage. The profile shows the temperature over only one day of storage (24 h); the temperature was maintained at −1.7 ± 0.3 °C for the entire storage time. After temperature equalization, i.e., one day of storage, the temperature at the centre of the samples was the same as that at the surface Fig. 4.

3.3. *Microscopy investigations*

3.3.1. *Evaluation of the microstructure size during superchilling process*

The macrographs of salmon fillet samples superchilled using different superchilling treatments are shown in Fig. 5. Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. The cross-section of the unprocessed sample exhibited a uniform distribution of regularly shaped fibres. The equivalent diameter of the muscle fibres was 99 ± 11 μm, which is close to the value reported by Zhu et al. (2003).

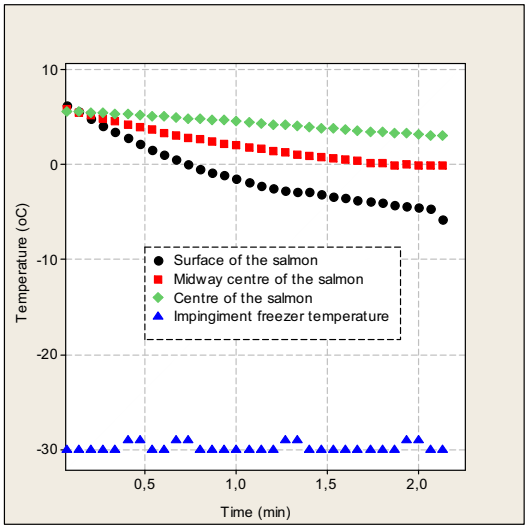


Fig. 3 – Time–temperature profile at different locations during superchilling process.

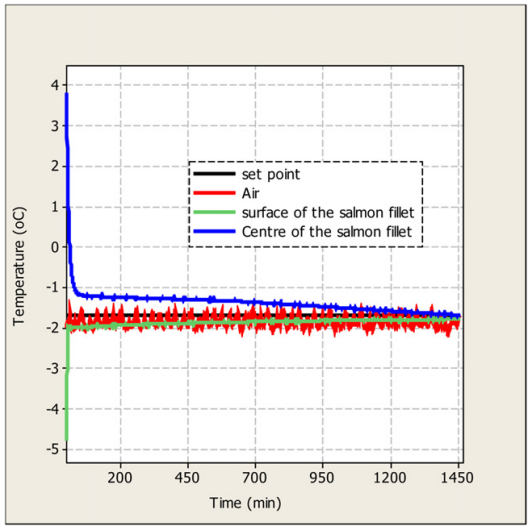


Fig. 4 – Time–temperature profile during superchilled storage.

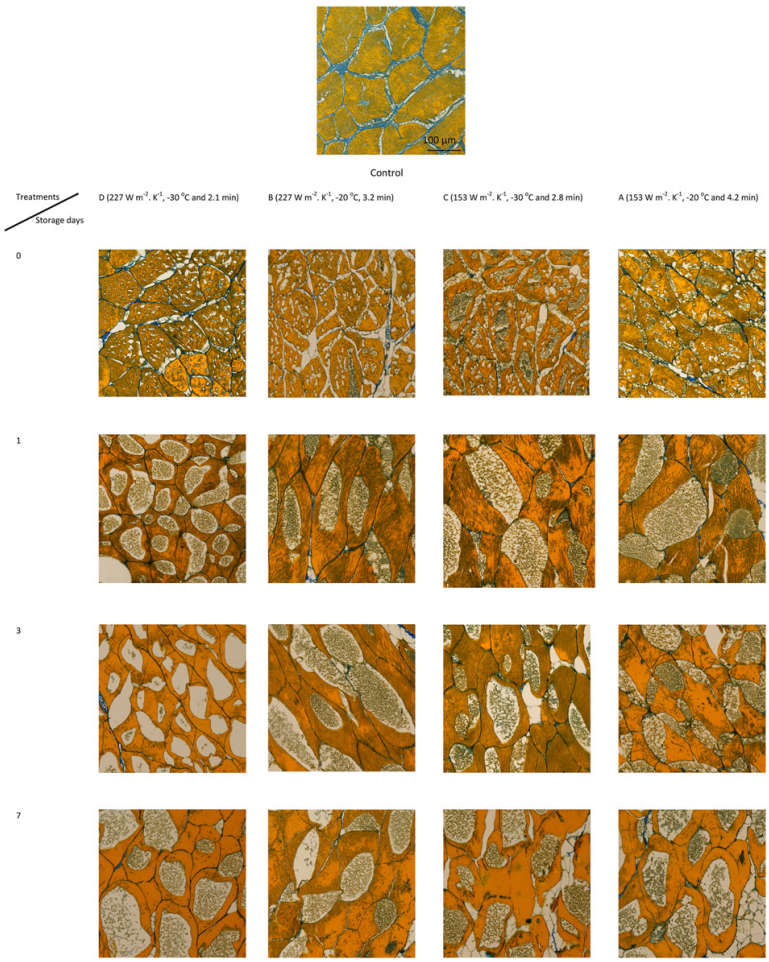


Fig. 5 – Micrographs of unsuperchilled and superchilled salmon muscles: surface layers.

Table 1 summarizes the statistical results of the ice crystals formed during the different superchilling processes and during superchilled storage. The ice crystals formed during processes A and C appeared to be large in size (Table 1, Figs. 6 and 7). Treatments A and C are slow superchilling processes, which are generally considered to form large and mainly extra-cellular ice crystals (Zhu et al., 2003), but it was very difficult to observe extra-cellular ice crystals in these samples. The statistical results indicated that there was no significant difference ($P < 0.05$) between the ice crystals formed during

Table 1 – Equivalent diameters of the ice crystals (Mean ± standard deviation) during superchilling.

Storage time (days)	Superchilling processes							
	A		B		C		D	
	Surface	Mid centre	Surface	Mid centre	Surface	Mid centre	Surface	Mid centre
0	60 ± 16	100 ± 23	45 ± 11	61 ± 9	54 ± 9	101 ± 18	25 ± 2	52 ± 7
1	124 ± 14	170 ± 19	110 ± 4	144 ± 5	114 ± 16	167 ± 14	95 ± 5	131 ± 3
3	148 ± 18	175 ± 9	132 ± 6	180 ± 10	147 ± 14	211 ± 20	108 ± 7	144 ± 23
7	170 ± 33	198 ± 44	140 ± 8	194 ± 14	159 ± 15	218 ± 28	117 ± 8	158 ± 17
14	189 ± 18	237 ± 27	160 ± 8	198 ± 9	169 ± 12	251 ± 12	129 ± 9	164 ± 4
21	207 ± 22	343 ± 4	196 ± 44	237 ± 20	224 ± 10	293 ± 41	184 ± 36	209 ± 25

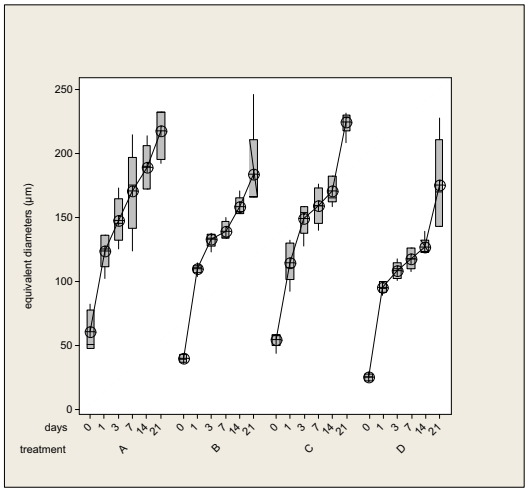


Fig. 6 – Boxplots showing equivalent diameters of the ice crystals at the surface layers of salmon fillets vs. storage days at different treatments.

treatments A and C (Table 1; Figs. 5–7). The equivalent diameter of ice crystals formed during treatment A was 60 ± 16 μm at the surface layer and 100 ± 23 μm midway to the centre layer, which was much larger than those formed during treatments B and D (Table 1, Figs. 5 and 6). Thus, slow superchilling processes such as A and C usually result in texture damage to real foods due to the formation of large ice crystals (Zhu et al., 2003, 2005). The damage induced during slow superchilling/partial freezing is the result of the increase in concentration of extra-cellular electrolytes, which in turn leads to an increase in the concentration of intracellular

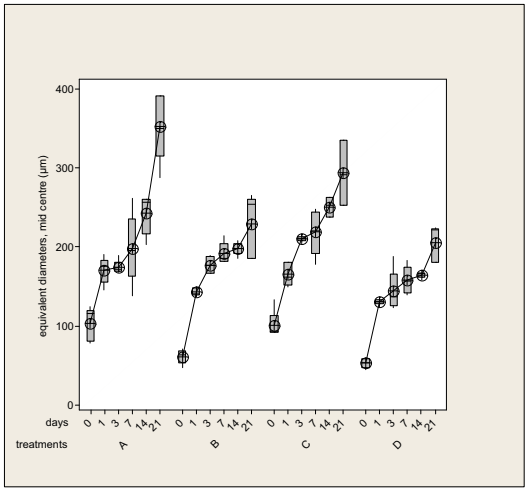


Fig. 7 – Boxplots showing equivalent diameters of the ice crystals at the midway centre layers of salmon fillets vs. storage days at different treatments.

electrolytes (Mazur, 1984). Cells also shrink osmotically in response to the increasing concentration of extra-cellular solutes during superchilling/freezing, i.e., it is a consequence of the inability of the cells to shrink to the extent required for osmotic equilibrium (Martino and Zaritzky, 1986; Martino et al., 1998; Mazur, 1984; Shenouda, 1980).

In treatment D, the system is able to remove heat quickly enough during the superchilling process to produce fine crystals that are evenly distributed both inside and outside the cells. Treatment D resulted in small ice crystals (Figs. 5–7, 10 and Table 1) with an equivalent diameter of $(25.15 \pm 2$ μm) at the surface layer and 52 ± 7 μm midway to the centre layer (Table 1) due to the rapid superchilling treatment (only 2.1 min). The treatment B ice crystals were larger, 45 ± 11 μm at the surface layer and 61 ± 9 midway to the centre layer; they were superchilled under the same SHTC as treatment D (227 W m^{-2} K^{-1}) but at different superchilling temperatures and times (Figs. 6 and 7 and Table 1). These differences between treatments can be explained by the faster freezing rate obtained at higher heat transfer coefficients and lower air temperatures. Because the rate of ice crystallization is a function of the speed of heat removal as well as the diffusion of water from the cell to the intercellular space (Dincer, 1997; Goransson and Londahl, 2005), at a lower superchilling rate (treatments A and C), fewer and larger ice crystals are formed in this space, while at a higher superchilling rate (treatments D and B), a large number of smaller ice crystals are formed both outside and within the cell.

3.3.2. *Ice crystal evolution during superchilled storage*

The evolution of the size of the ice crystals formed is important during superchilled storage. During the evaluation of the ice crystals, the statistical results indicated that the ice crystals that were formed at day zero (during the superchilling process) were significantly smaller ($p < 0.05$) than those observed during superchilled storage. The superchilling process is a highly transient process involving steep thermal gradients, which results in different layers with different ice crystal sizes during storage. From the microscopic analysis, we observed three different layers of different sizes of ice crystals within the salmon fillets. Fig. 8 shows the ice crystals under treatment D from day zero (superchilling process), day one, day three, and day seven (superchilling storage). In this study, we analysed two layers because the ice crystals at the top surface layer were different from those at the bottom surface layer, and it was not clear if this was due to lipid-content variation or due to the presence of the conveyor belt in the impingement freezer, which is situated at the bottom side of the samples during the superchilling process. To clarify our results, the top surface and midway-to-the-centre layers were analysed (Figs. 6–8).

After 1 day, the equivalent diameters of the intracellular ice crystals were 110 ± 4 and 95 ± 5 μm at the surface layer and 144 ± 5 and 131 ± 3 μm at the midway-to-the-centre layer for the samples treated using treatments B and D, respectively (Table 1), compared with 124 ± 14 and 114 ± 16 μm at the surface layer and 170 ± 19 and 167 ± 14 μm at the midway-to-the-centre layer for the samples treated using treatments A and C, respectively. Fig. 9 and Table 2 show the means of the ice crystals at the two locations within the superchilled

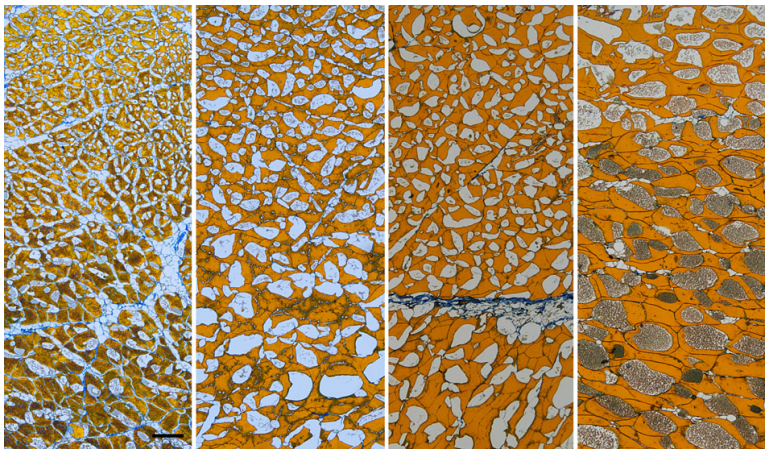


Fig. 8 – Micrographs of the salmon muscles for treatment D: surface and midway centre layers.

samples (surface layer and midway-to-the-centre layer) for the different treatments (A, B, C and D), which are displayed with individual 95% confidence intervals for a mean based on the pooled standard deviation. The statistical results indicated that, for the location data, there was no significant difference between surface layers A and C, at $P < 0.05$. Meanwhile, the midway to the centre layer D did not show significant difference between surface layers A and C (Table 2 and Fig. 9). There was also no significant difference between the midway-to-the-centre layer A and C and midway-to-the-centre layer B and surface layer A.

The growth of the size of ice crystals between day 0 and 1 (Figs. 5–7, 10 and Table 1) may be caused by two important factors during superchilled storage. The first factor is the large difference between the superchilling process temperature (−20, −30 °C) and the superchilled storage temperature (−1.7 ± 0.3 °C). This large temperature difference will cause the growth of the ice crystals, particularly the small ones that were formed at the surface of the salmon fillets. The second factor is the thermal gradient effect that was created during the superchilling process. It is well known that a temperature gradient causes ice recrystallization during superchilled storage. Temperature gradients, whether large or small, will result in recrystallization during superchilled storage. These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals. This process causes larger ice crystals to grow, which results in a reduction in the number of ice crystals at the surface layer of the superchilled salmon.

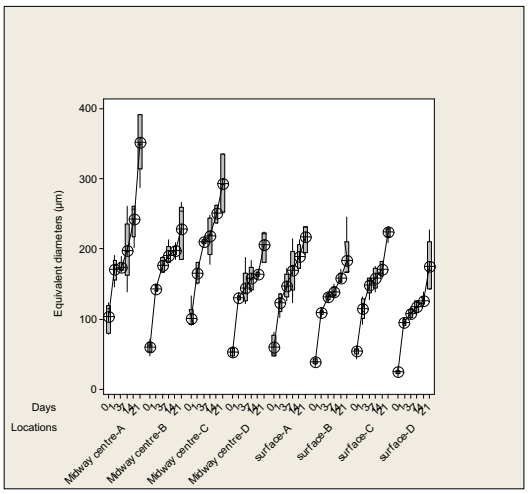


Fig. 9 – Boxplots showing equivalent diameters of the ice crystals vs. storage days at different locations and treatments.

After 1 day of storage, when temperature equalization was achieved within the samples, there was gradually growth of the intracellular ice crystals compared to that observed before temperature equalization (i.e. day 0 and 1). Conversely, spurt growth of the size of ice crystals were observed between 14 and 21 days of storage (Figs. 5–7, 10 and Table 1). It was not possible to analyse the samples on day 28 due to the spoilage odour, which may have been caused by oxidation or a problem involving microbiology (Table 1, Figs. 6 and 7).

The growth of ice crystals after temperature equalization (after 1 day of storage) specifically that observed during day 14 and 21(Fig. 10) may be associated with temperature fluctuation and Ostwald ripening. The present study indicated that the air-packed samples, the ice crystals were growing significantly after 14 days of storage. In the vacuum-packed samples (after one day of storage where the temperature equalization was achieved within the samples) there was no significant growth of ice crystals at any storage time (Kaale and Eikevik, 2012). The differences of the two studies can be explained as follows; during the air-packed study the samples were sliced into seven pieces each, i.e., equal to the number of sampling days, and stored in one bag for each treatment. During the sampling process the bags were opened and closed

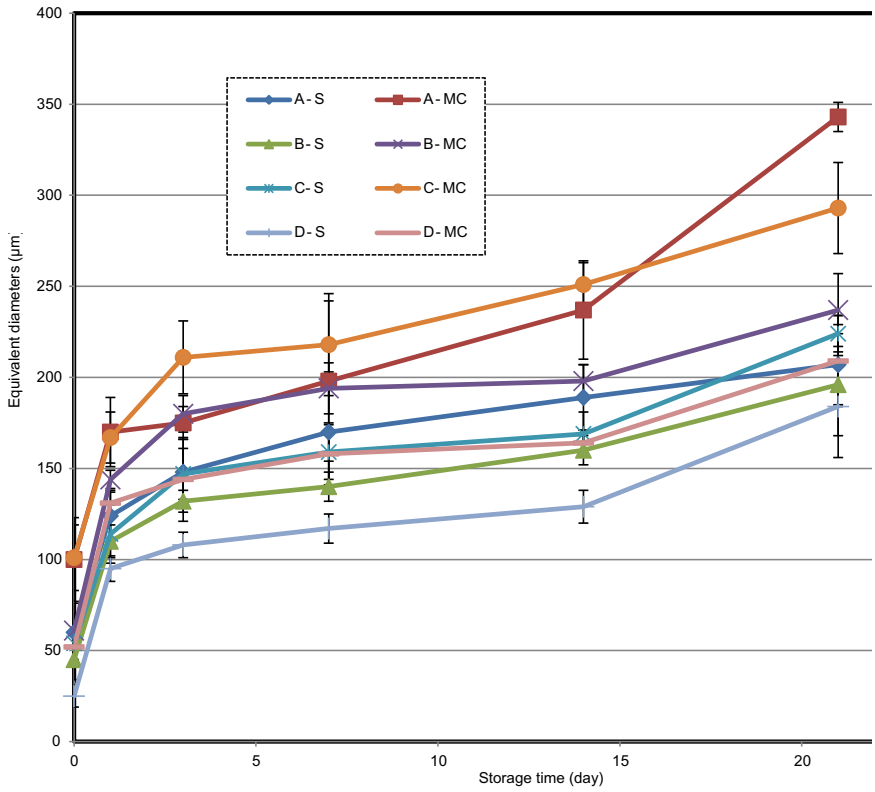


Fig. 10 – Equivalent diameters of the ice crystals vs. time in linear scale: S – surface and MC – mid centre.

in every sampling day. This could cause temperature fluctuations which can accelerate the increase in mean ice crystal size compared to that of vacuum-packed.

3.3.2.1. Other factors which cause ice crystals growth during superchilled storage. Ice crystals have a natural tendency to increase in size with increase storage time (Alvarez, 2009). The larger crystals become larger at the expense of the smaller ice crystals. The study of Hagiwara et al. (2011) reported that the ice crystals grew in size extensively with increasing storage time.

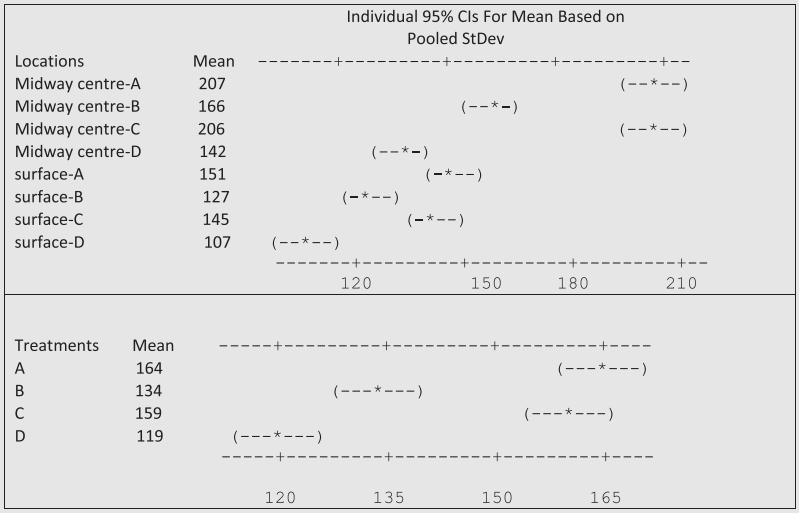
During superchilled storage, small ice crystals are thermodynamically unstable relative to large ice crystals (Mazur, 1984; Shenouda, 1980) and undergo changes in number, size, and shape, which phenomena are known collectively as recrystallization (Russell et al., 1999). During recrystallization, ice crystals can increase in average size and decrease in number through the redistribution of water from small to large crystals (Pham and Mawson, 1997). Some recrystallization occurs naturally at constant temperatures because water vapour will tend to transfer from regions of high vapour pressure (i.e., at the surface of small crystals) to regions of lower vapour pressure (at the surface of larger crystals), which is a phenomenon known as Ostwald ripening.

Temperature fluctuation, which is the most important factor to control during superchilled storage, can accelerate the increase in mean ice crystal size. If the temperature during superchilled storage increases, some of the ice crystals, particularly the smaller ones, melt completely, and consequently, the amount of unfrozen water increases. Conversely, in the period where the temperature decreases, no further nucleation will occur and free water will refreeze at the surface of large crystals, resulting in the reduction of the total number of crystals and the increase of the mean crystal size (Alizadeh et al., 2007; Russell et al., 1999; Shenouda, 1980).

3.3.3. Effects caused by ice formation on muscle during the superchilling process and superchilled storage

As demonstrated in this study, a high superchilling rate results in a high rate of heat removal, leading to the formation

Table 2 – Equivalent diameters of the ice crystals at different locations and treatments with individual 95% confidence intervals (CIs) based on the pooled standard deviation.



of a large number of small nuclei and thus a large number of small ice crystals that grow both within and outside cells, and consequently, the cells maintain their integrity which in turn minimizes drip loss during thawing (Smith, 2011). However, this advantage was reduced during superchilled storage by the rapid growth of the size of ice crystals in the salmon fillets. The growth of the size of ice crystals during superchilled storage may impart mechanical damage by physically rupturing cell walls, which may result in an increase in drip loss (a greater loss of liquid cellular components), protein denaturation, a reduction of the water-holding capacity and other quality parameters related to the damage of the cell structure.

The quality and shelf life of superchilled foods has been reviewed by Kaale et al. (2011), and many benefits of applying superchilling technology to food products were found compared to chilled and frozen food products. However, despite the benefits of the superchilled storage of food products, there is still a need to test the quality parameters separately at different locations within the superchilled product. It is unlikely that there is similar quality at both the surface and the centre of the superchilled product due to the significant differences of the microstructure sizes found in this study.

4. Conclusions

Significant differences were observed between the size of ice crystals formed during the superchilling process and superchilled storage. The results further indicated that, the growth of the intracellular ice crystal was significant at ($P < 0.05$) for the entire storage time. However, after temperature equalization (1 day of storage), there was gradually growth of the intracellular ice crystals compared to that observed before temperature equalization (i.e. day 0 and 1). Conversely, spurt growth of the size of ice crystals were observed between 14 and 21 days of storage.

It was also observed that the intracellular ice crystals in treatment D were significantly ($P < 0.05$) smaller than other treatments. In the future, knowledge of the behaviour of ice crystals could permit the improvement of the quality of superchilled products, and further work should therefore focus on the testing of quality parameters, such as drip loss, enzymatic degradation and proteins denaturation, in parallel with different degrees of superchilling levels (ice levels) within the food products.

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Paper IV

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Ice crystal development in pre-rigor Atlantic salmon fillets during superchilling process and following storage

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ABSTRACT

The objectives of this work were to compare ice crystal sizes of pre-rigor Atlantic salmon fillets subjected to two processes of superchilling and to assess the size changes during storage of superchilled samples at −1.7 ± 0.3 C. The fillets were superchilled in an impingement freezer at either a slow rate (−20 °C, 153 W/m^2 K, 4.2 min) which is referred to as process S or a fast rate (−30 °C, 227 W/m^2 K, 2.1 min) which is referred to as process F before storage for 29 days. Significantly smaller ($p < 0.05$) equivalent diameters of ice crystal occurred at faster superchilling rate when compared to slower superchilling rate. The influence of these processes on the microstructure of pre rigor salmon fillets was studied. The equivalent diameter of the intracellular ice crystals formed were 60 ± 5 and 23 ± 1 μm for the samples subjected to processes S and F, respectively. Significant differences were observed between the size of ice crystals formed during the superchilling process and during storage of superchilled samples. The formation of ice crystals within salmon muscle regardless of the superchilling rates was an important factor in reducing cell structure damage.

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1. Introduction

1.1. Superchilling process

Superchilling is the process of partial ice-crystallization from supercooled water in food products. During this process, a thin frozen layer of about 1–3 mm thick is achieved on the surface of food product depending on degree of superchilling required. The degree of superchilling is the amount of water (5–30%) which is partially frozen inside the food product and is one of the most important parameters which define the quality of the superchilled food product. Magnussen, Haugland, Torstveit Hemmingsen, Johansen, and Nordtvedt (2008), Stevik and Claussen (2011) and Stevik et al. (2010) have reported that the amount of ice crystals stored inside a superchilled product is one of the most important parameters which determine the quality of the end product. Also, it has been reported that a degree of superchilling between 5 and 30% is accepted and that a degree of superchilling higher than 30% will cause higher drip loss in food products (Stevik & Claussen, 2011).

1.2. Ice crystal formation in pre-rigor muscle

The quality of superchilled foods is mainly related to the properties of the ice crystals, such as their size, location (i.e. extracellular and intracellular) and shape during the superchilling process (Alizadeh, Chapleau, Lamballerie, & Bail, 2009; Martino, Otero, Sanz, & Zaritzky, 1998; Martino & Zaritzky, 1986; Petzold & Aguilera, 2009). These properties are influenced by the rate of superchilling, storage time, temperature fluctuation and physiological status of the muscle foods, i.e. pre-, in- or post-rigor muscle (Shenouda, 1980). Slow partial freezing/superchilling rates in post-rigor muscle usually cause texture damage due to the formation of large and extracellular ice crystals (Kaale, Eikevik, Bardal, Kjorsvik, & Nordtvedt, 2013; Shenouda, 1980) probably because the extracellular fluid has a lower osmotic pressure than the intracellular fluid. Rapid superchilling of post-rigor muscle also results in the initial formation of extracellular ice (Chevalier, Sequeira-Munoz, Bail, Simpson, & Ghoul, 2001; Dincer, 1997; Fernandez, Otero, Martino, Molina-Garciá, & Sanz, 2008; Kiani & Sun, 2011; Martino et al., 1998; Martino & Zaritzky,

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1986; Petzold & Aguilera, 2009). However, the extracellular crystals formed during rapid superchilling are much smaller and more finely distributed than those in slow superchilling (Kaale, Eikevik, Bardal, & Kjorsvik, 2013; Kaale, Eikevik, Bardal, et al., 2013). The formation of extracellular ice still dehydrates the cells to some extent, but as the temperature decreases rapidly, the cells become supercooled and the remaining intracellular water freezes before it has time to diffuse out of the cell.

In pre-rigor muscle, the cell fluids are tightly bound to the intracellular proteins and the diffusivity from inside to outside the cell is therefore limited resulting in the formation of intracellular ice crystals independent of superchilling/partial freezing rates (Shenouda, 1980). A large number of smaller ice crystals are formed within the cell. If very slow superchilling/partial freezing rates are used, the muscle can go into *rigor mortis* during the superchilling process and ice crystal formation will be extracellular.

There is pronounced interest for superchilling muscle in the pre-rigor state. Formation of the ice crystals within the cells regardless of the superchilling rates is the most important factor for reducing damage of food muscles and hence maintaining their quality. Freezing pre-rigor Atlantic salmon fillets has also been found to conserve more of the positive quality aspects than freezing of post-rigor muscle (Einen, Guerin, Fjæra, & Skjervold, 2002; Skjervold et al., 2001). An alteration of the freezing and thawing regime allowing for more rigor contraction might potentially conserve more of the positive quality aspects of pre-rigor muscle of the food products. The pre-rigor filleting allows the fish to be processed directly after slaughter; therefore no storage period before filleting is necessary (Bahuaud et al., 2008). Pre-rigor fillets reach the market 3–4 days fresher compared to post-rigor fillets and, as a matter of quality, show a reduction in the severity of gaping, firmer flesh texture, positive effect on color and increased thickness of the fillet (Bahuaud et al., 2008; Einen et al., 2002; Hansen, Mørkøre, Rudi, Langsrud, & Eie, 2009; Skjervold et al., 2001). This early processing also increases the fresh fillet value and reduces waste product transport by 20%, considerably decreasing transportation costs and energy wastage.

However, few studies have been conducted on how the superchilling rates and pre-rigor state will affect ice crystal sizes during the superchilling process and following storage. The size of ice crystals formed in pre-rigor fillets during the superchilling process and the change of the microstructure size during storage of superchilled samples should also be considered, as it is one of the main factors affecting the textural and physical properties of superchilled foods. Therefore, the objectives of this work were to compare the microstructure sizes of pre-rigor salmon fillets superchilled at slow and fast rates and to assess the change of these microstructure sizes during storage of superchilled products.

2. Materials and methods

2.1. Materials and superchilling process

Salmon fillets (0.9–1.2 kg) with thickness ranging from 26 to 28 mm were taken from the slaughtering plant, Salmar (Frøya, Norway). The fillets were vacuum packed and partially frozen pre-rigor (i.e. within 5–6 h of being caught) in an Impingement Advantec Lab Freezer (JBT Food - tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at −20 °C, 153 W/m^2 K for 4.2 min (S) and at −30 °C and 227 W/m^2 K for 2.1 min, (F) to achieve an ice content of 20%. The previously developed model (Kaale, Eikevik, Kolsaker, & Stevik, 2012) was used to predict the degree of superchilling and superchilling time.

Once superchilled, the salmon samples were analyzed at day zero (superchilling process) and other samples were stored in a cold room at −1.7 ± 0.3 °C for 29 days. Three fillets were analyzed at each sampling time.

2.2. Measurement of the temperature during the superchilling process and following storage

The temperature was measured at three different locations on the samples during the superchilling process: at the surface, midway to the center and in the center. Three thermocouples were used at each location. The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the

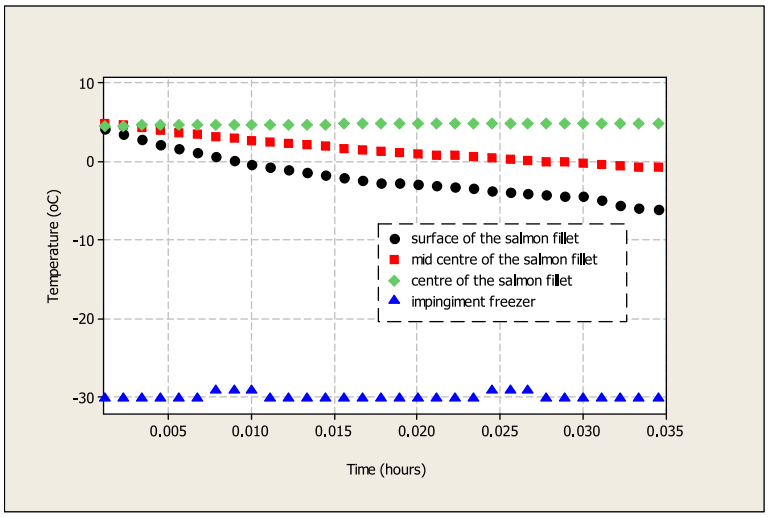

Fig. 1. Temperature–time profile at three places in the Atlantic salmon fillet and the air temperature in the impingement freezer during fast superchilling (−30 C, 227 W/m^2 K 2.1 min).

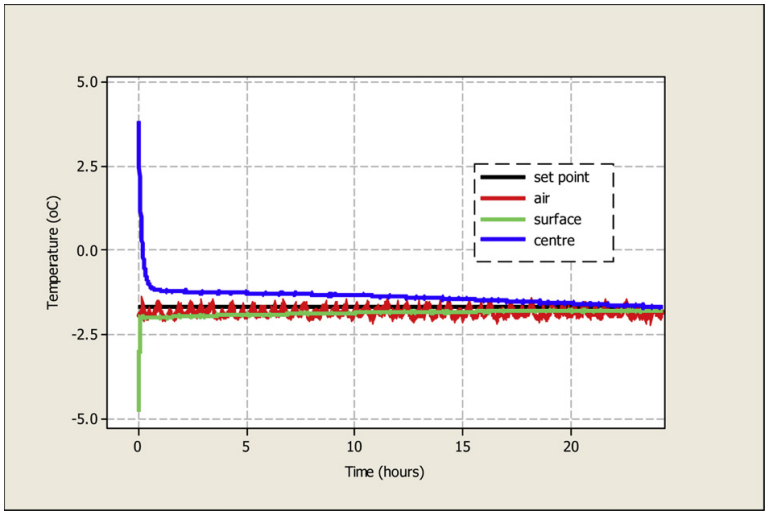

Fig. 2. Temperature–time profile during superchilling storage at −1.7 ± 0.3 °C.

sample were recorded every 4 s. The samples of 28 mm thickness were used. The temperature was measured by inserting thermocouples approximately 2 mm from the surface, 7 mm from the surface (midway to the center) and 14 mm from the surface (center).

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted in the storage box: one was used to measure the air temperature, and the other two were used to measure the surface and center temperatures of the superchilled salmon fillets. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately −5 °C (temperature outside the storage box).

2.3. Microscopic analysis

Two pieces were cut from the top surface to the bottom surface of each superchilled sample (−1.7 ± 0.3 °C) transversally to the muscle fiber using a standard knife blade that was previously stored at the same temperature. This procedure was conducted in a walk-in freezer to ensure a perfect cold chain. In this study, a fixation method similar to those proposed by Alizadeh, Chapleau, de Lamballerie, and Le Bail (2007) and Martino and Zaritzky (1988) was used to observe the spaces left by the formation of ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The control (unprocessed) samples were fixed using the same solution but at 4 °C. The fixed samples were then warmed to room temperature and were subsequently dehydrated using absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fiber using a microtome (Autocut 2055; Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Tissue Clear was used for rehydration, the samples were immersed in 1% blue aniline for 1 min, and xylene was used before mounting.

All the prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan). The images of the slides were recorded and treated using the stereological analysis program CAST2 (Olympus Inc., Denmark). Two parameters, namely the cross-sectional area and the equivalent diameter, were used in the evaluation. The cross-sectional area referred to the surface area of the cross-section of an object (ice crystal or fiber muscle). The equivalent diameter (D_{eq}) for each ice crystal was defined as the diameter of a circle having the equivalent area, (S_p) and mean crystal diameters were calculated. All analyses were performed for the six different specimens (three – surface and three – center) per fillet. For each case considered, more than 100 incidences of ice crystals were evaluated.

2.4. Statistical analysis

The observations of the microstructure sizes for different superchilling treatments and the locations with respect to the

Table 1
Equivalent ice crystal diameters (μm means ± standard deviations) for fast (F) vs. slow (S) superchilling of Atlantic salmon fillets.

Storage (days)	F		S	
	Top surface	Center	Top surface	Center
0	$23 \pm 1^{A/a}$	$0 \pm 0^{B/a}$	$60 \pm 5^{C/a}$	$0 \pm 0^{B/a}$
2	$93 \pm 3^{A/b}$	$312 \pm 2^{B/b}$	$117 \pm 2^{C/b}$	$310 \pm 1^{B/b}$
4	$101 \pm 2^{A/b}$	$307 \pm 4^{B/b}$	$122 \pm 6^{C/b}$	$317 \pm 6^{B/b}$
7	$103 \pm 1^{A/b}$	$305 \pm 4^{B/b}$	$118 \pm 1^{C/b}$	$314 \pm 6^{B/b}$
14	$110 \pm 1^{A/b}$	$323 \pm 2^{B/b}$	$131 \pm 4^{C/b}$	$326 \pm 2^{B/b}$
21	$105 \pm 3^{A/b}$	$329 \pm 1^{B/b}$	$130 \pm 4^{C/b}$	$332 \pm 5^{B/b}$
29	$116 \pm 4^{A/b}$	$343 \pm 4^{B/b}$	$133 \pm 1^{C/b}$	$341 \pm 4^{B/b}$

Capital letters (A, B, C) in the same line indicate significant differences ($P < 0.05$) of locations/processes.
Small letters (a, b) in the same column indicate significant differences ($P < 0.05$) of storage.

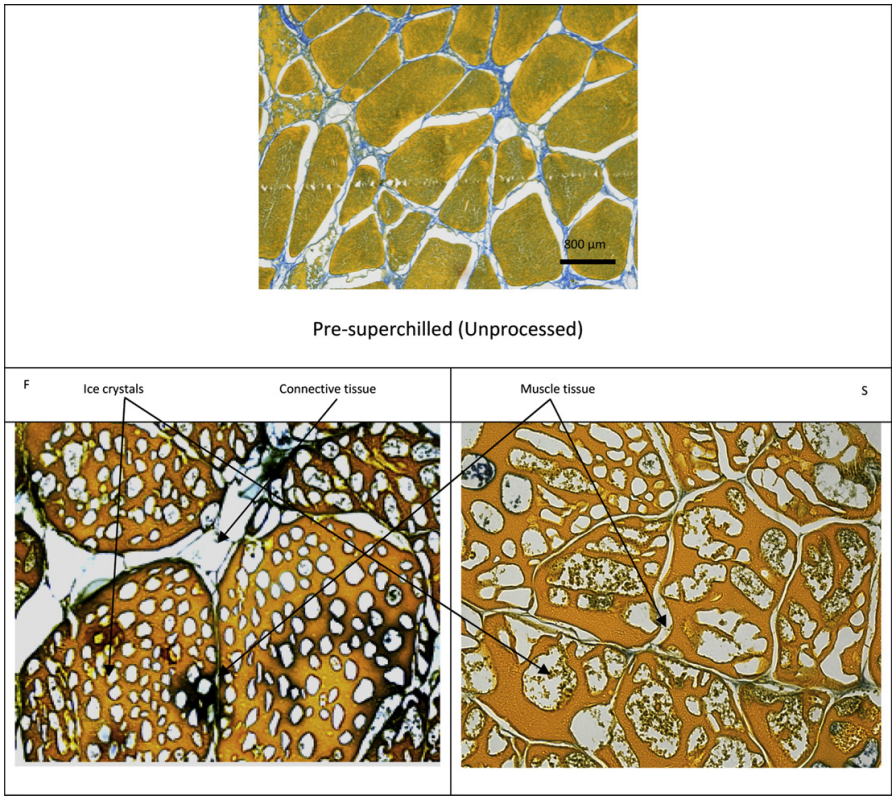

Fig. 3. Non-superchilled salmon fillets (upper micrograph) and after fast superchilling (left micrograph) and slow superchilling (right micrograph).

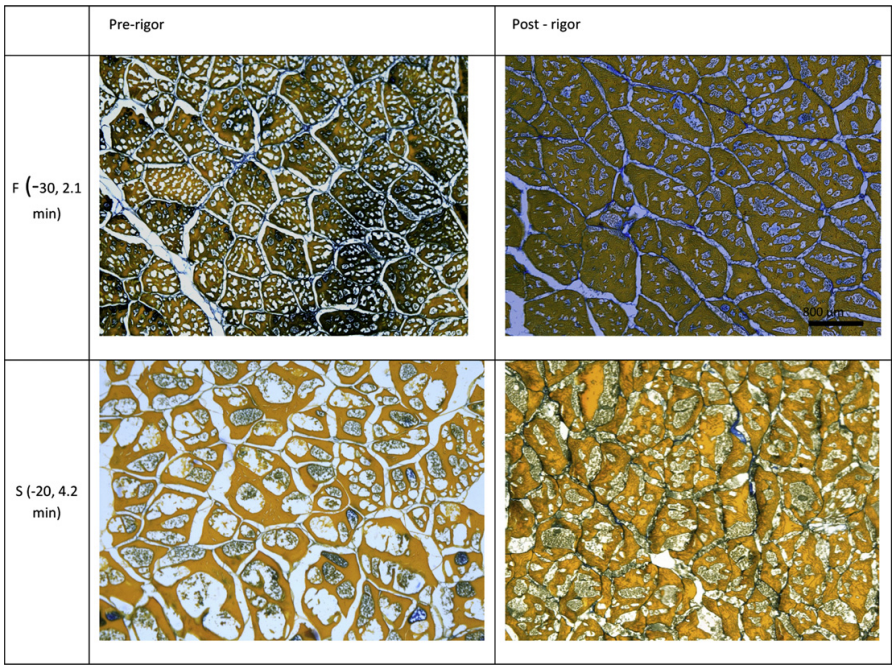

Fig. 4. Pre-rigor and post-rigor fillets after fast (F) or slow (S) superchilling.

number of storage days were determined by one- and two-way analyses of variance (ANOVA) using Minitab 16 software. A general linear model, (post-hoc test) under Tukey's simultaneous test, was applied whenever the ANOVA results were significant. The reason for performing the post-hoc test is to compare pairs of locations/processes with storage days simultaneously to understand why the significant results were obtained for the overall ANOVA. Data were expressed as the mean ± standard deviation, and the statistical significance of each experiment was $p < 0.05$.

3. Results and discussion

3.1. Thermal transition behavior of superchilling process

Changes in surface and center temperatures of the samples were recorded during superchilling experiments. Fig. 1 shows an example of the superchilling process at −30 °C for 2.1 min. The sample and superchilling medium temperatures were recorded every 4 s. The superchilling time–temperature profile consists of

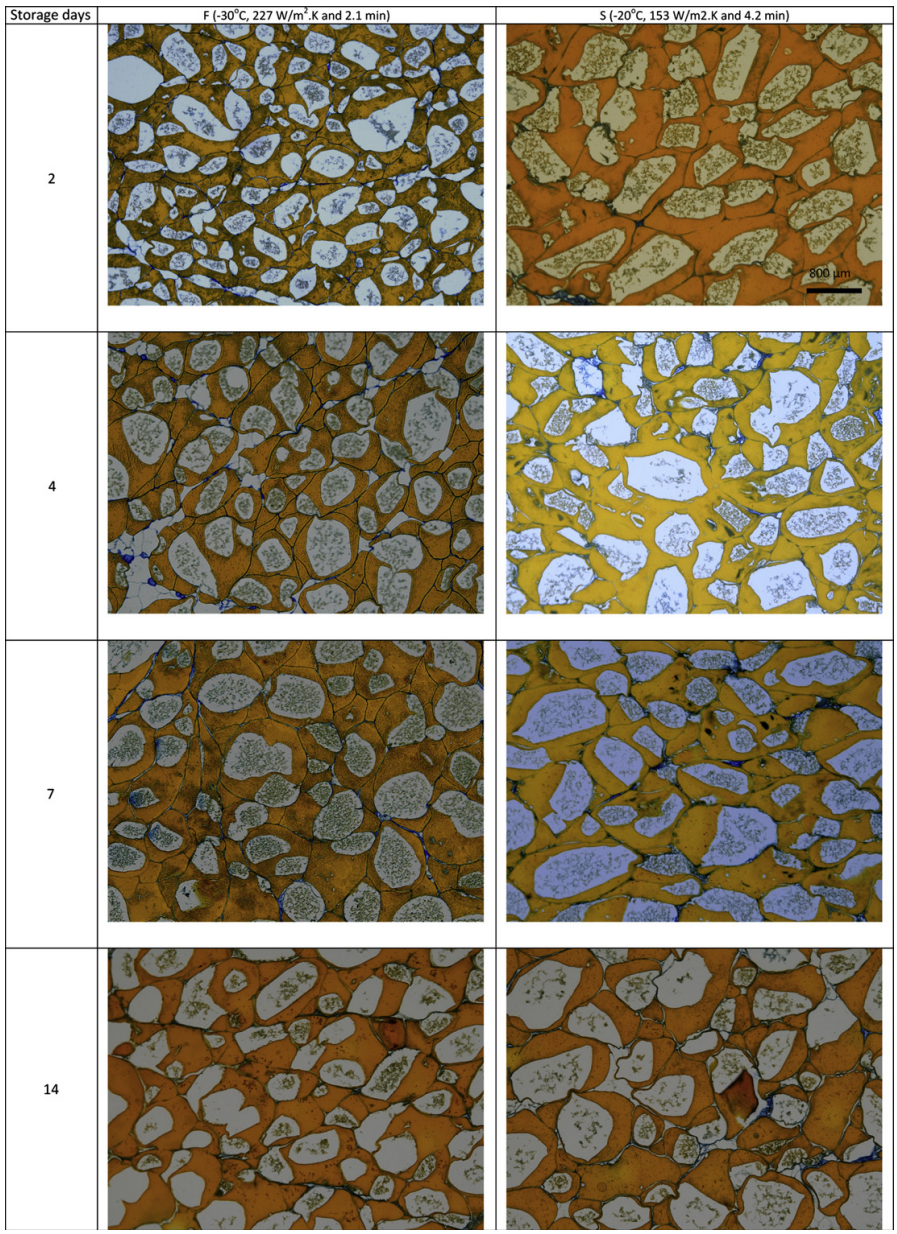

Fig. 5. Surface of salmon fillets after fast (F) or slow (S) superchilling and during −1.7 C storage.

2-stages: cooling the product to initial freezing point and removing the latent heat of crystallization, whereby about 20% of the water was frozen. These stages were mostly achieved on the surface of salmon fillet, approximately 2 mm from the surface of salmon fillet. The temperature at the midway center was about −0.7 °C, while temperature at the center of the sample was about +4.4 °C which was equal to the initial temperature of the sample before superchilling. In Fig. 2, the time–temperature profile during the storage of superchilled samples shows temperature equalization within one day of storage and was maintained at −1.7 ± 0.3 °C during storage.

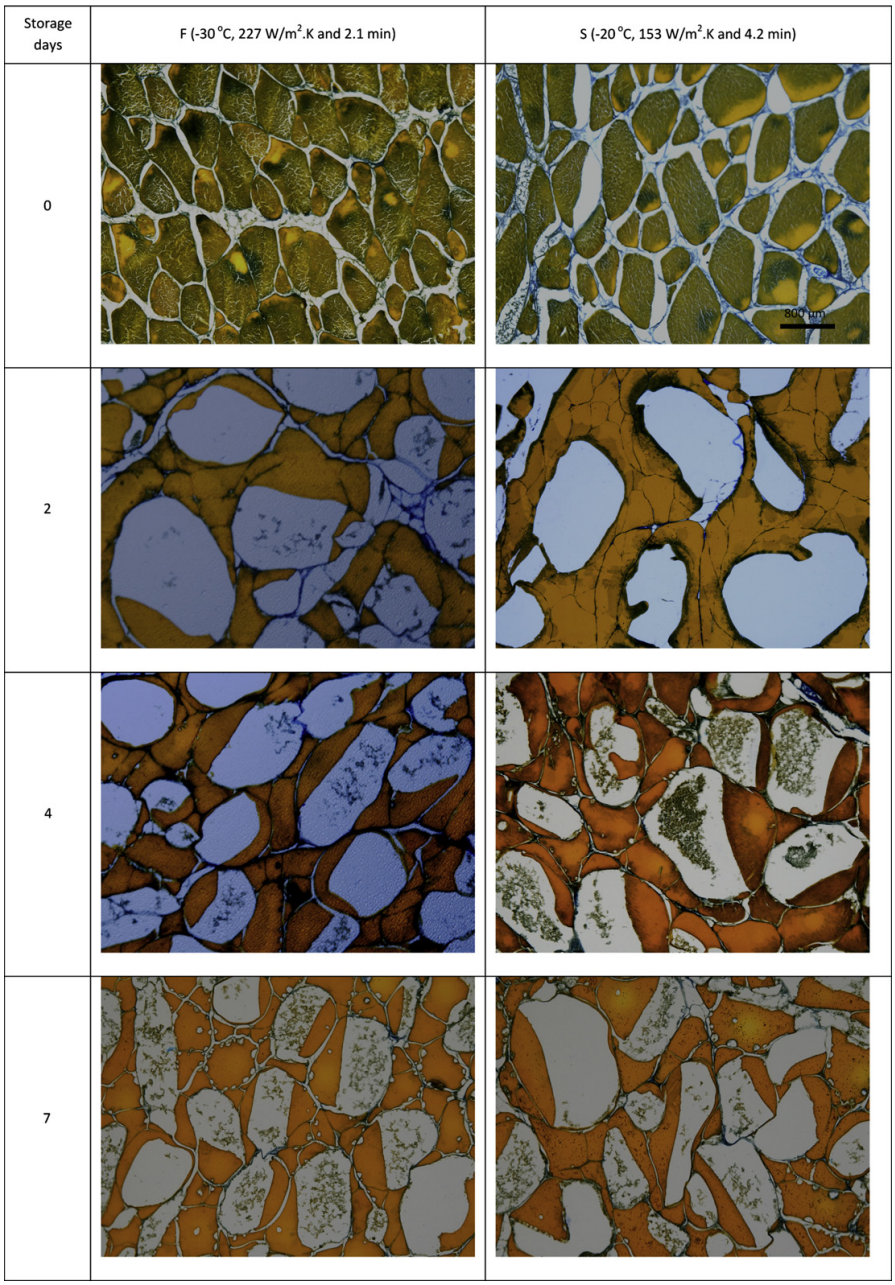

Fig. 6. Center of salmon fillets after fast (F) or slow (S) superchilling and during −1.7 C storage.

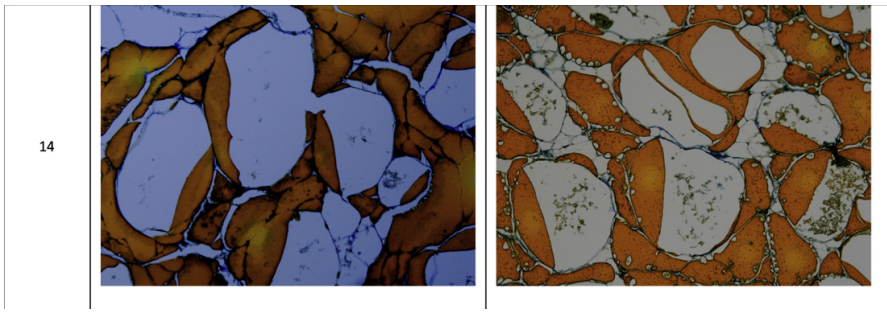

Fig. 6. (*continued*).

3.2. Ice crystal formation during the superchilling process

Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. The cross-section of the unprocessed sample exhibited a uniform distribution of regularly shaped fibers. The equivalent diameter of the muscle fibers was 95 ± 11 μm, which is close to the value reported by Zhu, Bail, and Ramaswamy (2003). The ice crystals formed during the slow superchilling process (S) appeared larger ($p < 0.05$) in size than those from process F (Table 1, Figs. 3 and 4). Treatment S is a slow superchilling process, which is generally considered to form large and mainly extracellular ice crystals. Since the salmon muscle was superchilled pre-rigor, large extracellular ice crystals were not observed. The intracellular ice crystals were formed regardless of superchilling rates. There was no significant difference ($p > 0.05$) between sizes of ice crystals formed pre-rigor compared to post-rigor after fast superchilling. Post-rigor fillets showed fewer ($p > 0.05$) intracellular ice crystals (Fig. 4) while larger and mainly extracellular ice crystals were formed in post-rigor fillets after slow superchilling. There was no significant difference ($p > 0.05$) between sizes of intracellular ice crystals formed pre-rigor compared to post-rigor after slow superchilling.

3.3. Ice crystal evolution at the surface layers during storage of superchilled samples

The evolution of the size of ice crystals formed is important during storage of superchilled products. Significant differences ($p < 0.05$) in ice crystal sizes during the superchilling process and following storage were observed. The equivalent diameter, (D_{eq}) for the outline of the microstructure sizes during storage of superchilled samples were 117 ± 2 and 93 ± 3 μm, for processes S and F, respectively. For the surface layers (Fig. 5), after only 2 days of storage, the ice crystals were 2 times larger than crystals at day 0 during process S and 4 times larger than crystals day 0 during process F. This is because the superchilling processes (S and F) were performed at a low temperature of −30 °C, or −20 °C and the samples were then stored at a higher temperature (superchilling storage temperature) of -1.7 ± 0.3 °C. These results are in agreement with that observed by Kaale et al. (2013), Kaale, Eikevik, Bardal, et al. (2013) and Kaale and Eikevik (2013).

Additionally, the superchilling process is a highly transient process that develops steep thermal gradients in the product near the surface. It is well known that a temperature gradient causes ice recrystallization during superchilled storage. Temperature gradients, whether large or small, will result in recrystallization during superchilled storage (Chevalier et al.,; Payne, Sandford, Harris, & Young, 1994). These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals. This process causes larger ice crystals to grow, resulting in a reduction in the number of ice crystals (Alizadeh et al., 2007; Russell, Cheney, & Wantling, 1999; Shenouda, 1980) at the surface layer of the superchilled salmon fillets during storage at -1.7 ± 0.3 °C. When temperature equalization was achieved within the samples, the growth of the intracellular ice crystals at the surface layer was not significant ($p < 0.05$).

3.4. Microstructure sizes at the center of the superchilled samples

Prior to temperature equalization, ice crystal growth progresses from the surface to the center of the superchilled food products. There was no significant difference ($p > 0.05$) between the ice crystals formed at the fillet centers for both processes. There was no ice crystals formed at the centers in both processes on day zero (Table 1 and Fig. 6). Therefore, the formation of ice crystals in both centers had the same temperature rate that resulted in the same size of ice crystals. The results further indicated that once temperature equalization was achieved within the samples, the growth of the intracellular ice crystals at the center was not significant ($P > 0.05$) during storage. The new crystals that are formed at the center during storage were large, due to the slow process, and could therefore damage the integrity of the superchilled product. In fish and meat tissues the destructive effect of ice crystal formation is minimized due to the elasticity of the cellular structure in muscle (Smith, 2011, chap. 11). Nevertheless, the loss of quality in fish and meat is associated largely with loss of functionality of proteins. When water forms ice, there is an increased concentration of enzymes and a build up of salt concentration in the remaining water, which may cause protein denaturation and affect the protein functionality (Shenouda, 1980; Smith, 2011, chap. 11). During superchilling, the protein denaturation may be minimal because only small amount of water is frozen (5–30%) which will result in less enzyme and salt concentration in the remaining water. Thus, superchilling results in extended shelf life of stored food compared to conventional chilling and better quality compared to freezing. However, despite the benefits of the superchilled storage of food products, there is still a need to study quality parameters separately at different locations within the superchilled product. The significant differences in ice crystal sizes found in this study may result in differences in quality at different locations within the superchilled product.

4. Conclusions

Smaller ice crystal formation was due to the faster superchilling process of pre-rigor Atlantic salmon fillets. Quality benefits may have been lost during -1.7 ± 0.3 °C storage due to recrystallization.

Drip loss, protein denaturation and water holding capacity need to be determined to completely evaluate Atlantic salmon fillet quality.

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Paper V

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A study of the ice crystals in vacuum-packed salmon fillets (*Salmon salar*) during superchilling process and following storage

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ABSTRACT

The aim of this work was to study the microstructure of vacuum-packed salmon fillets superchilled in an impingement freezer at −30 °C (air temperature) and 227 W/m^{2}K (surface heat transfer coefficient, SHTC) for 2.1 min prior to storage at a superchilling storage temperature of −1.7 ± 0.3 °C for 28 days. The microstructure of vacuum-packed salmon fillets were analysed at the surface, mid-centre and centre layers. Significant differences were observed between the ice crystals formed at the surface, mid-centre and centre layers. The size of ice crystals at the centre of the superchilled fillets was 3 times larger than those at the surface layer. Significant differences were observed between the size of ice crystals formed during the superchilling process and following storage. The results further indicated that, after temperature equalisation (1 day of storage) the growth of the intracellular ice crystal was not significant at ($P < 0.05$) at any storage time.

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1. Introduction

1.1. Degree of superchilling

The degree of superchilling (ice fraction) which represents the amount of water that is partially frozen (5–30%) inside the food products, is one of the most important parameters that define the quality of the finished product during superchilled storage. Bahuaud et al. (2008), Chevalier et al. (2001), Dincer, (1997), Fernández et al. (2008), Hagiwara et al. (2002), Kiani and Sun, (2011), Martino and Zaritzky, (1986), Martino et al. (1998); Petzold and Aguilera, (2009) found that the properties of the ice crystals, such as their size, distribution and shape, have a major influence on the quality of food products. Understanding these properties of ice crystals is critical not only for the quality control of superchilled foods but also for the proper design and development of superchilling/partial freeze-related processes.

1.2. Packaging during superchilled storage

Packaging is a particularly important element of the superchilled products. Packaging materials intended for superchilled/frozen food applications should meet minimum requirements, many of which have a direct influence on the quality of the superchilled/frozen food (George, 1998). Proper packaging like vacuum-packaging whereby the foods are sealed within a skin-tight package prevents dehydration and evaporative water loss from the surface of the food, and can minimize the effects of freezer burning (excessive hydration loss from the product surface) and postthaw exudates (drip loss) that often limit the quality and shelf life of partially frozen foods (Fernández et al., 2010; Pham and Mawson, 1997; Pornchai and Chitsiri, 2011). It is therefore important to understand that, packaging is one of the most important factors to consider during storage of superchilled food products.

There is, however, few published studies describing the size of ice crystals formed during superchilling process and the change of the microstructure of vacuum-packed foods during superchilled storage. The study of the size of ice crystals formed during superchilling process and the change of the microstructure on vacuum-packed products during superchilled storage should also be considered because these factors greatly affect the textural quality and physical properties of superchilled foods. For example, the growth of ice crystals during storage of superchilled products may be related to protein denaturation, low water holding capacity or enzymatic degredation. Therefore, the objectives of this work were to analyse the ice crystals in salmon fillets after the superchilling process and to assess the change in microstructure of vacuum-packed salmon fillets during storage of superchilled products.

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2. Materials and methods

2.1. Materials and superchilling process

Salmon fillets (*Salmon salar*) 0.9–1 kg, were delivered by Lerøy Midnor (Hitra, Norway). The samples were vacuum packed and stored at 4 °C for 24 h before the superchilling process to ensure a constant temperature in all samples. Superchilling was performed in an Impingement Advantec Lab Freezer (JBT Food – tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at −30 °C and 227 W/m^2 K (at 2.5 kPa pressure differences of the fan at the impingement freezer)) for 2.1 min to achieve an ice content of 20%. The previous developed model (Kaale et al., 2012) was used to predict the degree of superchilling. The experiments for measuring surface heat transfer coefficient (SHTC) value were performed in an Impingement Advantec Lab Freezer. Details on experiments set up and equation used to calculate SHTC explained elsewhere (Kaale et al., 2012). Once superchilled, the salmon samples were stored in a cold room at −1.7 ± 0.3 °C for 28 days. Three fillets were used in each daily analysis.

2.2. Temperature trend during superchilling process and following storage

The temperature was measured at three different locations on the samples during the superchilling process: the surface, mid centre and the centre. Three thermocouples were used at each location (3 – surface, 3 – mid centre and 3 – centre). The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the sample were recorded every 4 s. The thickness of each sample was approximately 26 mm. The temperature was measured by inserting thermocouples approximately 2 mm from the surface, 7 mm from the surface (midway to the centre) and 13 mm from the surface (centre).

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensor were inserted in the storage box: one was used to measure the air temperature, and the other two were used to measure the surface and centre temperatures of the superchilled salmon fillets. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately −5 °C (temperature outside the storage box).

2.3. Microscopic analysis

Four pieces were cut from the surface layer to the centre layer of each superchilled sample (−1.7 ± 0.3 °C) transversally to the muscle fibre using a standard knife blade that was previously stored at −1.7 ± 0.3 °C. This procedure was conducted in a walk-in freezer to ensure a perfect cold chain. In this study, a fixation method similar to those proposed by Alizadeh et al. (2007), Martino and Zaritzky, (1988) was used to observe the spaces left by the ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The control (unprocessed) samples were fixed with the same solution but at 4 °C. The fixed samples were then brought to room temperature and were dehydrated with absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fibre using a microtome (Autocut 2055, Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Tissue Clear was used for rehydration, the samples were immersed in 1% blue aniline for 1 min, and xylene was used before mounting.

All the prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan). The images of the slides were recorded and treated using the stereological analysis program CAST2 (Olympus Inc., Denmark). Two parameters, namely the cross-sectional area and the equivalent diameter, were used in the evaluation. The cross-sectional area refers to the surface area of the cross-section of an object (ice crystal or fibre muscle). The equivalent diameter for each ice crystal is defined as the diameter of a circle having the equivalent area S_p. From the data set of each equivalent diameter, the mean crystal diameter, D_{eq}, was calculated. All analyses were done for the 9 different specimens (three-surface layer, three – mid centre layer and three – centre layer) per fillet. For each case considered, more than 100 incidences of ice crystals were evaluated.

2.4. Statistical analysis

The observations of the ice crystal size at different locations with respect to storage days were analysed by one- and two-way analyses of variance using Minitab 16 software. A general linear model, (post-hoc test) under Tukey's simultaneously test was applied whenever the ANOVA results were significant. Data were expressed as the mean ± standard deviation, and the statistical significance of each experiment was $p < 0.05$.

3. Results

3.1. Ice crystal evolution during superchilling process

The superchilling process was done at high rate which allowed removing enough heat to produce fine crystals that were evenly distributed both inside and outside the cells. Fig. 1 shows the temperature–time profile and the thermal gradients developed during superchilling process. The initial freezing point was −1.1 °C, which

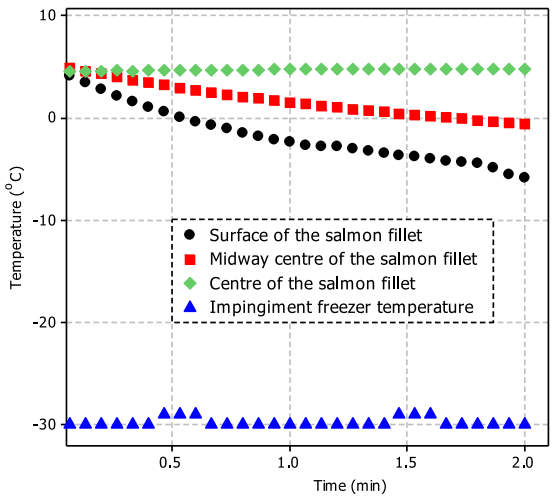

Fig. 1. Temperature–time profile: surface, mid centre and centre layers during superchilling process.

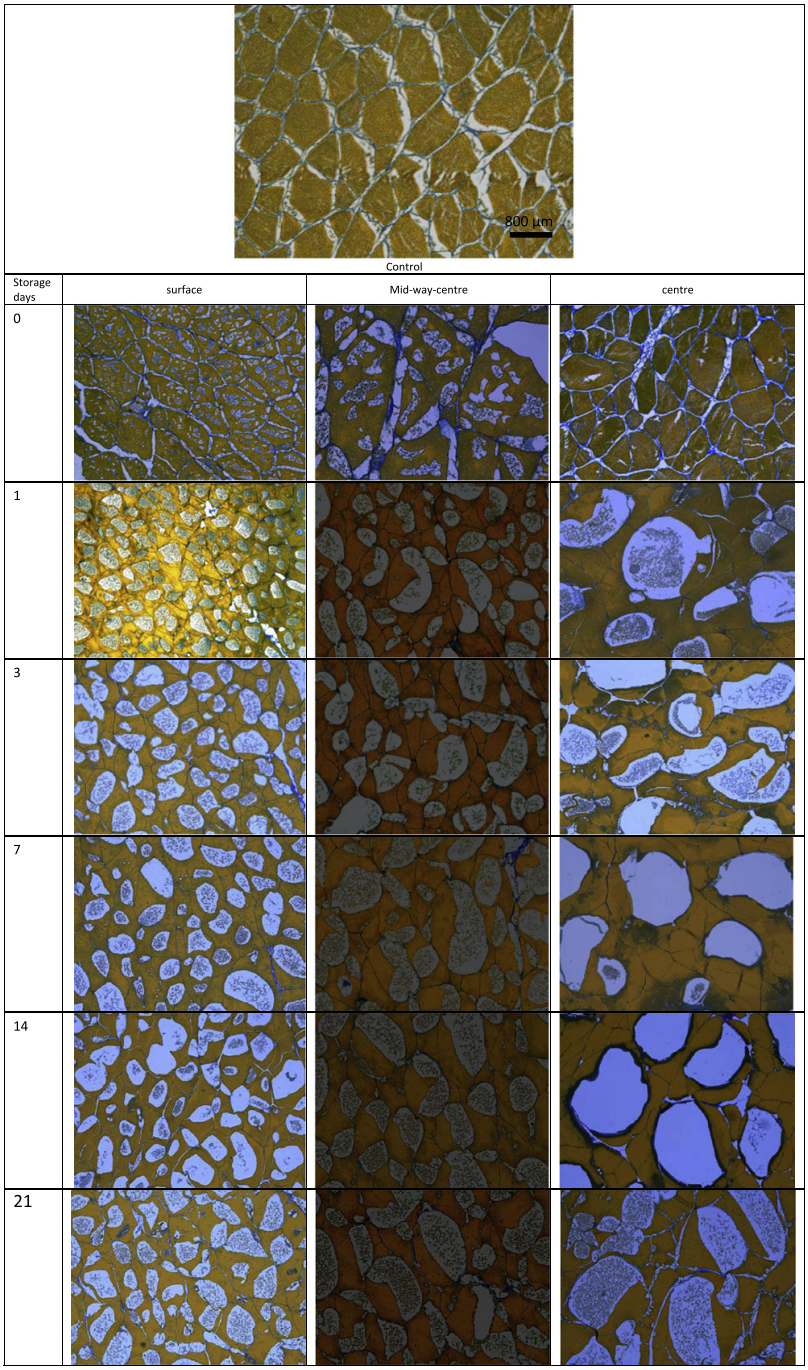

Fig. 2. Micrographs of unsuperchilled and superchilled salmon tissues: surface mid centre and centre layers.

was indicated by the beginning of the freezing plateau at the centre of the sample. The initial freezing point of the salmon was determined using separate samples that were totally frozen in the impingement freezer for approximately 30 min, and these samples were not used for any other analysis. The present work explained how these thermal gradients created ice crystals with different size in salmon fillets (Fig. 2). The microstructure of vacuum-packed salmon fillets was investigated at the surface, mid centre and centre

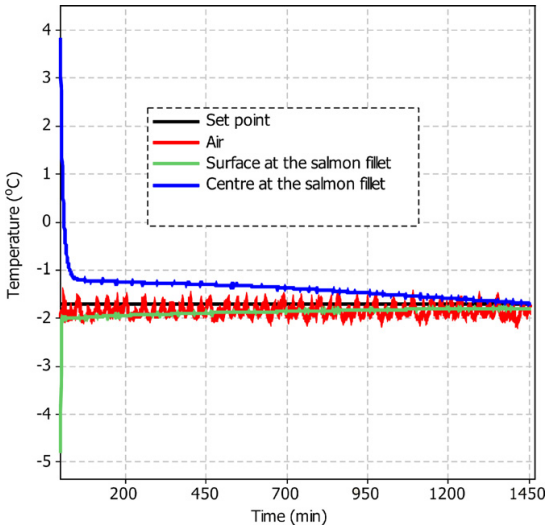

Fig. 3. Temperature–time profile during storage of superchilled samples.

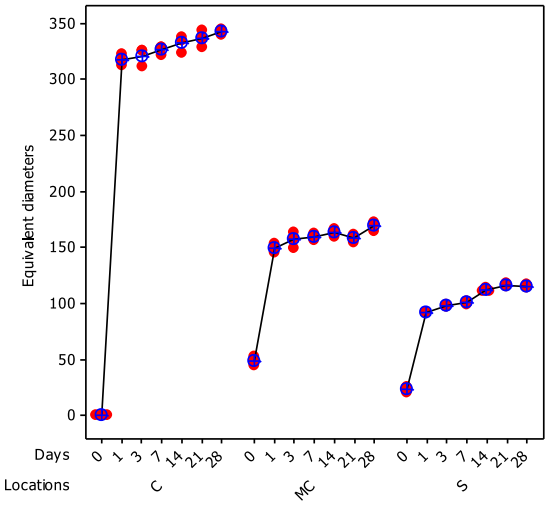

Fig. 4. Boxplot showing equivalent diameters of the ice crystals versa storage days: surface, mid centre and centre layers.

layers. The macrographs of salmon fillet samples are shown in Fig. 2. Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. The cross-section of the unprocessed sample showed a uniform distribution of regularly shape fibres. The equivalent diameter of muscle fibres was 96 ± 9 μm, which is close to the value reported by Zhu et al. (2003).

3.2. Ice crystal evolution during storage of superchilled samples

Significant differences in ice crystal sizes during the superchilling process and following storage were observed here, as in our previous study (Kaale and Eikevik, 2012) (Figs. 2, 4 and 5 and Table 1). Table 1 summarises the results of the ice crystal sizes during superchilling process and storage of superchilled samples. The

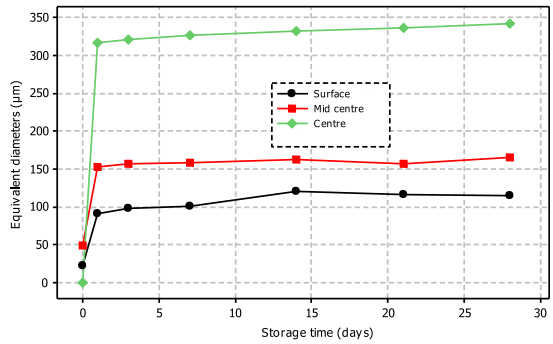

Fig. 5. Equivalent diameters of the ice crystals vs time in linear scale: surface, mid centre, and centre.

equivalent diameter, D_{eq}, for the outline of the ice crystal size during storage of superchilled samples was 92 ± 0.3 μm, at the surface layer after only one day of storage, which means 4 times larger than crystals during superchilling process Table 1. This is because the superchilling process was performed at a very low temperature of −30 °C, and the samples were then stored at a higher temperature (superchilling storage temperature) of −1.7 ± 0.3 °C. Additionally, superchilling process is a highly transient process that develops steep thermal gradients in the product near the surface. It is well known that a temperature gradient causes ice recrystallization during superchilled storage. Temperature gradients, whether large or small, will result in recrystallization during superchilled storage (Chevalier et al., 2001; Payne et al., 1994). These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals. This process causes larger ice crystals to grow, which results in a reduction in the number of ice crystals (Alizadeh et al., 2007; Russell et al., 1999; Shenouda, 1980) at the surface layer of the superchilled salmon. Results showed that after one day of storage, when temperature equalisation was achieved within the samples, the growth of the intracellular ice crystals at the surface layer was not significant ($P < 0.05$) at any storage times (Fig. 2 and Table 1). In Fig. 3, the profile shows the temperature over one day of storage (24 h); the temperature was maintained at −1.7 ± 0.3 °C for the other days of storage.

3.3. Superchilling process (partial freezing) and ice crystal size at the surface layer of the superchilled samples

Superchilling process (partial freezing) is the preservation process that depends on the amount of water which is frozen (5–30%) inside the food product. The degree of superchilling 5–30% is low enough that makes the products taste just like fresh food. During this process, an even ice zone was formed to a depth of about 2 mm (Fig. 2). It has been reported that superchilling/freezing rates influence the size, distribution and location of the ice crystals within the food product (Chevalier et al., 2001). These characteristics of ice crystals (size, distribution and location) have big influence on food quality such as protein functionality, water holding capacity and texture. Texture is one of the critical quality factor influenced by freezing/partial freezing (Kerr et al., 2004). A rapid superchilling, small and mostly intercellular ice crystal is formed within the product. This will lead to a superchilled product with a smooth texture when consumed in a partial frozen state (Heldman and Hartel, 1999), high water holding capacity and protein functionality. There are a variety of other quality attributes such as pigments establishing product colour, flavours and nutrients which are not influenced much during partial freezing process but may be influ-

Table 1
Equivalent diameters of the ice crystals (Mean ± standard deviation) μm during superchilling: surface and centre layers.

Storage time (days)	Superchilling process (-30 °C, 227 W/m^2.K and 2.1 min)		
	surface	mid centre	centre
0	23 ± 2.9	49 ± 4	No ice crystals
1	92 ± 0.3	153 ± 4	318 ± 5
3	98 ± 0.9	157 ± 7	321 ± 8
7	101 ± 1.4	159 ± 3	327 ± 4
14	112 ± 1.4	163 ± 2	333 ± 7
21	117 ± 1.3	158 ± 3	337 ± 8
28	116 ± 1.2	166 ± 4	343 ± 3

enced by conditions maintained during storage of the partial frozen food. On the other hand, slow superchilling/freezing rate large and extracellular ice crystals is formed which generally result in disrupt cell and cause the loss of product structure that does not recover when thawed (Heldman and Hartel, 1999; Zhu et al., 2003; Shenouda, 1980). In this study, the size of the ice crystals which were observed at the surface layer of the superchilled food did not observed to destroy the integrity of the muscle both during the superchilling process and following storage because the size of the ice crystals 23 ± 2.9 and 92 ± 0.3 in days 0 and1, respectively were smaller than the size of the muscle tissue, 96 ± 11 μm.

3.4. Ice crystal size at the mid centre and centre of the superchilled samples

The present study showed that, prior to temperature equalisation, ice crystal growth progresses from the surface to the centre of the superchilled food products. This is due to temperature gradients during superchilling process. Results indicated that the ice crystals at the mid centre and centre were 153 ± 8 and 318 ± 4 μm, respectively after only one day of storage. The ice crystals at the centre were 3 times larger than the crystals at the surface layer (Fig. 4 and Table 1). In addition to temperature gradients, during superchilling processes there was no ice crystals formed at the mid centre and centre of the superchilled samples (Figs. 1 and 2). The formation of the ice crystals at the mid centre and centre took place during the storage of the superchilled samples whereby the superchilling rate was so low .i.e. at −1.7 ± 0.3 °C which result in large size of the ice crystals. The results further indicated that once the temperature equalisation was achieved within the samples, the growth of the intracellular ice crystals at the centre was not significant ($P < 0.05$) at any storage time. Fig. 5, equivalent diameters vs. time in linear scale. This Figure clearly showing that after temperature equalisation there was no significant growth of the ice crystals Fig. 4.

The large ice crystals at the centre of the product may have a major effect on morphological changes and cell destruction (Bahuaud et al., 2008), which may result in microstructure changes during storage of sueprchilled foods. However, the quality and shelf life of superchilled foods has been reviewed by Kaale et al. (2011), and many benefits of applying superchilling technology to food products were found compared to chilled and frozen food products. This may be due to, in fish and meat the destructive effect of ice crystal formation is minimised due to the elasticity of the cellular structure in muscle (Smith, 2011). Furthermore, the loss of quality in fish and meat is associated largely with loss of functionality of proteins. When water form ice, there is an increased concentration of enzymes and builds up of salt concentration in the remaining water, which both causes protein denaturation and therefore effect the protein functionality (George, 1993; Shenouda, 1980; Smith, 2011). In superchilling the protein denaturation may be minimal because only small amount of water is frozen 5–30% which will results in less enzyme and salt concentration in the remaining water. It has also been reported by George (1993) that, the superchilling temperature range is cold enough to suppress bacterial growth and enzymatic but does not cause cellular damage. However, despite the benefits of the superchilled storage of food products, there is still a need to test the quality parameters separately at different locations within the superchilled product. It is unlikely that there is similar quality at both the surface and the centre of the superchilled product due to the significant differences of the microstructure sizes found in this study.

3.5. Factors to consider during superchilling process and following storage

In order to obtain high-quality superchilled foods, high-quality raw materials are necessary, and processing, distribution and storage must be carefully controlled (George, 1993). The quality of superchilled fish or meat is particularly affected by the loss of moisture during superchilling process, and even if the factors influencing the quantity of drip losses are numerous, properties of ice crystals such as shape, size, and distribution are most important in determining textural and physical properties of many superchilled products (Chevalier et al., 2001; Hagiwara et al., 2002). In this study small and well distribution of the ice crystals both inside and outside the cells were observed which can be concluded that the fish muscles were not destroyed or the destruction was minimized during shell freezing (superchilling process). It is also important to control temperature fluctuation which is often unavoidable during storage of superchilled products. This will assist to minimize recrystallisation during storage of the superchilled samples. Temperature fluctuation can be controlled under laboratory conditions, but under industrial conditions can be difficult. In this study temperature was strictly controlled at −1.7 ± 0.3 °C (Fig. 3). This has been proved during the analysis of the ice crystals whereby after temperature equalisation within the samples there was no significant growth of the ice crystal at any storage time. In additional to process and control of temperature during storage of the superchilled products, packaging is also one of the most important factors to consider during superchilled storage. The main purpose of packaging like vacuum-packaging whereby the foods are sealed within a skin-tight package is to keep food from drying out, to preserve nutritive value, taste, flavor, texture and colour and to prevent freezer burn and oxidation, where air or oxygen comes into contact with food (Pham and Mawson, 1997; Pornchai and Chitsiri, 2011). However, none of these parameters were analysed in this study apart from observation of freezer burn in the samples. Freezer burn as one of the main causes of deterioration during storage of superchilled products (Kolbed and Kramer, 2007) occurs through a process called sublimation (Goff, 1995) whereby the evaporation of the ice crystals during storage occurred resulting in brown spots on the surface of the food products (excessive hydration loss from the product surface). This is a visible effect of severe dehydration on the surface of the fish. The spots cause the tissue to become dry and tough and are very likely to develop off-flavours (Barbosa-Cánovas et al., 2005). In this study the samples did not exhibit any concerning features related to freezer burn during storage. To summarize, a combination of high-quality raw materials, good superchilling process, stable temperature during storage of the superchilled products and good packaging will result in high-quality superchilled foods.

4. Conclusions

The microstructure of vacuum-packed salmon fillets were analysed at the surface, mid-centre and centre layers. Significant

differences were observed between the ice crystals formed at the surface, mid-centre and centre layers. The size of ice crystals at the centre of the superchilled fillets was 3 times larger than those at the surface layer. Significant differences were observed between the size of ice crystals formed during the superchilling process and following storage. The results further indicated that, after temperature equalisation (1 day of storage) the growth of the intracellular ice crystal was not significant at ($P < 0.05$) at any storage time.

Further studies should be carried out to test the quality parameters, such as drip loss, water holding capacity, protein denaturation and enzymatic degradation. These parameters should be evaluated at both the surface and centre layers of the superchilled product due to the size differences in the ice crystals at each location, as observed in this study.

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Paper VI

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A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (*Salmo salar*) fillets during superchilling process and storage

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ABSTRACT

Atlantic salmon (*Salmo salar*) fillets were partial frozen in an impingement freezer at −30 °C and 227 W/m2.K for 2.1 min prior to storage at a superchilling storage temperature of −1.7 ± 0.3 °C for 28 days. The aim of this article is to study the microstructure of the red and white muscles during superchilling process and during superchilled storage. The histology and microscopic analysis of the red and white muscles were carried out. It was found that the size of the ice crystals formed in the red muscles was smaller than those formed in the white muscles. The equivalent diameters of the intracellular ice crystals obtained upon superchilling (day 0) were 17 ± 2 and 29 ± 1 μm for the red and white muscles, respectively. Significant differences were initially observed between the size of the ice crystals formed during the superchilling process and after 1 day of storage. However, after temperature equalisation (day 1), there was no significant change in the size of the ice crystals.

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1. Introduction

The superchilling process (partial/shell freezing) is simply the partial crystallisation of ice in muscle tissue and involves the consecutive processes of nucleation and growth. The superchilling/partial-freezing process has two stages: (1) cooling the product to initial freezing point and (2) removing the latent heat of crystallisation (phase transition stage), whereby 5–30% of the water is frozen and stored within the product. During this process, a thin frozen layer of about 1–3 mm thick is achieved on the surface of food product depending on degree of superchilling required. Degree of superchilling (ice fraction) is amount of water (5–30%) which is frozen inside the food product, is one of the most important parameters which define the quality of the superchilled food product (Magnussen et al., 2008; Stevik and Claussen, 2011; Stevik et al., 2010). The degree of superchilling, 5–30% is low enough that makes the products taste just like fresh food. It has been reported that a degree of superchilling between 5% and 30% is accepted and that a degree of superchilling more than 30% will cause higher drip loss in food products (Stevik and Claussen, 2011). Superchilling storage is when the partially frozen food product is stored at 1–1.5 °C below its initial freezing point.

Different researchers have been defined/performed superchilling of foods in different ways: Ando et al. (2004) defines it as the temperature zone below 0 °C but where ice crystals are not generated. Beaufort et al. (2009) defined superchilling as a technology where food is stored just below the initial freezing temperature (superchilled storage of foods without shell/partial freezing). Duun and Rustad (2008), Stevik et al. (2010), and Bahuaud et al. (2008) have been performed superchilling by doing shell/partial freezing of food products followed by temperature equalisation during the superchilled storage. Recent research has shown that, this process previously thought to be negative to food products, but now is actually a viable way to rapidly cool food products, provided the freezing is extremely fast and even. In fact, using the impingement technique to shellfreeze food products can result in better production yields, improved product quality and a longer shelf life than was possible using old-fashioned bulk storage rooms and traditional fast-cooling systems (Goransson and Londahl, 2005). The impingement freezer is a tunnel freezing system which employs a multiple high velocity refrigerated air impingement jets to quick freeze food products. Another advantage of doing shell freezing of fresh foods is that the ice formed on the surface of the product acts as an internal ice reservoir during storage and transportation. During storage, the ice distribution equalises and the product obtains a uniform temperature at which it is maintained during storage and distribution. This will provide the food product an internal ice reservoir so that no external ice is required during transportation or storage (Kaale et al., 2011).

Superchilling is an efficient food preservation process because water in the partially frozen state is immobilised as ice, and the

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rates of deterioration are much slower at lower temperatures than at higher temperatures (Do et al., 2004; George, 1993). Food products are multicomponent systems of uneven properties containing many substances; water is the most abundant component (50–95%), and it exists in different forms in tissues (Dincer, 1997; Do et al., 2004; Kiani and Sun, 2011). The superchilling of food products entails the conversion of a minor part of this water (5–30%) into ice crystals. It is the formation of these ice crystals that affects the quality of superchilled (partially frozen) food products. The formation of fine crystals that are evenly distributed both inside and outside the cells leads to better preservation of the quality of the product due to less tissue damage (Chevalier et al., 2001; Dincer, 1997; Fernandez et al., 2008; Kiani and Sun, 2011; Martino and Zaritzky, 1986; Martino et al., 1998; Petzold et al., 2009).

Fish muscle has a unique arrangement of muscle fibres. It is divided into a number of segments called myotomes, which are separated from one another by a sheath of connective tissue called the myocomma (Jiang and Lee, 2007). There are two major types of fish skeletal muscles: red and white. The red muscle lies along the side of the body next to the skin, particularly along the lateral lines, and may comprise up to 30% of the fish muscle, depending on the species (Ayala et al., 2005; George, 1962; Jiang and Lee, 2007; Rabah, 2005). Kiessling et al. (2006) reported that Atlantic salmon was comprised of 10% red muscle and 90–95% white muscle. Red muscle is usually slow, with low contractive power, and is used for prolonged activity sustained by aerobic metabolism. White muscle is faster, more powerful, and capable of bursts of activity that may be anaerobic (Lindsey, 1978).

Studies on muscle fibres have been conducted over the years. Muscle fibre diameter has been reported to be an important factor in the textural characterisation of the flesh (Ayala et al., 2005; Johnston et al., 2000a, b). There is a direct relationship between average muscle fibre size and the firmness of the raw flesh, such that species with a firmer texture have relatively smaller fibres than species with a softer texture (Ayala et al., 2005). George (1962) found that the size of the white muscle in mackerel is two times larger than that of the red muscle fibres. However, the microstructure sizes of the two types of muscles subjected to the superchilling process and storage have not been studied yet. Studies of the microstructure sizes of the red and white muscles during superchilling process and storage are important in the determination of a better scientific basis for the evaluation of chilling methods and a comparison of these technologies. Thus, the present work is focused on describing the microstructure sizes of the red and white muscles during the superchilling process and superchilled storage.

2. Materials and methods

2.1. Materials and superchilling processes

Salmon fillets (0.9–1 kg) were delivered by Lerøy Midnor (Hitra, Norway). The samples were taken at the middle part of the salmon fillets. The samples were cut from the top side to the bottom side of the salmon fillet in order to obtain samples that possessed both red and white muscles. The salmon fillets were vacuum packed and stored at 4 °C for 24 h before the superchilling process to ensure a constant temperature in all samples. Shell freezing was performed in an Impingement Advantec Lab Freezer (JBT Food – tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at −30 °C and 227 W/m2.K for 2.1 min to achieve a degree of superchilling (ice content) of 20%. The previous developed model (Kaale et al., 2012) was used to predict the degree of superchilling. The experiments for measuring surface heat transfer coefficient (SHTC) value were performed in an Impingement Advantec Lab Freezer. Details on experiments set up and equation used to calculate SHTC explained elsewhere (Kaale et al., 2012). Once partial frozen, the salmon samples were stored in a cold room at −1.7 ± 0.3 °C for 28 days. Three fillets were used in each daily analysis.

2.2. Measurement of the temperature during the superchilling process and superchilled storage

The temperature was measured at three different locations on the samples during the superchilling process: the surface, midway to the centre and the centre. Three thermocouples were used at each location (3 – surface, 3 – midway centre and 3 – centre). The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the sample were recorded every 4 s. The thickness of each sample was approximately 26 mm. The temperature was measured by inserting thermocouples approximately 2 mm from the surface, 7 mm from the surface (midway to the centre) and 13 mm from the surface (centre).

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted in the storage box: one was used to measure the air temperature, and the other two were used to measure the surface and centre temperatures of the superchilled salmon fillets. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately −5 °C (temperature outside the storage box).

2.3. Microscopic analysis

Four pieces were cut from the centre of each superchilled sample (−1.7 ± 0.3 °C) transversally to the muscle fibre using a standard knife blade that was previously stored at −1.7 ± 0.3 °C. This procedure was conducted in a walk-in freezer to ensure an intact cold chain. In this study, a fixation method similar to those proposed by Alizadeh et al. (2007) and Martino and Zaritzky (1988) was used to observe the spaces left by the ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The control (unsuperchilled) samples were fixed with the same solution but at 4 °C. The fixed samples were then brought to room temperature and were dehydrated with absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fibre using a microtome (Autocut 2055, Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Tissue Clear was used for rehydration, the samples were immersed in 1% blue aniline for 1 min, and xylene was used before mounting.

All the prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan). The images of the slides were recorded and treated using the stereological analysis program CAST2 (Olympus Inc., Denmark). Two parameters, namely the cross-sectional area and the equivalent diameter, were used in the evaluation. The cross-sectional area refers to the surface area of the cross-section of an object (ice crystal or fibre muscle). The equivalent diameter for each ice crystal is defined as the diameter of a circle having the equivalent area S_p. From the data set of each equivalent diameter, the mean crystal diameter, D_{eq}, was calculated. All analyses were performed for six different specimens (three red – surface and three white – surface samples) of each

fillet. For each case considered, more than 100 incidences of ice crystals were evaluated.

2.4. Statistical analysis

The observations of the microstructure sizes of the red and white muscles with respect to storage days were determined by one- and two-way analyses of variance using Minitab 16 software. Data were expressed as the mean ± standard deviation, and the statistical significance of each experiment was $p < 0.05$.

3. Results

3.1. Thermal transition behaviour of superchilling process

Fig. 1 is showing time–temperature profile of the superchilling process at −30 °C for 2.1 min. In this study, the surface part of the salmon fillet will be considered because the red muscle is situated at the surface (lies along the side of the body next to the skin) of the salmon fillet. The superchilling time–temperature profile consists of two stages: cooling the product to initial freezing point and phase change whereby about 20% of water was frozen. These stages were achieved on the surface of salmon fillet, approximately 2 mm from the surface of salmon fillet. The temperature at the centre was about +3.8 °C which were high than initial freezing point of salmon. This shows that, during the superchilling process there were no ice crystals formed at the centre of the salmon fillet. Fig. 2 shows the time–temperature profile during superchilled storage. The profile shows that, the temperature equalisation was achieved within 1 day of storage (24 h); the temperature was maintained at −1.7 ± 0.3 °C for the other days of storage (Figs. 1 and 2).

3.2. Ice crystallisation during superchilling process and storage

The superchilling process was performed at a high rate that was able to remove enough heat to produce fine crystals that were evenly distributed both inside and outside the cells. During this process, an even ice zone was formed to a depth of about 2 mm (Fig. 3). The microstructure sizes of the red and white muscle fibres

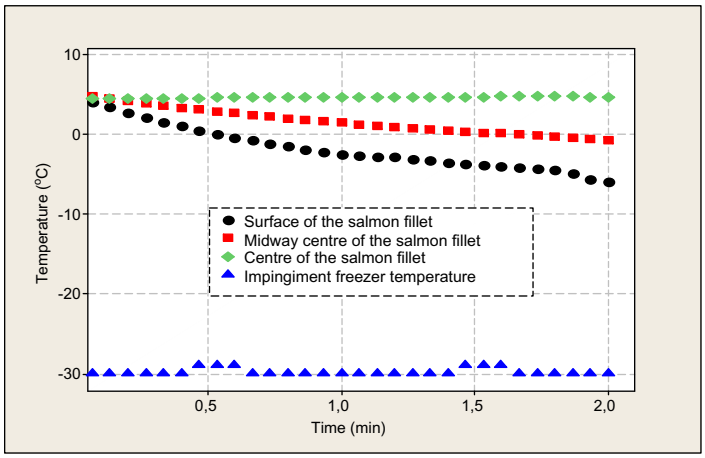

Fig. 1. Time–temperature profile at different locations during superchilling process.

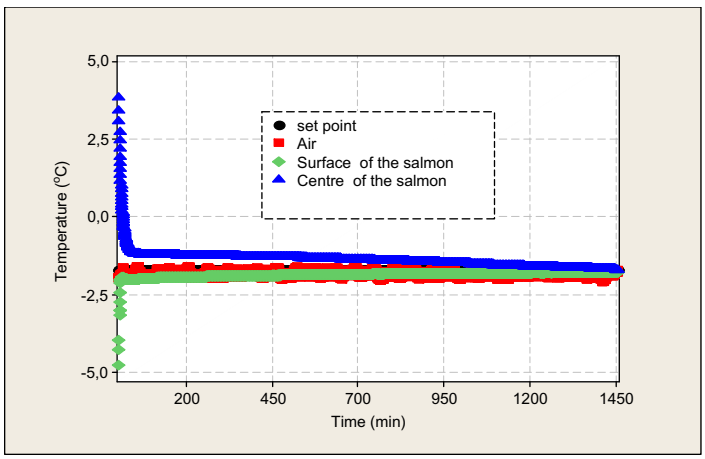

Fig. 2. Time–temperature profile during superchilled storage.

at the surface of the salmon fillets were analysed. Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the partial frozen samples. The cross-section of the unprocessed sample showed a uniform distribution of regularly shaped fibres. The red muscle, concentrated along the lateral line, consisted of narrow fibres with an average diameter of 53 ± 9 μm, whereas the white muscle on either side had broad fibres with an average diameter of 96 ± 9 μm, which is close to the value reported by Kiessling et al. (2006). Rabah (2005) reported that the red muscles in salmon are characterised by smaller fibre diameters, high capillary density and a profusion of lipid droplets. On the other hand, the white

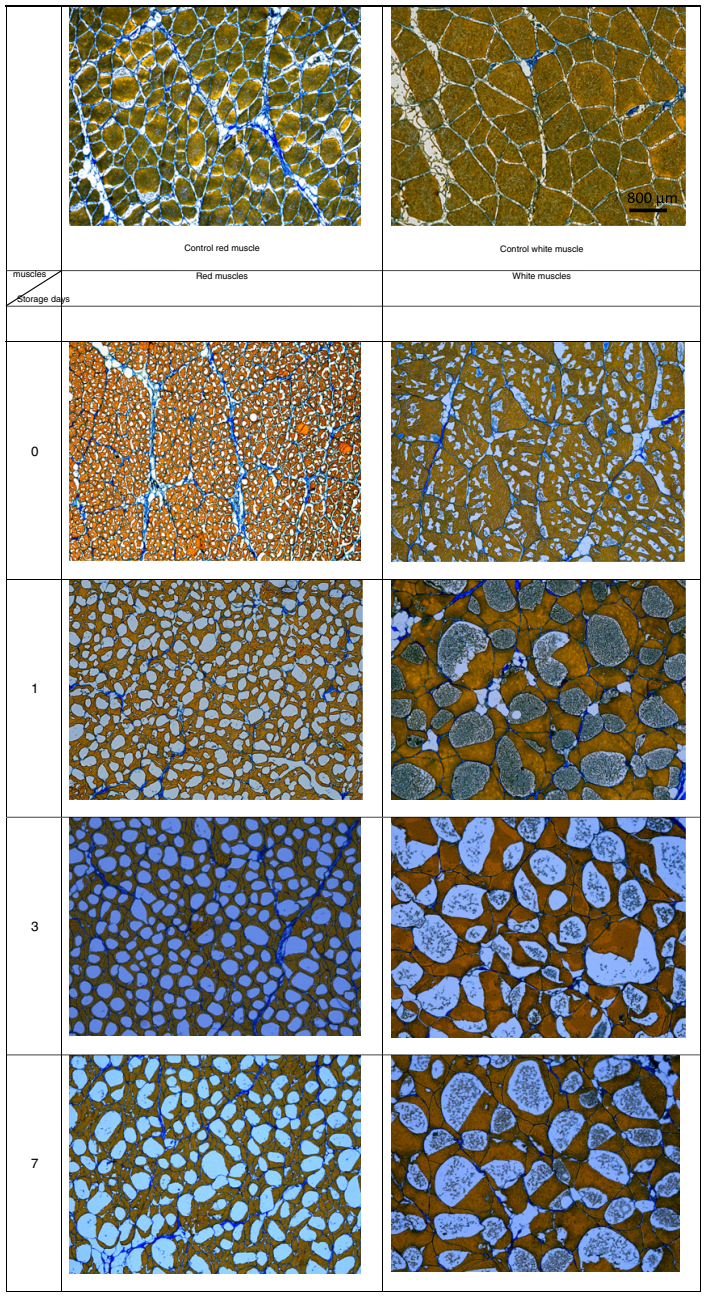

Fig. 3. Micrographs of unsuperchilled and superchilled red and white of the salmon muscles: bottom surface layers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

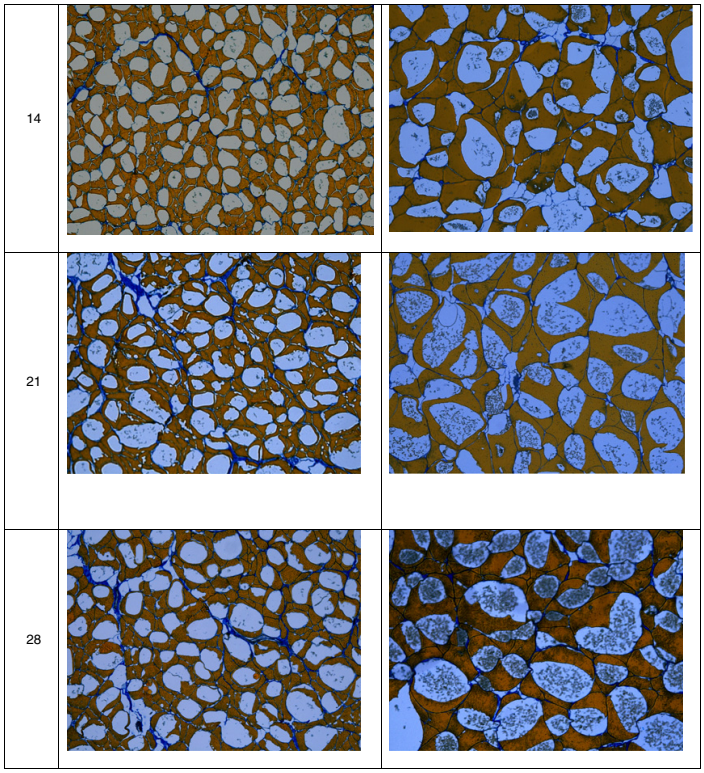

Fig. 3. (*continued*)

muscles had larger fibre diameters and few capillaries. A histophysiological study of the red and white muscles of mackerel (George, 1962) showed that the red muscle consisted of narrow fibres with an average diameter 32 μm and white muscle of broad fibres of 65 μm.

The evolution of the microstructure sizes of the red and white muscles of food products is important during the superchilling process and during superchilled storage. The information about the microstructure size will give industrial food technologists necessary scientific information on the handling, processing and storage conditions of different types of food products. During the evaluation of the ice crystals, the results showed that the size of the ice crystals formed in the red muscle during the superchilling process (day 0) was smaller than those formed in the white muscle, with average equivalent diameters of 17 ± 2 μm and 29 ± 1 μm, respectively (Fig. 3 and Table 1).

The size of the ice crystals increased significantly after 1 day of superchilled storage. This is due to two important factors during superchilled storage: Firstly, the large difference between the superchilling process temperature (−30 °C) and the superchilled storage temperature (−1.7 ± 0.3 °C). This large temperature difference will cause the growth of the ice crystals, particularly the small ones that were formed at the surface of the salmon fillets. Secondly, the thermal gradient effect that was created during the superchilling process. It is well known that a temperature gradient causes ice recrystallization during superchilled storage (Payne et al.,1994). Temperature gradients, whether large or small, will result in recrystallization during superchilled storage. These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals. This process causes larger ice crystals to grow, which results in a reduction in the number of ice crystals at the surface layer of the superchilled salmon. Interestingly, this large and rapid change of temperature between day 0 and day 1 of storage resulted in equivalent diameters of the intracellular ice crystals of 52 ± 1 in red muscles, which is half the equivalent diameter in white muscles, 105 ± 9 μm (Table 1). Additionally, the ice crystals in the red muscles seemed to be well arranged and more spherical than those in the white muscles, as may be seen in Fig. 3. Results showed that after 1 day of storage, when tempera-

Table 1
Equivalent diameters of the ice crystals (Mean ± standard deviation) of the red and white muscle during superchilling.

Muscles	Storage days						
	0	1	3	7	14	21	28
Red muscle surface	17 ± 2	52 ± 1	55 ± 3	58 ± 2	60 ± 5	62 ± 4	63 ± 2
White muscle surface	29 ± 1	105 ± 9	116 ± 8	121 ± 2	129 ± 5	133 ± 3	134 ± 4

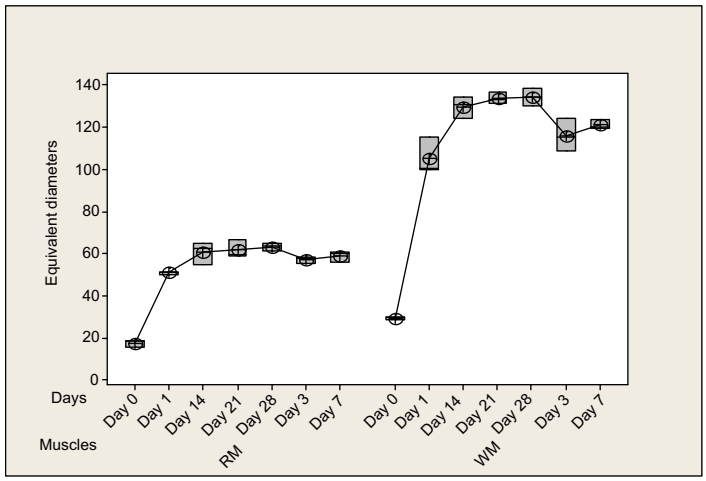

Fig. 4. Boxplot showing equivalent diameters of the ice crystals in the red muscle (RM) and white muscle (WM) vs storage time during superchilling.

ture equalisation was achieved within the samples, the growth of the intracellular ice crystals in both the red and white muscles was not significant ($P < 0.05$) at any storage times (Figs. 3 and 4 and Table 1).

3.3. Quality and shelf life of food product during superchilling process and storage

The loss of quality in fish and meat is associated largely with loss of functionality of proteins. When water form ice, there is an increased concentration of enzymes and builds up of salt concentration in the remaining water, which both causes protein denaturation and therefore effect the protein functionality (George, 1993; Shenouda, 1980; Smith, 2011). In superchilling process the protein denaturation may be minimal because only small amount of water is frozen 5–30% which will result in less enzyme and salt concentration in the remaining water. In additional to loss of functionality of proteins, the recrystallization during superchilled storage is important for the determination of shelf life and quality of food. This may impart mechanical damage by physically rupturing cell walls, which may result in an increase in drip loss (a greater loss of liquid cellular components), protein denaturation, a reduction of the water-holding capacity and other quality parameters related to the damage of the cell structure. However, the size of the ice crystals which were observed in this study at the surface of the superchilled food did not observed to destroy the integrity of the muscle because the size of the ice crystals (52 ± 1, 105 ± 9 μm, for red and white muscle, respectively) were smaller or equal to the size of the muscle tissue (53 ± 9 μm, 96 ± 9 μm red and white muscle, respectively). Furthermore, in fish and meat the destructive effect of ice crystal formation is minimised due to the elasticity of the cellular structure in muscle (Smith, 2011). It has also been reported by George (1993) that, the superchilling temperature range is cold enough to suppress bacterial growth and enzymatic but does not cause cellular damage. The information above can be supported with results which has been reported in literature that; superchilling results in extends shelf life of stored food compared to conventional chilling and better quality compared to freezing.

4. Conclusion

Based on the discussion above and available information on the change of microstructure size during the superchilling process and superchilled storage, the results of this study will be of interest to the food industry. This study clearly showed the differences in the fibres and the microstructure sizes of the red and white muscles of salmon fillets.

Although the muscle fibre diameter has been previously reported to be an important factor in determining the textural characteristics of the flesh (i.e., species with smaller fibres have firmer textures than species with large fibres), there was no information on how the microstructure of the two types of muscle would be affected during the superchilling process and superchilled storage. This study aimed to address this lack of information and progress should therefore focus on analysing the quality and shelf life of food products, based on red and white muscle fibres, during the superchilling process and superchilled storage.

The results of this study may be used to scale up improved industrial processes with this new understanding of ice crystal behaviour in both red and white muscles during the superchilling process and storage of food products. Future research should focus on tests of the quality parameters such as drip loss, enzymatic degradation and protein denaturation.

Acknowledgements

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A study of the ice crystal sizes of red muscle of pre-rigor Atlantic salmon (*Salmo salar*) fillets during superchilled storage

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ABSTRACT

Pre-rigor salmon fillets were superchilled in an impingement freezer and stored at −1.7 ± 0.3 °C for 29 days. The objective of this work was to study the ice crystal sizes in red muscle of pre-rigor salmon fillets that were partially frozen at fast (−30 °C, 227 W/m^2 K, 2.1 min) which is referred to as process F and slow (−20 °C, 153 W/m^2 K, 4.2 min) which is referred to as process S during superchilled storage. It was observed that the size of intracellular ice crystals in pre-rigor muscles at faster superchilling rate was significantly ($p < 0.05$) smaller than that at slower superchilling rate. The size of ice crystals formed in pre-rigor muscle was significant ($p < 0.05$) smaller than that formed in post-rigor muscle. It was also observed that the size of intracellular ice crystals formed in pre-rigor red muscles was significant smaller than that in white muscle. In addition, a large number of small ice crystals are formed within the muscle during partial freezing of pre-rigor muscle compared to post-rigor muscle. Future research should focus on tests of the quality parameters separately in red and white muscles (pre- and post-rigor) during superchilled storage of food products in order to understand more about their characteristics (quality and shelf life).

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1. Introduction

Superchilling is a method of preserving the food products by partial crystallisation. The main objective of superchilling is to prolong the shelf-life of food products compared to conventional chilling and to maintain the quality compared to frozen foods. The selection of the optimum method by which food should be partially frozen is the subject of many studies. Recent studies (Kaale and Eikevik, 2013; Kaale et al., 2013b, 2013c, 2013d) showed that partially freezing food products using an impingement freezer results in suitable properties of ice crystals with regard to size, distribution and shape, provided that the partial freezing occurs at a high rate. The study by Kaale et al. (2013b) showed that the superchilling rate is one of the most important parameters to consider. In that work, samples partially frozen at slow superchilling rates contained larger extracellular ice crystals, during the superchilling process (partial freezing) and following storage of the superchilled food products, compared to the samples that were partially frozen at fast rates.

In pre-rigor muscle, the cell fluids are tightly bound to the intracellular proteins and the diffusivity from the inside to the outside of the cell is therefore limited, resulting in the formation of intracellular ice crystals independent of the superchilling/partial freezing rates (Hyldig, 2012; Shenouda, 1980). The formation of ice crystals within the cells, regardless of the superchilling rate, is important for the quality of food products.

Fish myotomes contain two types of muscle, red and white. Red muscle is found as a thin superficial layer below the skin along the mid-lateral aspect of the fish (Hudson, 1973; Pritchard et al., 1971).

Red muscle contains more lipids than white muscle (Ayala et al., 2005; George, 1962; Jiang and Lee, 2007; Pritchard et al., 1971). In addition, red muscle has more mitochondria but less sarcoplasmic reticulum than white muscle. Red muscle is well oxygenated and contains a high content of myoglobin, which is the colored compound that gives the muscle its red color (Jiang and Lee, 2007). The amount of red muscle varies considerably depending on the species. In species such as tuna and small fatty pelagic fish, red muscle can constitute up to 48% of the muscle as a whole, whereas in lean fish such as cod and flounder, red muscle constitutes only a small percentage of the muscle (Nielsen and Nielsen, 2012). Pink muscle (mosaic muscle) is intermediate between red and white muscles (Fig. 1). In some fish, pink muscle is a thin layer of muscle that separates the red muscle from the white muscle. In other fish, such as salmon, carp and trout pink muscle is scattered throughout the body of the fish.

The characteristics of red muscle are coupled with the presence of a large amount of lipids, particularly in the fatty species (Jiang and Lee, 2007; Pritchard et al., 1971). Triglycerides are deposited

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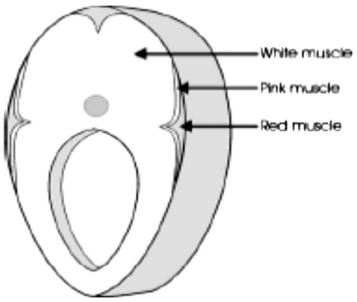

Fig. 1. A cross-section of a fish muscle showing the location of red and white muscle (Kiessling et al., 2006).

primarily as a substrate, providing fatty acids for aerobic metabolism, whereas phospholipids represent most of the lipid fraction of white muscle (Nielsen and Nielsen, 2012). This can cause preservation problems because of the increased susceptibility of red muscle to lipid oxidation (Jiang and Lee, 2007). Kiessling et al. (2006) reported that Atlantic salmon was composed of 5–10% red muscle and 90–95% white muscle. Therefore, it is clear that some species, such as salmon and lean fish, which are primarily composed of phospholipids, can be much easier to preserve compared to tuna and other species, which are composed of a large percentage of red muscle. It has also been reported that the ice crystals formed in white muscle are twice as large as those formed in red muscle (Kaale and Eikevik, 2013). In addition, species with a firmer texture have relatively smaller fibers than species with a softer texture (Ayala et al., 2005). However, there is no study showing ice crystal size in red muscle partially frozen in a pre-rigor state. The objective of this work was to study the ice crystal sizes of red muscle in pre-rigor salmon fillets partially frozen at slow and fast rates during superchilled storage.

2. Materials and methods

2.1. Materials and superchilling processes

Salmon fillets (0.9–1.2 kg) were taken from a slaughterhouse located in Salmar, Frøya, Norway. The samples were cut from the top to the bottom side of the salmon fillet to obtain samples that contained red muscles. The fillets were vacuum packed and partially frozen in a pre-rigor condition (i.e., within 5–6 h of being caught) in an Impingement Advantec Lab Freezer (JBT Food – tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at −20 °C, 153 W/m^2 K (pressure differences of the fan at the impingement freezer) for 4.2 min, which is referred to as process S (slow superchilling), and at −30 °C and 227 W/m^2 K for 2.1 min, which is referred to as process F (fast superchilling), to achieve an ice content of 20%. The surface heat transfer coefficient values, ice content and the superchilling time were established in previous work (Kaale et al., 2013a). Once superchilled, the salmon samples were stored in a cold room at −1.7 ± 0.3 °C for 29 days. Six fillets were analysed at each sampling time (i.e. 3-fast superchilling and 3-slow superchilling).

2.2. Measurement of the temperature during the superchilling process and superchilled storage

The temperature was measured at three different locations on the samples during the superchilling process: the surface, midway to the center and the center. Three thermocouples were used at each location. The thermocouples were connected to a temperature recorder while the sample was cooled in the impingement freezer. The temperatures of the cool air and of the sample were recorded every 4 s. The samples of 28 mm thickness were used. The temperature was measured by inserting thermocouples approximately 2 mm from the surface, 7 mm from the surface (midway to the center) and 14 mm from the surface (center).

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted in the storage box: one was used to measure the air temperature, and the other two were used to measure the surface and center temperatures of the superchilled salmon fillets. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately −5 °C (temperature outside the storage box).

2.3. Microscopic analysis

The samples were cut from the top to the bottom of each superchilled sample (−1.7 ± 0.3 °C) transversal to the muscle fiber using a standard knife blade that was previously stored at −1.7 ± 0.3 °C. This procedure was conducted in a walk-in freezer to ensure a perfect cold chain. In this study, a fixation method similar to those proposed by Alizadeh et al. (2007) and Martino and Zaritzky (1988) was used to observe the spaces left by the ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The control (not superchilled) samples were fixed with the same solution but at 4 °C. The fixed samples were then brought to room temperature and were dehydrated with absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fiber using a microtome (Autocut 2055, Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Tissue Clear was used for rehydration, the samples were immersed in 1% blue aniline for 1 min and xylene was used before mounting.

The prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan). The images of the slides were recorded and treated using the stereological analysis program CAST2 (Olympus Inc., Denmark). Two parameters, namely the cross-sectional area and the equivalent diameter, were used in the evaluation. The cross-sectional area refers to the surface area of the cross-section of an object (ice crystal or fiber muscle). The equivalent diameter for each ice crystal is defined as the diameter of a circle having the equivalent area S_p. From the data set of each equivalent diameter, the mean crystal diameter, D_{eq}, was calculated. All analyses were performed for three different specimens of each fillet. For each case, more than 50 incidences of ice crystals were evaluated.

2.4. Statistical analysis

The observations of the microstructure sizes of the pre-rigor red muscles with respect to storage days were analysed using one- and two-way analyses of variance using Minitab 16 software. The data were expressed as the mean ± standard deviation, and the statistical significance of each experiment was $p < 0.05$.

3. Results and discussion

3.1. Thermal transition behaviour of superchilling process

Thermal transition behaviour of superchilling process and storage in this study is the same as that reported in our previous work Kaale et al. (2013d). However, in this study analysed ice crystal sizes of the red muscles along the lateral lines of salmon fillets while (Kaale et al., 2013d) analysed ice crystal sizes of the white muscles at the top surface and center part of salmon fillets. The initial freezing point was −1.1 °C, which was indicated by the beginning of the freezing plateau at the center of the sample. The initial freezing point of the salmon was determined using separate samples that were totally frozen in the impingement freezer for approximately 30 min, and these samples were not used for any other analysis.

Fig. 2 shows an example of the superchilling process at −30 °C for 2.1 min. The sample and superchilling medium temperatures were recorded every 4 s. The temperatures were recorded at surface, mid center and center layers. The temperature at the frozen surface layer, approximately 2 mm from the surface was about −6 °C, while temperature at the mid center and center were about −0.7 and 4.4 °C, respectively. Fig. 3, the time–temperature profile during the superchilled storage shows temperature equalization within one day of storage and maintained at −1.7 ± 0.3 °C during storage.

3.2. Evaluation of ice crystal sizes during superchilled storage

Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. The cross-section of the unprocessed sample exhibited a uniform distribution of regularly shaped fibers Fig. 4.The red muscle concentrated along the lateral line consisted of narrow fibers of average diameter (46 ± 11 μm), whereas the white muscle on either side consisted of broad fibers of 95 ± 11 μm.

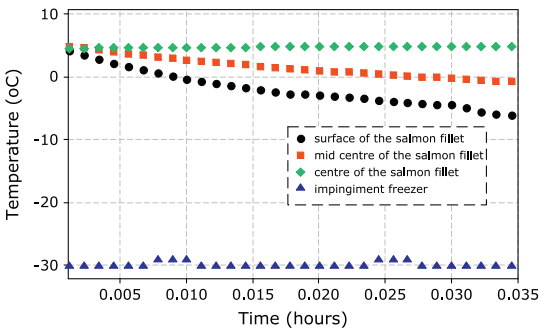

Fig. 2. Temperature–time profile: surface, mid center and center layers during superchilling process.

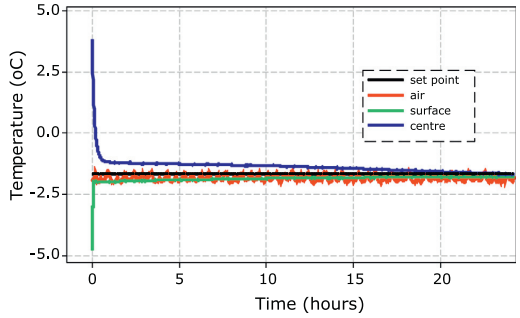

Fig. 3. Temperature–time profile during storage of the superchilled samples.

The study Kaale and Eikevik (2013), showed that the size of ice crystals formed in red muscle during the superchilling process (day zero) was significant $p < 0.05$ smaller than those during superchilled storage. In this study, we will not discuss ice crystal sizes during the superchilling process (i.e., partially frozen) because we did not perform an analysis at day zero. However, microscopically these results are clearly distinguishable from the previous study. The development of ice crystals in red muscle of pre-rigor salmon fillets during superchilled storage (days 2–29) are therefore presented.

The results indicated that the size of ice crystals at the faster superchilling rate (F) was smaller compared to the slower superchilling rate (S) (Figs. 4 and 5). The size of ice crystals was 42 ± 7 μm at the faster superchilling process and 55 ± 3 μm at the slower superchilling process in red muscles after 2 days of storage. The results indicated that the increase of ice crystal sizes in the fast and slow superchilling rate samples was not significant ($P < 0.05$) at any storage time.

However, it is well known that, recrystallization is a temperature-dependent process, which is enhanced by temperature fluctuations (Roos, 1995; Syamaladevi et al., 2012). In addition, small ice crystals are thermodynamically unstable, having a high surface–volume ratio and therefore a high excess of surface free energy (Alizadeh et al., 2007; Mazur, 1984; Russell et al., 1999; Shenouda, 1980; Syamaladevi et al., 2012; Zaritzky, 2012). In superchilling technology, recrystallization is a main challenge particularly between day 0 and 1 of storage (before temperature equalization). The recrystallization process should be expected during superchilled storage due to isothermal conditions and accounting that we have both ice at the surface and water at the center of the samples. Fig. 6 shows the development of ice crystals in white muscles both at the centers and surfaces of the superchilled salmon. The results of ice crystal in white muscle presented in this study were taken from our previous study (Kaale et al., 2013d). This work reported a significant ($p < 0.05$) increase of the ice crystal sizes between superchilling process (day 0) and superchilled storage. The significant increase of ice crystal sizes in superchilled products may be due to two main factors: The first factor is the large difference between the superchilling process temperature (−30 °C or −20 °C) and the superchilled storage temperature (−1.7 ± 0.3 °C) (i.e. temperature fluctuation).

This large temperature difference affected the growth of ice crystals, particularly the small ones that were formed at the surface of the salmon fillets during shell freezing. The second factor is the thermal gradient effect that was created during the superchilling process. These gradients were observed to result in slight melting of the small ice crystals formed at the surface layer and the subsequent water diffusion to larger ice crystals, resulting in a reduction in the number of ice crystals at the surface layer of the superchilled salmon (Kaale et al., 2013d; Payne et al., 1994).

In the present study, this large increase of ice crystal sizes (recrystallization) was not observed because the samples were analyzed after 2 days of storage. Since, the same superchilling process and storage were applied in Kaale et al. (2013d) and the present work, we believe that the recrystallization in red muscle of pre-rigor salmon fillets would be observed between day 0 and 1. The recrystallization in red muscle of post-rigor salmon fillet was also observed by Kaale and Eikevik (2013) between day 0 (superchilling process) and day 1 (superchilled storage). It should also be noted that, after temperature equalization (temperature at the surfaces equal to temperature at the centers of the superchilled samples)

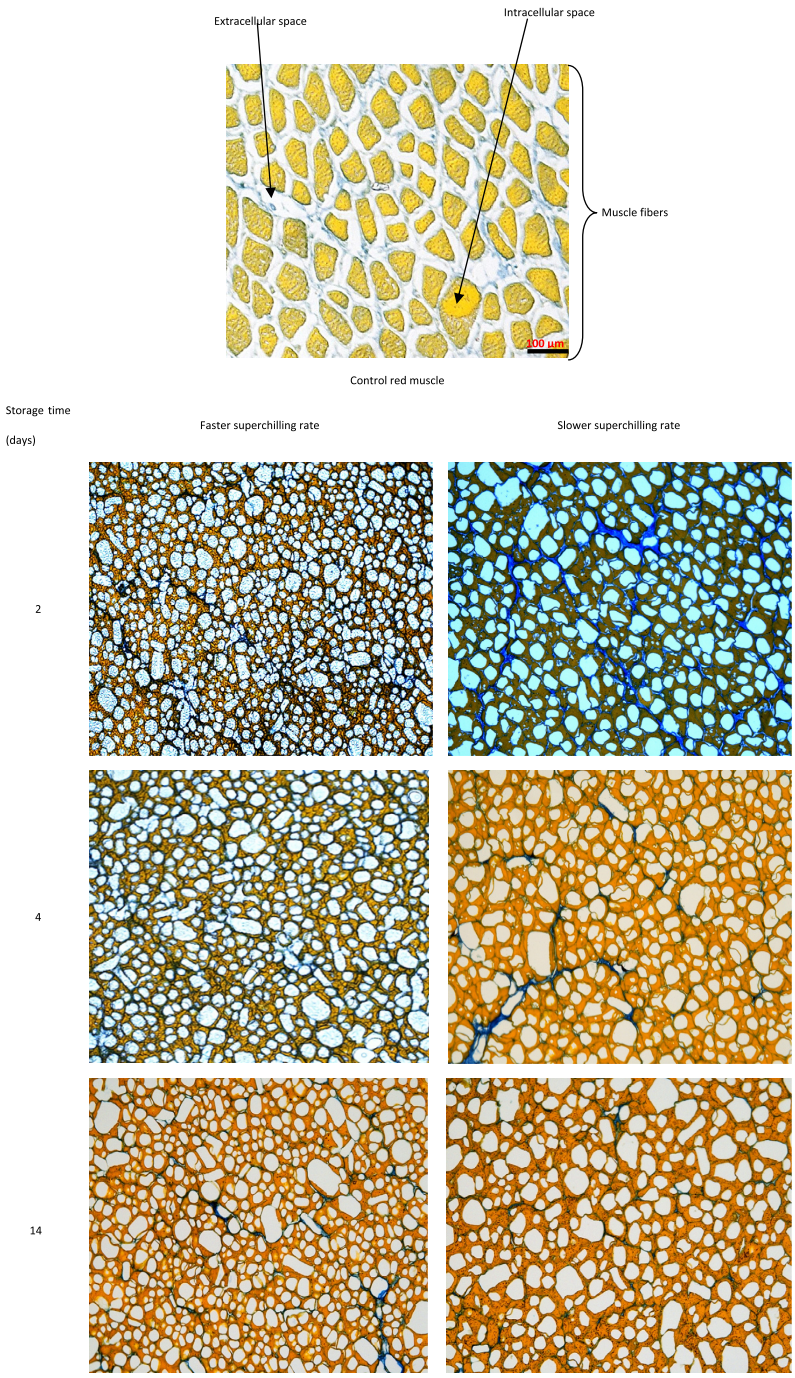

Fig. 4. Micrographs of unsuperchilled and superchilled salmon tissues (faster and slower superchilling rates).

and control of the temperature fluctuation during storage the increase of ice crystal sizes is not significant ($P < 0.05$) at any storage times (Kaale et al., 2013c, 2013d; Kaale and Eikevik, 2013). Bevilacqua and Zaritzky (1982) reported that when temperature is constant, the recrystallisation occurs at significant rate only when the specimen contains crystals with diameters less than

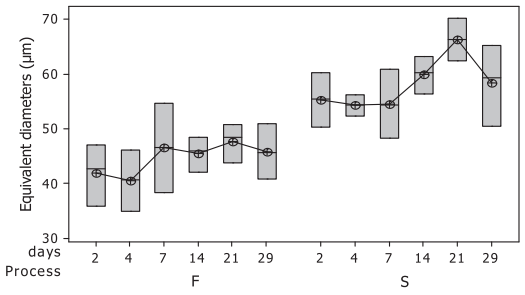

Fig. 5. Boxplots showing equivalent diameters of the ice crystals in red muscles at faster rate (F) and slower rate (S).

24µm. However, temperatures fluctuation enhance recrystallisation even if have larger diameters.

3.3. Microstructure sizes in pre- and post-rigor muscles

Table 1 shows the means of ice crystals in pre- and post-rigor of salmon fillets during superchilled storage (days 7, 14 and 21) which are displayed with individual 95% confidence intervals for a mean based on the pooled standard deviation. The post rigor results presented in this study for comparison were taken from our previous study (Kaale and Eikevik, 2013). The comparison was done between 3 days because were the only corresponding days between pre- and post-rigor, (i.e. in post rigor (Kaale and Eikevik, 2013) the analysis was performed at 0, 1, 3, 4, 7, 14, 21 and 28 days while in pre-rigor (this study) was performed at 2, 4, 7, 14, 21 and 29 days). The result indicated that, the size of ice crystals in pre-rigor red muscle was significant $p < 0.05$ smaller than in post-rigor red muscle during superchilled storage of salmon fillets (Fig. 7 and Table 1). Meanwhile, the storage days did not show significant difference $p < 0.05$. In addition, a larger number of small ice crystals were observed in pre-rigor muscles compared to post-rigor muscles, which can be said that, large amount of water is inside the muscle cells during superchilling process of pre-rigor muscle and the water is both inside and outside the cells in the post-rigor muscle. More details on post rigor muscles can be found elsewhere (Kaale and Eikevik, 2013). These results are similar to those reported by Kaale et al. (2013d), who observed that at the fast superchilling rate, a large number of small ice crystals were present within the muscles during pre-rigor compared to post-rigor muscles. Thus, the location, size and distribution of ice crystals in muscle tissue is the function of superchilling rate (slow versa fast) and state of the muscle tissue (pre- and post-rigor muscle).

Figs. 8 and 9 show the micrographs in red and white muscles of the salmon fillets. A significant difference between the size of ice crystals formed in red and white muscles was observed in this study, as well as in the previous study (Kaale and Eikevik, 2013). Figs. 8 and 9 show the ice crystals in the red muscles concentrated along the lateral lines with white muscle on either side. It is clearly shown that the size of ice crystals formed in red muscles is significantly smaller than that in white muscles. The size of ice crystals was 40 ± 11 µm and 121 ± 2 in red and white muscles, respectively, after 4 days of storage (Fig. 8) and 46 ± 5 and 119 ± 7 µm in red and white muscles, respectively, after 7 days of storage (Fig. 9).

Summary of the red muscle: The red muscle is characterized by the following points: (1) the smallness and uniformity of size of the fibers (2) smallness of size of the ice crystals formed in the muscles and (3) most important of all, its enormous loading of fat (Greene, 1913; George, 1962). The study of George (1962) clearly shows that the red muscle, narrow fibers (NF) is more loaded with fat than white muscle, broad fibers (BF) in mackerel fish. Greene (1913) use different muscles in salmon fish to study the fat deposition and discovered that the red muscle contains enormous loading of fat. The storage fat is both inter- and intramuscular. It is present between the fibers in a relatively small number of medium-sized drops (Greene, 1913). In this study the samples did not exhibit any concerning features related to fat during storage. We believe that, the fat droplets would not appear in our samples due to the following reason: In the histology experiment two main processes are taking place; fixation and tissue processing. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at −1.7 ± 0.3 °C for 24 h. The fixed samples were then brought to room temperature and were proceed to the tissue processing stages. Tissue processing is concerned with the diffusion of various substances into and out of stabilizes porous tissues. Dehydration is the first step in tissue processing. In this study ethanol was used as dehydrant. During this stage various cellular components are dissolved by dehydrating fluids for example fat (lipids) in the muscle tissue are extracted out by alcohol solutions. Fats are long chains with a carbon backbone able to dissolve into nonpolar such as ethanol. Fats are massive long carbon chains, the charge is relatively well distributed, and therefore they are nonpolar. On the other hand, ethanol has both polar and nonpolar characteristics due to presence of the hydrogen chain (nonpolar group) and the OH (polar) and therefore dissolves fats. Generally polar substances dissolve in polar substances, and nonpolar substances dissolve in nonpolar substances (like dissolves like). Therefore, fat in the salmon tissues (our samples) would extract out by ethanol.

These findings are significant information for the superchilling industries because small ice crystals indicate better quality while large crystals often produce damage during partial freezing (Farouk et al., 2013; Pham and Mawson, 1997; Roos, 1995; Zaritzky, 2012). The information about the microstructure sizes of red and white muscles will give industrial food technologists necessary scientific information on the handling, processing and storage conditions of different types of food products during superchilled storage. These findings might also provide scientific information to other researchers working in the same area. They may want to explore more about the characteristics (quality and shelf life) of food products which composed of large or less percentage of red muscles during superchilling processes and storage and of course during freezing processes and frozen storage.

4. Conclusions

It was observed that smaller ice crystal formation was due to the faster superchilling process of pre-rigor Atlantic salmon fillets. A large number of small ice crystals are formed within the muscle during partial freezing of pre-rigor muscle compared to post-rigor muscle which can be confirmed that large amount of water is inside the muscle cells during superchilling process of pre-rigor muscle and the water is both inside and outside the cells in the post-rigor muscle.

Certain characteristics of red muscle are known to influence preservation quality. Muscle fiber diameter is another important factor determining the textural characteristics of the flesh. However, there was no information on how the microstructure of the red muscle partially frozen in pre-rigor state at slow and fast rates would be affected during superchilled storage. This study aimed to address this lack of information and progress should therefore focus on analysing the quality parameters, such as water holding capacity, protein denaturation and enzymatic degradation based on red and white muscle during storage of the superchilled food products.

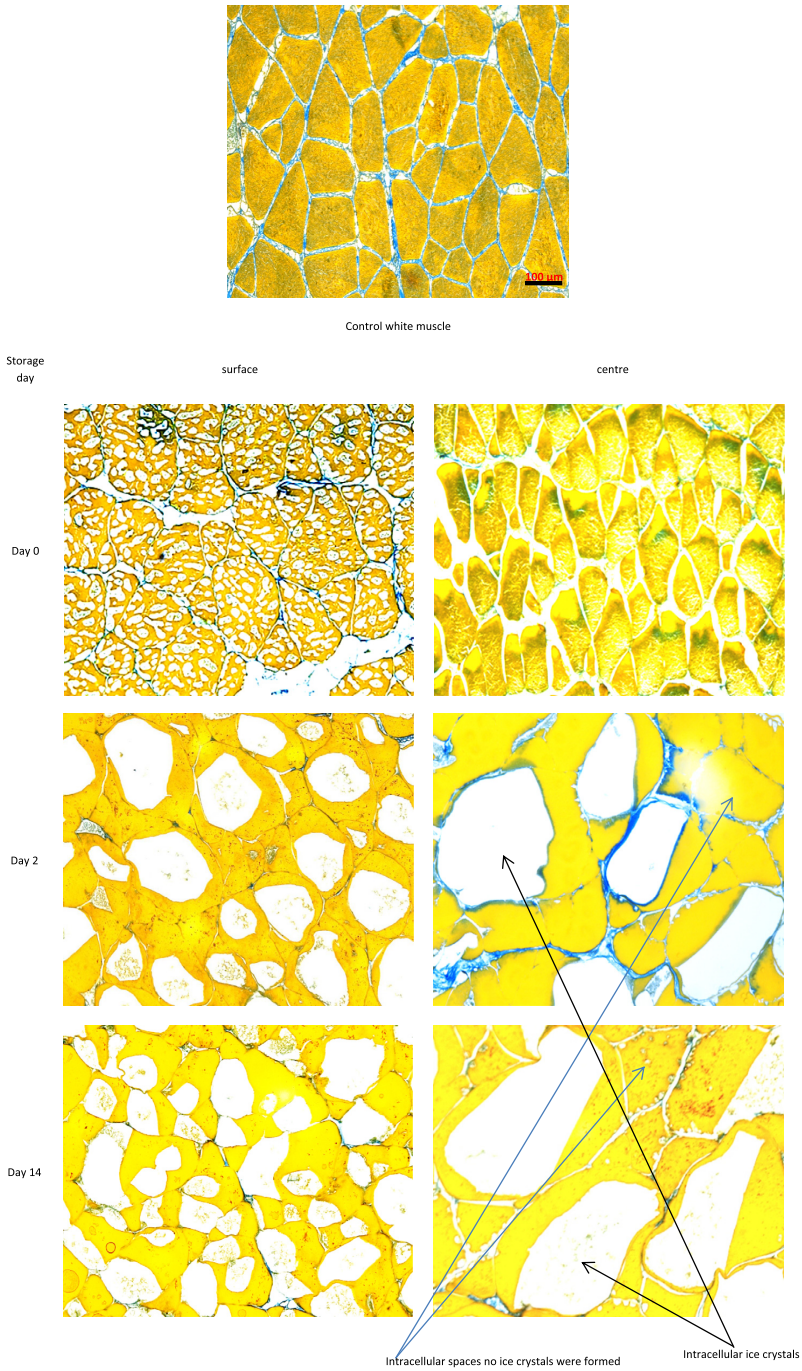

Fig. 6. Micrographs during superchilling process and storage of pre-rigor white muscle of salmon fillets (faster superchilling rates) (from Kaale et al., 2013d study).

Table 1
Equivalent diameters (μm) of the ice crystals of pre and post-rigor of salmon fillets with 95% confidence intervals (CIs) based on the pooled standard deviation.

Muscles	Mean				
F-post rigor	60.26			(----*-----)	
F-pre rigor	46.57	(-----*----)			
		48.0	54.0	60.0	66.0

Days	Mean				
7	52.64	(----------*----------)			
14	52.96	(----------*----------)			
21	54.65		(----------*----------)		
		50.0	52.5	55.0	57.5

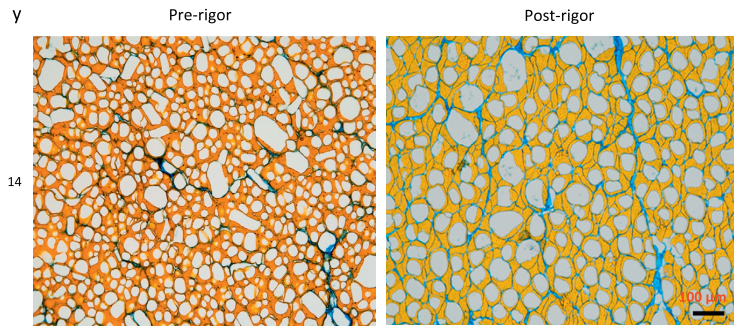

Fig. 7. Micrographs of pre- and post-rigor at fast superchilling rate.

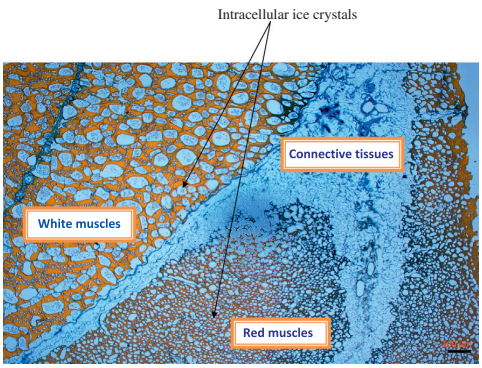

Fig. 8. Showing microstructure (day 4) in red and white muscles of pre-rigor salmon fillet.

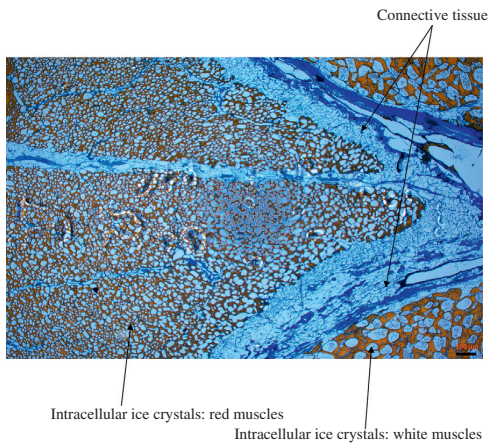

Fig. 9. Showing microstructure of white muscles both sides and red muscles (day 7) of pre-rigor salmon fillet.

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Paper VIII

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Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage

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Drip loss

ABSTRACT

Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) fillets during superchilled storage were studied. Due to the significant differences in ice crystal sizes observed in our previous study, the liquid loss (LL) was analysed separately, at the surface and centre of the superchilled samples. No significant differences were found in LL between surface and centre parts of the superchilled samples. No significant difference in the LL was observed from surface samples between 1 and 14 days of storage. There was a significant difference in the LL at day 1 of the centre samples, but no significant differences were observed between 3 and 14 days of storage. In contrast, the LL was significantly decreased at day 21 both at the centre and the surface of the superchilled samples. No significant difference ($p < 0.05$) was found in drip loss between 1 and 14 days of storage for the superchilled samples. A significant increase in drip loss for the superchilled samples was observed at day 21. These findings are significant for the industry because it provides valuable information on the quality of food in relation to ice crystallisation/recrystallisation during superchilled storage.

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1. Introduction

1.1. Superchilling process

Fresh and high quality food is becoming increasingly important. Many studies have been done to find the good preservation technologies. Superchilling technology is an alternative for preserving the freshness and maintaining the quality of food compared to conventional chilling and freezing technologies (Kaale, Eikevik, Rustad, & Kolsaker, 2011). Several different performances/definitions for superchilling are used, and have shown beneficial effects in storage of foods: Ando, Nakamura, Harada, and Yamane (2004) define it as the temperature zone below 0 °C but where ice crystals are not generated. Beaufort, Cardinal, Le-Bail, and Midelet-Bourdin (2009) defined superchilling as a technology where food is stored just below the initial freezing temperature. Bahuaud et al. (2008), Duun and Rustad (2008), Kaale, Eikevik, Bardal, Kjorsvik, and Nordtvedt (2013), Kaale, Eikevik, Bardal, and Kjorsvik (2013), Kaale, Eikevik, Rustad, et al. (2013), and Stevik et al. (2010) have performed superchilling by shell freezing food products and then letting the temperature equalise during storage at a temperature below the initial freezing point. The advantage of shell freezing is to facilitate temperature equalisation (enhance heat transfer) within the food and hence good mechanism of ice crystal formation. The ice formed in the food product acts as an internal ice reservoir during distributions or storage for short periods.

The main potential disadvantage of partially freezing foods is the risk of damage caused by ice crystal formation. The size and location of ice crystals formed during partial freezing (1–3 mm) from the surface is dependent on the superchilling rate. Furthermore, these crystals affect important quality parameters such as texture, water holding capacity and drip loss upon thawing (Mittal & Griffiths, 2005).

The question of the optimum method by which food should be shell frozen has been the subject of many studies. Recent studies Kaale and Eikevik (2013) and Kaale, Eikevik, Bardal, Kjorsvik, and Nordtvedt (2013), Kaale, Eikevik, Bardal, and Kjorsvik (2013), Kaale, Eikevik, Rustad, et al. (2013) indicated that shell/partial frozen food products using impingement freezer result in suitable properties of ice crystal with regard to size, distribution and shape provided that is done at a high rate of superchilling. Bahuaud et al. (2008), Chevalier, Sequeira-Munoz, Bail, Simpson, and Ghoul (2001), Dincer (1997), Fernandez, Otero, Martino, Molina-García, and Sanz (2008), Hagiwara, Wang, Suzuki, and Takai (2002), Kiani and Sun (2011), Martino and Zaritzky (1986), Martino, Otero, and Sanz (1998), and Petzold and Aguilera

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(2009) found that the size, distribution and shape of ice crystals have a major influence on the quality of food products.

1.2. Water holding capacity (WHC) and drip loss

A useful tool for describing the quality in muscle foods post-mortem is to measure the WHC of the muscle (Olsson, Ofstad, Lodemel, & Olsen, 2003). The WHC of food products is an important quality parameter as it affects both profitability and quality, because it affects the weight change during transport and storage, the drip loss during thawing, the weight loss and shrinkage during cooking, and the juiciness and tenderness of the meat (Duun & Rustad, 2007; den Hertog-Meischke, van Laack, & Smulders, 1997; Huff-Lonergan, 2002; Irie, Izumo, & Mohri, 1996; Shaviklo, Thorkelsson, & Arason, 2010). The WHC is closely related to textural properties, and a 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 (Duun, 2008). Myofibrils are long rod-like organelles found in skeletal and cardiac muscle that constitute approximately 80% of the volume of the muscle cell (Huff-Lonergan, 2002; Huff-Lonergan & Lonergan, 2005). Moreover, approximately 85% of the water in a muscle cell is held in the myofibrils (den Hertog-Meischke et al., 1997; Huff-Lonergan, 2002; Huff-Lonergan & Lonergan, 2005).

Drip loss, or the release of water during thawing, implies nutrient loss (Duun, 2008; Turan, Kaya, & Erkoyuncu, 2003). Drip loss is usually expressed as a percentage of the initial weight of the product (Huff-Lonergan & Lonergan, 2005). Most of the proteins found in drip are water-soluble, sarcoplasmic proteins. It is noted that, in general, muscle proteins in fish and shellfish are more susceptible to partial freeze denaturation compared with land animal proteins (Benjakul & Visessanguan, 2010).

Our previous studies Kaale, Eikevik, Bardal, Kjorsvik, and Nordtvedt (2013), Kaale, Eikevik, Bardal, and Kjorsvik (2013), Kaale, Eikevik, Rustad, et al. (2013) demonstrated that a high superchilling rate results in a high rate of heat removal, which leads to the formation of a large number of small nuclei and thus a large number of small ice crystals that grow both within and outside cells. Consequently, the cells maintain their integrity which in turn minimises drip loss; maintain water holding capacity and other quality parameters during thawing (Smith, 2011). However, this advantage was reduced during superchilled storage by the rapid growth in ice crystal size in the salmon fillets. Moreover, there were large differences between the ice crystal sizes at the surfaces and centres of the superchilled salmon fillets (Kaale, Eikevik, Bardal, & Kjorsvik, 2013). An increase in the size of ice crystals during superchilled storage may impart mechanical damage by physically rupturing cell walls, which may result in an increase in drip loss, a reduction of the WHC and changes in other quality parameters related to the damage of the cell structure.

Nevertheless, there are few studies showing the relationship between ice crystal development and quality of food during superchilled storage. Most of the studies on superchilling have focused on the physical, chemical and microbiological analysis (Ando et al., 2004, Bao, Arason, & Thórarinsdóttir, 2007; Duun & Rustad 2007, 2008; Gallart-Jornet, Rustad, Barat, Fito, & Escriche, 2007). Therefore, the objective of this study was to analyse the WHC at both the surfaces and centres of salmon fillets and drip loss. Taken together these results help to clarify the effect of ice crystal development during the superchilled storage.

2. Materials and superchilling process

Salmon (*Salmo salar*) fillets (0.9–1 kg), were delivered by Lerøy Midnor (Hitra, Norway). The samples were vacuum-packed and stored at 4 °C for 24 h before the superchilling process to ensure a constant temperature in all samples. Superchilling was performed in an Impingement Advantec Lab Freezer (JBT Food Tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at −30 °C and 227 W/m^2 K (at 2.5 kPa pressure differences of the fan in the impingement freezer) for 2.1 min to achieve an ice content of 20%. A previously developed model (Kaale, Eikevik, Kolsaker, & Stevik, 2013) was used to predict the degree of superchilling. The experiments for measuring the surface heat transfer coefficient (SHTC) were also performed in an Impingement Advantec Lab Freezer. A detailed experimental set up and the equations used to calculate the SHTC are explained elsewhere (Kaale, Eikevik, Kolsaker, et al., 2013). Once superchilled, the salmon samples were stored in a cold room at −1.7 ± 0.3 °C for 28 days. Three fillets were analysed at each sampling time.

2.1. Temperature trend during storage

The temperature, one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box (92 × 73 × 54.5 cm) was designed with an internal heating element to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted into the storage box. One sensor was used to measure the air temperature, and the other two were used to measure the temperatures at the surface and centre of the superchilled sample. The set-point temperature was −1.7 °C. The box was placed inside the storage room, which was at a temperature of approximately 5 °C (temperature outside the storage box).

2.2. Microscopic analysis

A fixation method similar to that proposed by Alizadeh, Chapleau, de Lamballerie, and Le-bail (2007) and Martino and Zaritzky (1988) was used to observe the spaces left by the ice crystals in the tissue. Detail information in the method used will not presented here because the micrographs presented in this study were taken from the previous study (Kaale, Eikevik, Bardal, & Kjorsvik, 2013). However, the superchilling process and storage were the same in both studies except, this study analysed WHC and drip loss while (Kaale, Eikevik, Bardal, & Kjorsvik, 2013) analysed ice crystal sizes of the salmon fillets.

2.3. Water holding capacity and drip loss

The liquid loss was determined for minced muscle by low speed centrifugation as described by the WHC method of Eide, Børresen, & Strøm (1982). A centrifugal force of 270 *g* was used instead of 1500 *g* (Hultmann & Rustad, 2002). The LL is expressed as the percentage of weight lost during the centrifugation of 2 *g* of minced sample for 5 min. The analyses were run in quadruplicate. The water content in the mince was determined by drying a 2 g minced sample at 105 °C for 24 h. These analyses were run in duplicate.

For the quantification of drip loss, the sample was removed from the vacuum bag after thawing at 4 °C for approximately 24 h and the remaining liquid in the bag was weighed. The calculation of the drip loss was based on the initial sample weight after thawing. Mean values were calculated from three triplicates.

2.4. Statistical analysis

The observations for the WHC at the two locations (surface and centre) and the drip loss with respect to the storage days were analysed by one- and two-way analyses of variance using Minitab 16 software. A general linear model, (post-hoc test) under Tukey's

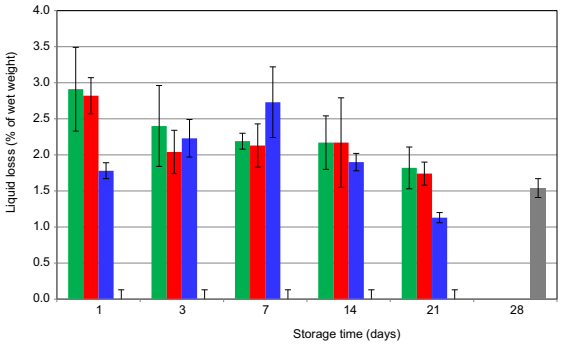

Fig. 1. Changes in liquid loss of salmon fillets during storage. Superchilled-surface [green], superchilled-centre [red], chilled reference [blue] and frozen reference [grey]. Standard error of the mean is shown as *y*-error bar ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

simultaneously test was applied whenever the ANOVA results were significant, $p < 0.05$.

3. Results and discussion

3.1. Thermal transition behaviour during the superchilled of food products

The thermal transition behaviour of the superchilling process and subsequent storage in this study is the same as that reported in our previous work (Kaale, Eikevik, Bardal, & Kjorsvik, 2013) (results not shown). However, in this study we analysed WHC and drip loss while (Kaale, Eikevik, Bardal, & Kjorsvik, 2013) analysed ice crystal sizes in the salmon fillets during superchilled storage. The changes in the surface and centre temperatures of the samples were recorded after every 4 s during the superchilling experiments. The initial freezing point of salmon fish was −1.1 °C, which was designated by the beginning of the freezing plateau at the centre of the sample. The initial freezing point of the salmon was determined using separate samples that were completely frozen in the impingement freezer for approximately 30 min.

After partial freezing (superchilling process), the surface and core temperatures were approximately −6 and +3.8 °C, respectively. The temperature at the core was higher than the initial freezing point of the salmon which indicates that, during the superchilling process there were no ice crystals formed at the centre of the salmon fillet.

The temperature–time profile for superchilled storage was also shown over one day and the temperature equalisation was achieved after 1 day and it was maintained at -1.7 ± 0.3 °C for the entire storage time explained elsewhere (Kaale, Eikevik, Bardal, & Kjorsvik, 2013).

3.2. WHC during the storage of superchilled salmon fillets

The WHC was analysed at the surface (approximately 2–3 mm from the surface) and the centre of the superchilled samples. The results showed that the liquid loss decreased with storage time (i.e. WHC increased with storage time) both at the surface and in the centre of the superchilled samples (Figs. 1 and 2). The larger ice crystals at the centre of the superchilled samples were expected to give higher LL, but interestingly the opposite was found in this study. The LL was slightly lower at the centre than at the surface, however there was no significant difference ($p < 0.05$) between the surface and centre of the superchilled salmon fillet. There were no significant differences ($p < 0.05$) in the LL between 1 and 14 days of storage for the surface samples. There was a significant difference in the LL at day 1 for the centre samples, but no significant differences were observed between 3 and 14 days of storage. In contrast, the decrease in LL was significant at day 21, both at the centre and surface of the superchilled samples.

In an earlier study of Atlantic salmon stored at −1.4 or −3.6 °C a decrease in liquid loss was observed during the first two weeks (16 days of superchilled storage) (Duun & Rustad, 2008). However, the LL in their samples were significantly higher, 9.3–3.8% at −1.4 °C and 7.3–5.4% at −3.6 °C, than in our samples, 2.9–1.8% at the surfaces and 2.8–1.7% at the centres. The large LL in their study could be due to the higher degree of superchilling in their samples, which were 32 ± 2 and 49 ± 5% at −1.4 and −3.6 °C, respectively. It has been reported that the degree of superchilling between 5% and 30% is acceptable and that a degree of superchilling above 30% will result in reduced quality in food products (Stevik & Claussen, 2011). Furthermore, the superchilling rate was generally much slower compared to the superchilling rate applied in this study. A recent study by Kaale, Eikevik, Bardal, Kjorsvik, and Nordtvedt (2013) reported that salmon fillets that were shell/partially frozen using an impingement freezer had suitable ice crystal properties like size, distribution and shape provided that the superchilling is executed

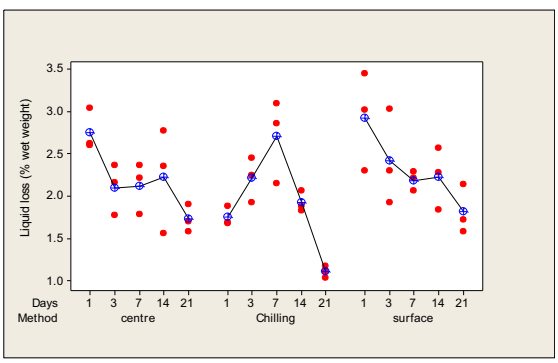

Fig. 2. Individual plot showing variation of liquid loss vs. storage time: mean values and standard deviation are shown ($n = 3$).

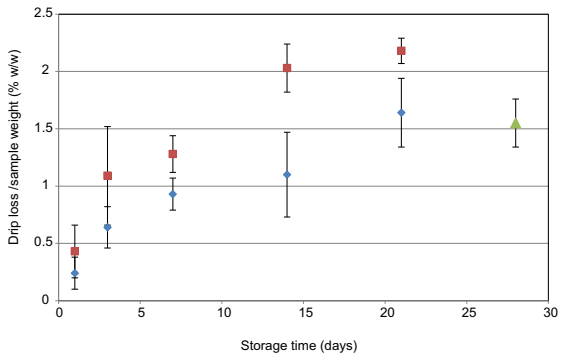

Fig. 3. Variation of drip loss in salmon fillets during storage. Superchilled (♦), chilled reference (■) and frozen reference (▲). Mean values and standard deviation are shown ($n = 3$).

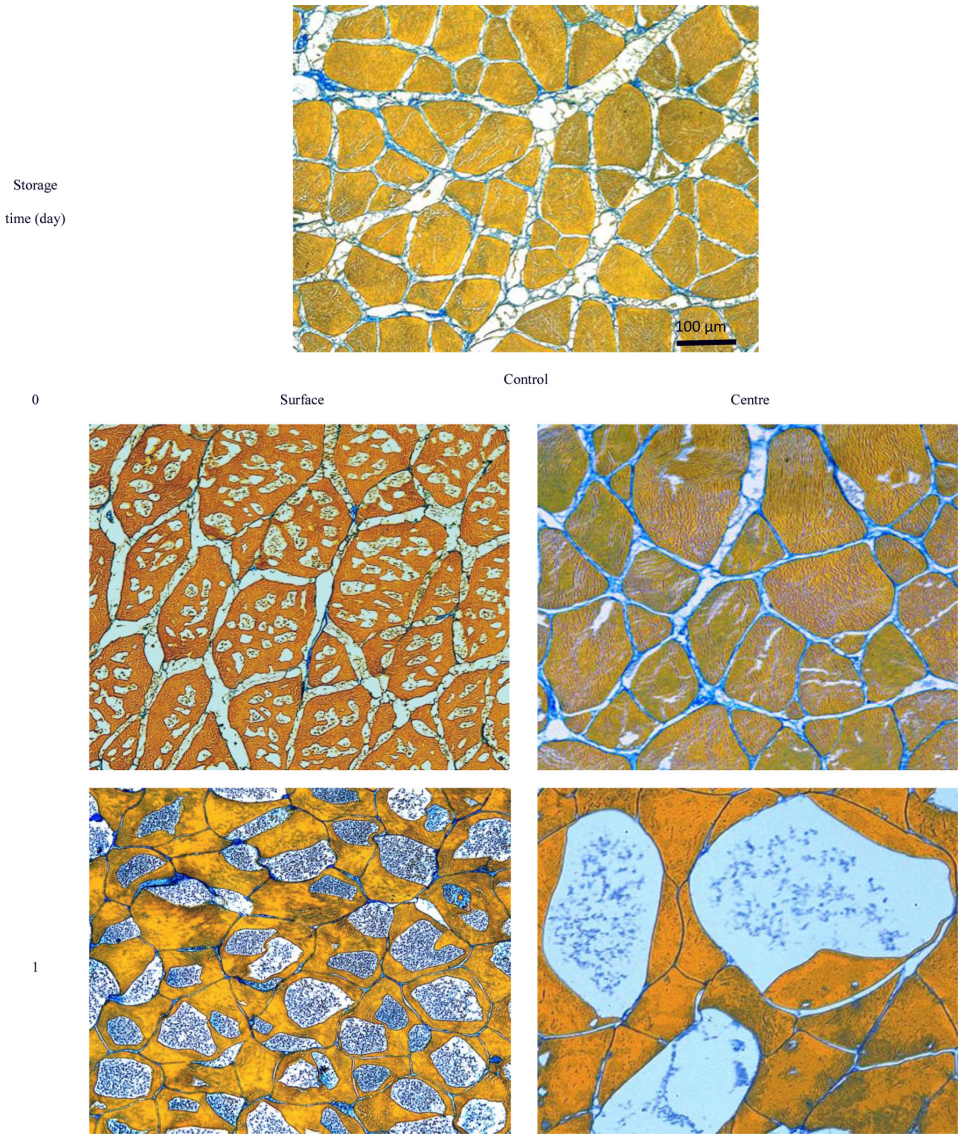

Fig. 4. Micrographs of unsuperchilled and superchilled salmon tissues.

at a high rate. These ice crystal characteristics have a major influence on food quality such as WHC, drip loss and textural changes. In addition, impingement freezers have been identified as an alternative to conventional freezing methods because of their high turbulence characteristics, which enhance heat transfer and therefore the quality of food product (Salvadori & Mascheroni, 2002).

An increase in WHC with storage time has been observed in several other studies: on pork (Kristensen & Purslow, 2001), halibut (Olsson et al., 2003) and arctic charr (*Salvelinus alpinus*) (Bao et al., 2007). Bao et al. (2007) claimed that decreases in the LL (increases in the WHC) may partly have been caused by a higher ratio of loosely bound water that was released as drip over time. Increases in WHC with storage time may also originate from proteolytic activity in the muscle during storage.

The WHC is a useful tool for describing quality in muscle foods. Since LL is highly related to structural changes in the muscle/flesh microstructure (Erikson, Misimi, & Gallart-Jornet, 2011; Olsson et al., 2003), the results from our study indicated that no significant change in the protein network occurred between day 1 and 14, both at the surfaces and centres of the salmon samples during superchilled storage. The significant decrease in LL at day 21 (Figs. 1 and 2) might be due to proteolytic activity in the muscle during superchilled storage.

In the chilled samples (stored at 4 °C), the LL increased significantly from day 0 to day 7 $p < 0.05$. Then, there was a significant

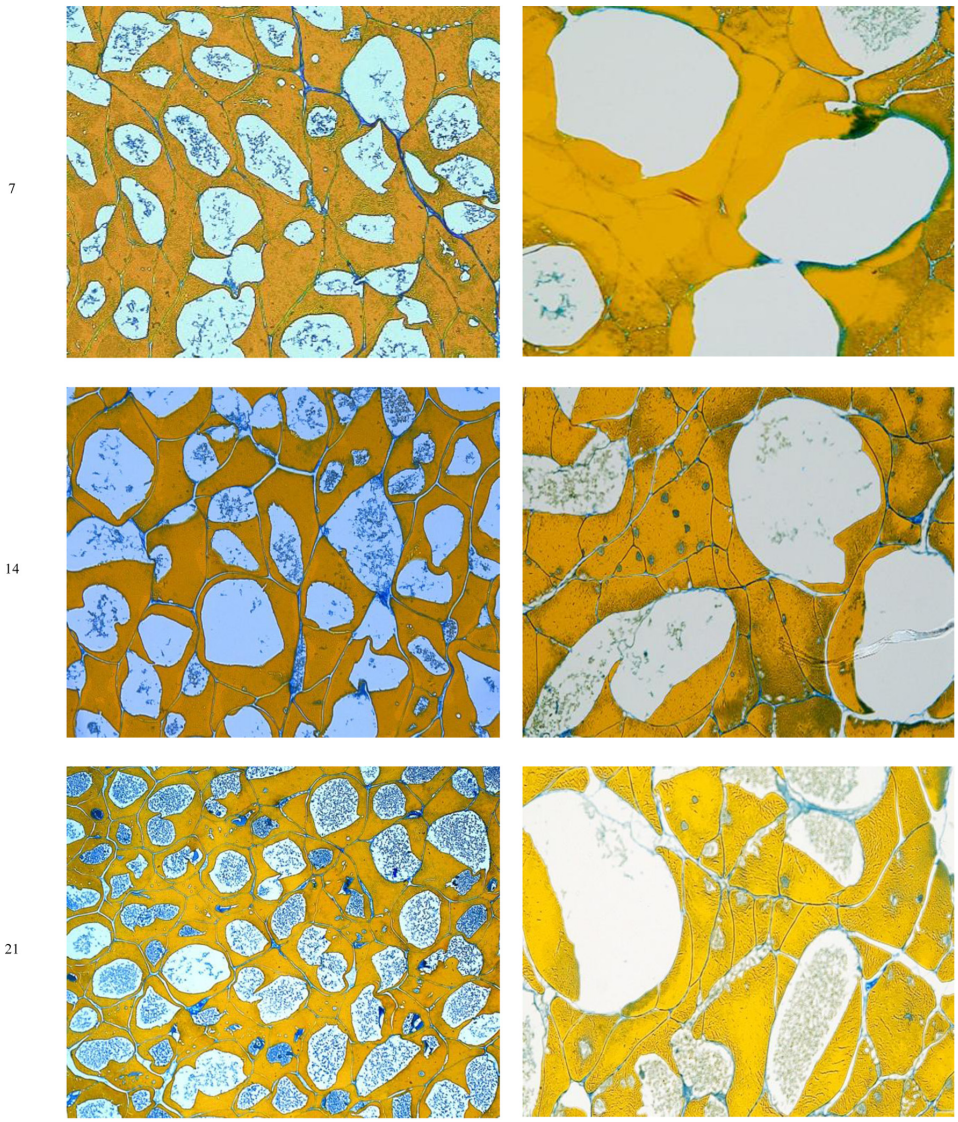

Fig. 4. (*continued*).

decrease in LL from 14 to 21 days of storage compared to day 7. An initial increase and subsequent decrease in LL has also been reported by Olsson et al. (2003) and Kristensen and Purslow (2001). It has been suggested that the increase in the WHC during storage is due to reduced water content described as the "leaking out" effect (Olsson et al., 2003). In this study, however, the water content in the salmon was unchanged (71%) during the entire storage time.

3.3. Drip loss during storage of superchilled salmon samples

The drip loss directly quantifies the loss of saleable weight and/or the deterioration of appearance, and further facilitates surface microbial growth (Duun, 2008). Between day 1 and 14 days of storage the drip loss was significantly lower in superchilled samples compared to chilled and frozen samples (Fig. 3). No significant difference $p < 0.05$ was found in drip loss between 1 and 14 days of storage. A significant increase in drip loss for the superchilled samples was observed at day 21. However, drip loss values between 1 and 2% cannot regard as high (Duun & Rustad 2008; Einen, Guerin, Fjæra, & Skjervold, 2002) therefore this cannot be considered as a major problem in the superchilled samples. The drip loss in the chilled samples increased significantly ($p < 0.05$) after 7 days of storage and the drip loss was higher than 2% after 14 days of storage.

These results clearly demonstrate that the superchilling temperature is low enough to substantially maintain the quality of

food products but high enough to avoid significant levels of ice crystal growth that can cause structural damage. In the study by Kaale, Eikevik, Bardal, and Kjorsvik (2013), a significant increase in the ice crystal sizes was observed. The ice crystal size in the superchilling process (day 0) was significantly smaller compared to that during the storage of superchilled samples, in fact the ice crystals in storage were 4 times larger than at day 0 (Kaale, Eikevik, Bardal, & Kjorsvik, 2013). This is due to thermal behaviour within the superchilled sample and accounting that we have both ice at the surface and water at the centre, an important diffusion process is expected during the storage of the superchilled food. This should produce recrystallisation. In addition, temperature fluctuation between superchilling process at −30 °C and superchilled storage temperature at −1.7 ± 0.3 °C should also contribute to recrystallisation. The influence of the storage temperature is well known, Martino and Zaritzky (cited by Blond & Meste, 2004) reported that the mean ice crystal diameter, which was 10 μm in beef muscle frozen at −40 °C, becomes equal to 40 μm after 150 h storage at −5 °C. Syamaladevi, Kalehiwot, Balasingam, and Shyam (2012) reported a significant increase in ice crystal size during the storage of salmon which was also attributed to temperature fluctuation.

Prior to temperature equalisation, ice crystal growth progresses from the surface to the centre of the superchilled food products (Fig. 4). No ice crystals were formed at the centre during the superchilling process (on day zero) (Fig. 4). The new ice crystals formed at the centre during storage were large, due to slow superchilling rate and these crystals could therefore damage the integrity of the superchilled product. It was reported that the ice crystals at the centre were 3 times larger than those at the surface (Kaale, Eikevik, Bardal, & Kjorsvik, 2013). However, it should also be noted that after temperature equalisation (temperature at the surfaces equal to temperature at the centres of the superchilled samples) and control of the temperature fluctuation during storage the increase of ice crystal sizes was not significant ($p < 0.05$) at any storage time (Kaale & Eikevik, 2013; Kaale, Eikevik, Bardal, & Kjorsvik, 2013; Kaale, Eikevik, Rustad, et al., 2013). Bevilacqua and Zaritzky (1982) reported that when temperature is constant, the recrystallisation occurs at significant rate only when the specimen contains crystals with diameters less than 24 μm. However,

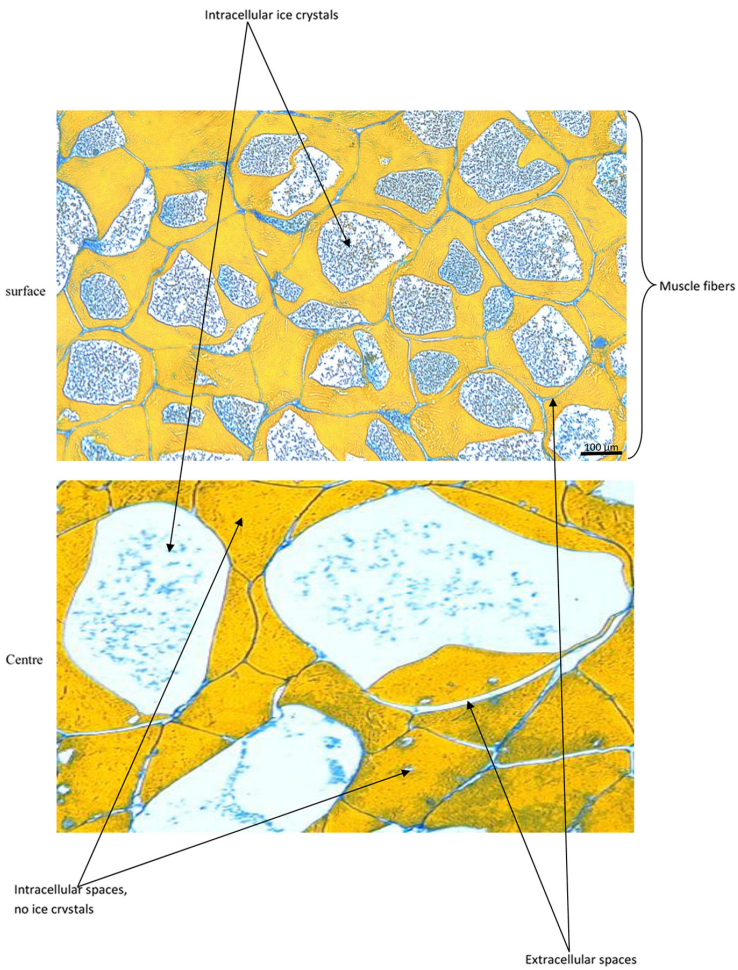

Fig. 5. Micrographs of superchilled salmon showing intracellular, extracellular spaces and distribution of ice crystals after 1 day of storage.

temperatures fluctuation enhance recrystallisation even if have larger diameters. The statistical analysis results for the ice crystal sizes are explained elsewhere (Kaale, Eikevik, Bardal, & Kjorsvik, 2013).

The results found in this study might be due to; in fish and meat tissues, the destructive effect of ice crystal formation is minimised due to the elasticity of the cellular structure in muscle (Smith, 2011). Nevertheless, the loss of quality in fish and meat is largely associated with the loss of protein functionality. When ice is formed, there is an increased concentration of enzymes and an increase in salt concentration in the remaining water, which cause protein denaturation and affect protein functionality (Shenouda, 1980; Smith, 2011). During superchilling, protein denaturation should be minimal because only a small amount (5–30%) of the water is frozen which would result in less enzyme and salt concentration in the remaining water.

Superchilling technology comprises the: shell freezing of food products followed by temperature equalisation during the superchilled storage. This technology allowed for a suitable mechanism of ice crystal formation (Figs. 4 and 5) that did not seem to affect the quality of food products compared to unprocessed product (chilled samples). The results from this study corroborate by previous studies which have been reviewed by Kaale et al. (2011). The review summarises the quality and shelf life of superchilled foods and the numerous benefits of applying superchilling technology to food products were found.

4. Conclusion

No significant differences were found in LL between surface and centre parts of the superchilled samples. There were no significant differences in the LL for the surface samples between 1 and 14 days of storage. For the centre samples, there was a significant difference in the LL at day 1, but no significant differences were observed between 3 and 14 days of storage. In contrast, a significant decreased in LL was observed at day 21 both at the centre and surface of the superchilled samples. No significant difference ($p < 0.05$) was found in drip loss between 1 and 14 storage days for the superchilled samples. A significant increase in drip loss for the superchilled samples was observed at day 21.

The small changes in LL and drip loss found in this study during superchilled storage of salmon (*S. salar*) fillets might be due to the required degree of superchilling (approximately 20%) and high superchilling rate used in this study. These findings are significant for the industry because it provides valuable information on the quality of food products in relation to ice crystallisation/recrystallisation during superchilled storage.

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Paper IX

The influence of superchilling storage methods on the characteristics of ice crystal during storage of Atlantic salmon (*Salmo salar*).

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Abstract

The objective of this work was to study the influence of superchilling storage methods, shell freezing (i.e. initial surface freezing of salmon and the followed storage at -1.7 ± 0.3℃) and non - shell freezing (i.e. storage of salmon at -1.7 ± 0.3℃ without initial surface freezing/shell freezing) on the characteristics of ice crystal (location/distribution) during the superchilled storage of salmon. Physical measurements, water holding capacity and drip loss were also studied. Ice crystals were mainly formed in the extracellular space in non-shell frozen samples. Fine and well-distributed ice crystals were formed both intracellular and extracellular spaces in shell frozen samples. Liquid loss (LL) decreased with storage time in both superchilling storage methods. There was no significant difference $p<0.05$ in LL between shell and non-shell frozen samples. There was a significant difference of LL in day 1 for the non-shell samples, and no significant differences were observed between 7 and 21 days of storage. There was significant difference $p<0.05$ in the drip loss between shell frozen samples (1.62% as maximum) and non-shell frozen samples (1.41 as maximum). It is however, well known that the values between 1-2% of drip loss cannot be regarded as high. Nevertheless, since most of the ice crystals were formed in extracellular spaces in non-shell frozen samples, the cells in the surface layer might have been destroyed. The samples in this study were taken randomly from surface to the centre and therefore it was not possible to detect the destructive effect in this layer. In the future it is necessary to study quality parameters separately at different locations (surface and centre) within the superchilled product in order to see if the formation of extracellular ice at the surface layer has an effect on the quality of superchilled food. It is also necessary to perform biochemical and microbiological analyses in both methods in order to compare their capabilities of storing foods in relation to shelf life and the quality of the final superchilled food.

Key words: Shell freezing/initial surface freezing, superchilling, salmon fish, liquid loss, drip loss

1. Introduction

1.1. Superchilling processes

The superchilling process is defined as a method of preserving food by partial ice-crystallization. The superchilling involve the conversion of some water to ice, the amount depending on the degree of superchilling required. The degree of superchilling in this context has been defined as the amount of free water frozen (5 – 30 %) inside the food product. Provided that the temperature is not permitted to go below (-2℃), superchilled food will not become rigidly frozen (Ronsivalli and Baker, 1981). The superchilling process is a slow process which allows the formation of large extracellular ice crystals which may affect the quality of the superchilled product. However, this depends on how one defines the superchilling. The concept of superchilling has been under continuous development for the last 10-20 years (Claussen, 2011). Even today, superchilling of foods is performed in different ways. Ando et al. (2004) defines it as the temperature zone below 0℃, but where ice crystals are not generated. Beaufort et al. (2009) defined superchilling as a technology where food is stored just below the initial freezing temperature. Bahuaud et al. (2008); Duun and Rustad (2008); Kaale et al. (2013b); (2013c); (2013d); Stevik et al. (2010) have performed superchilling by shell freezing the food products (initial surface freezing), followed by the storage of food at 1 – 1.5℃ below its freezing point. The latter case, the purpose of the initial surface freezing is to enhance heat transfer which facilitates temperature equalization within the food and hence a good mechanism of ice crystals growth. After shell freezing (frozen layer 1 – 3 mm from the surface) the ice distribution equilibrates, and the product obtains a uniform temperature at which it maintains during storage and distribution. Figure 1 shows the temperature equalization during the superchilled storage of salmon (Kaale et al., 2013c). This provides the food product an internal ice reservoir so that no external ice is required during transportation or short term storage.

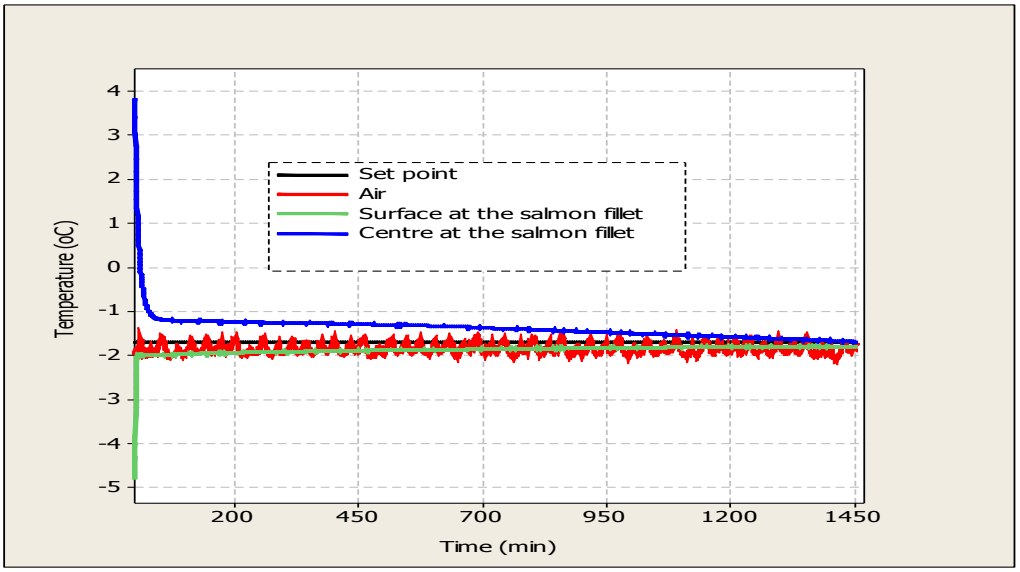


Figure 1: Temperature-time profile during superchilling storage at -1.7 ± 0.3 ℃

1.2. Water holding capacity and drip loss

During the superchilled storage of food products changes on the quality of food products may occur, such as the reduction of water holding capacity, the excessive increase of drip loss and other related quality parameters. These changes that occur in superchilled food products are the major concern for consumers. It is therefore important to assess the quality parameters, such water holding capacity, drip loss and other quality parameters during superchilled storage. The water-holding capacity (WHC) of food products is one of the most important factors as it not only affects economics but also their quality, because it affects the weight change during transport and storage, drip loss during thawing, weight loss and shrinkage during cooking, and the juiciness and tenderness of the meat (den Hertog-Meischke et al., 1997; Duun and Rustad, 2007; Gholam et al., 2010; Huff-Lonergan, 2002; Irie et al., 1996). 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 (Duun, 2008). Figure 2 shows the overview of muscle structure.

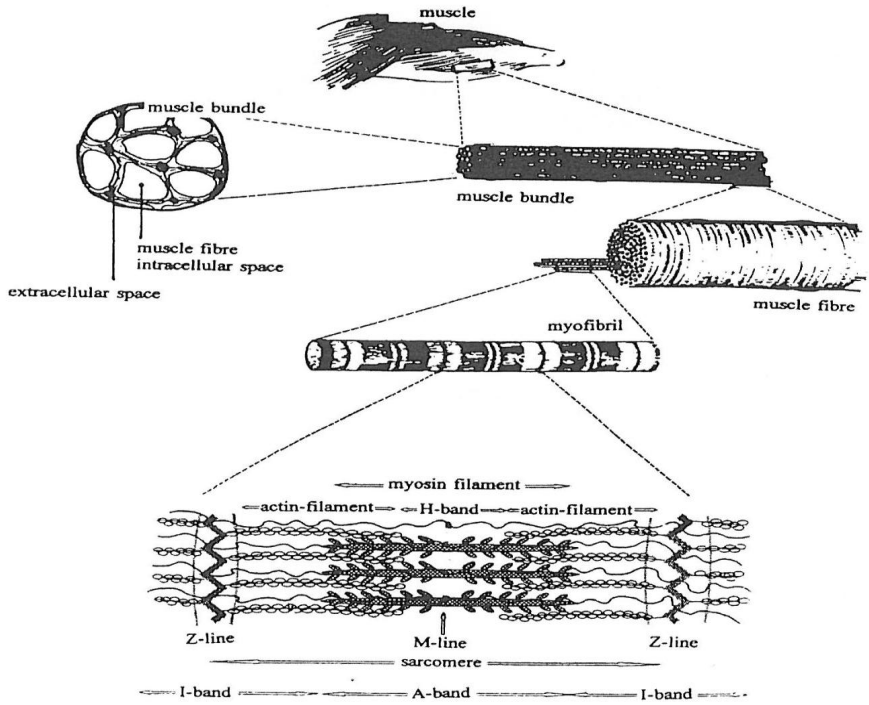


Figure 2: Schematic overview of muscle structure (den Hertog-Meischke et al., 1997)

Drip loss is also an important quality parameter to determine during the superchilled storage of the food products. High drip losses are undesirable because they are visually unattractive, lose soluble nutrients and flavour compounds, lose value due to weight loss, and results in dry, stringy texture (Duun, 2008; Kolbe and Kramer, 2007). The factors that affect the amount of drip include formation and size of ice crystals in the tissue, **location of ice crystals**, rate of thawing, internal pressure during freezing, irreversibility of water removal from cells (extent of water resorption), physiological status of tissue prior to partial freezing, and intrinsic water binding prior to partial freezing (Kolbe and Kramer, 2007).

The location is one of the characteristics of ice crystal, and is designated by the formation of ice crystals either extracellularly or intracellularly depending on the superchilling rate. The characteristics of ice crystals have a strong influence on the quality of the final superchilled food. Therefore, the objective of this work was to study the influence of superchilling storage methods, (shell freezing verses non - shell freezing) on the characteristics of ice crystals (location/distribution) during the superchilled storage of salmon. Physical measurements, water holding capacity and drip loss were also studied.

2. Materials and superchilling process

Salmon fillets (*Salmon salar*) 1–1.2 kg, were delivered by Lerøy Midnor (Hitra, Norway). The samples were vacuum-packed and stored at 4℃ for 24 h before the superchilling process to ensure a constant temperature in all samples. For the shell frozen samples, the superchilling process (initial surface freezing) was performed in an Impingement Advantec Lab Freezer (JBT Food - tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at -30℃ and 227 $W/m^2.K$ (at 2.5 kPa pressure differences of the fan at the impingement freezer) for 2.1 min to achieve an ice content of 20 %. The previous developed model (Kaale et al., 2013a) was used to predict the degree of superchilling. The experiments for measuring the surface heat transfer coefficient (SHTC) value were performed in an Impingement Advantec Lab Freezer. Details on the set-up of the experiments and the equation used to calculate SHTC is explained elsewhere (Kaale et al., 2013a). Once superchilled, the salmon samples were stored in a cold room at -1.7 ± 0.3℃ for 21 days. The non-shell frozen samples were also stored in the same temperature for comparison. Six fillets were analyzed at each sampling time (3 for shell freezing and 3 non-shell freezing).

2.1. Temperature trend during storage

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 x 73 x 54.5 cm) with a heating element inside to ensure adequate temperature regulation. The Pt100 temperature sensor was used to measure the air temperature in the storage box during storage of the superchilled salmon fillets. The set-point temperature was -1.7℃. The box was placed in a storage room, which holding a temperature of approximately -5℃.

2.2. Water holding capacity and drip loss

Liquid loss (LL) was determined on minced muscle by low speed centrifugation as described by the water holding capacity method of Eide et al. (1982). A centrifugal force of 270 g was used instead of 1500 g (Hultmann and 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℃ for 24 h. The analyses were run in duplicate.

For quantification of the drip loss, the sample was removed from the vacuum bag after thawed at 4℃ for approximately 24 hours and the liquid left in the bag was weighed. Calculation of the drip loss was based on the initial sample weight after thawing. Mean values were calculated from three triplicates.

2.3. Microscopic analysis

Two pieces were cut from the surface layer to the bottom layer of each superchilled sample (-1.7 ± 0.3℃) transversally to the muscle fibre using a standard knife blade that was previously stored at -1.7 ± 0.3℃ This procedure was conducted in a walk-in freezer to ensure a perfect cold chain. In this study, a fixation method similar to those proposed by Alizadeh et al. (2007); Martino and Zaritzky (1988) was used to observe the spaces left by the ice crystals in the tissue. The samples were fixed by immersion in Clarke's solution (absolute ethanol and glacial acetic acid, 3:1) at -1.7 ± 0.3℃ for 24 h. The control (unprocessed) samples were fixed with the same solution but at 4℃. The fixed samples were then brought to room temperature and were dehydrated with absolute ethanol. The dehydrated samples were then embedded in paraffin. The embedded samples were cut transversally to the muscle fibre using a microtome (Autocut 2055, Leica Microsystems, Germany) into 4 μm thick slices. The sliced samples were then stained according to a method developed by Alizadeh et al. (2007) with some modifications: Neo-clear (Tissue Clear) was used for rehydration, the samples were immersed in 1% blue aniline for 1 min, and xylene was used before mounting. All the prepared slides were observed with a microscope (Zeiss Axioskop 2 plus, Zeiss Inc., Germany) fitted with a digital camera (Nikon DS-5M, Nikon, Japan).

2.4. Statistical analysis

The observations of the water holding capacity and drip loss with respect to storage days were analysed by one- and two-way analyses of variance using Minitab 16 software. A general linear model, (post- hoc test) under Tukey's simultaneously test was applied whenever the ANOVA results were significant, $p < 0.05$.

3. Results and discussion

3.1. Thermal transition behavior during superchilled storage of food products

Figure 3 shows the time - temperature profile during storage of the superchilled salmon. The temperature in the storage box was strictly controlled at -1.7 ± 0.3℃. The temperature in the samples was measured at each sampling time. After 1 day of storage (24 h) the temperature in non-shell frozen samples was -1.4 and -0.3℃ at the surface and centre, respectively. The temperature at the centre of non-shell frozen samples decreased slowly and after 21 days of storage, was about -0.78℃. In shell frozen samples, the temperature equalization was achieved within the samples after 1 day of storage (i.e. the surface and centre temperatures were the same after 1 day of storage). This result (in shell frozen samples) is similar with that reported by Kaale et al. (2013b; 2013c; 2013d).

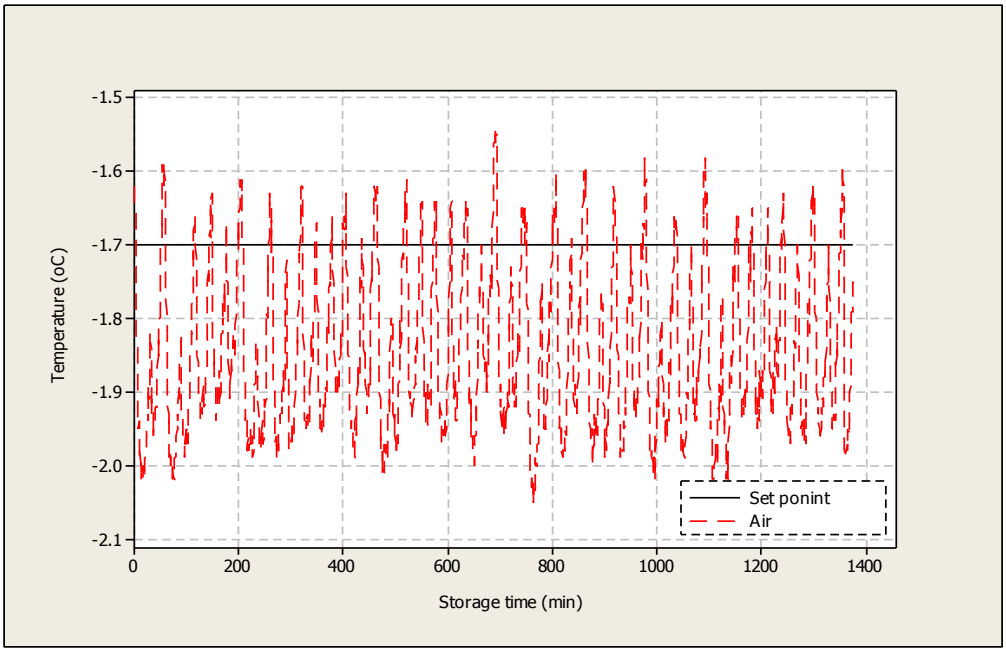
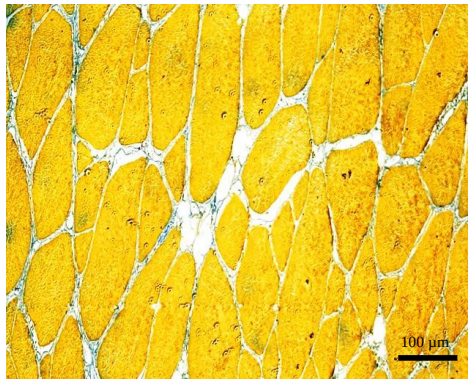


Figure 3: Time - temperature profile during storage of the superchilled salmon fillet.

3.2. Ice crystal evolution during superchilling process and following storage of the superchilled products.

Pre-superchilled (unprocessed) salmon muscle was used as a control for the purpose of microstructure comparison with the superchilled samples. In non- shell frozen samples, the ice crystals were mainly formed in extracellular spaces (Figures 4) during superchilled storage. The location of the ice crystals depends mainly on the superchilling rate. The extracellular ice crystals observed in non-shell frozen samples are due to the slow superchilling rate, which is generally considered to form extracellular ice crystals. There were no ice crystals formed at the centres of non-shell frozen samples for the entire storage time (after 21 days of storage) Figure 4. However, it was not clear how deep the ice crystals were formed from the surface.

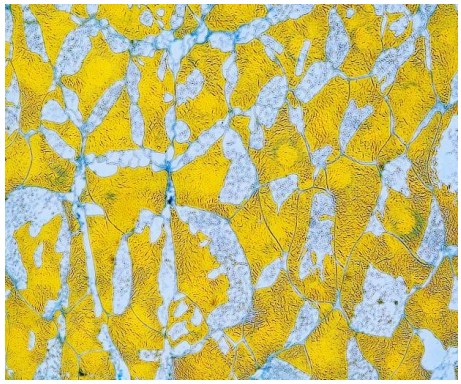


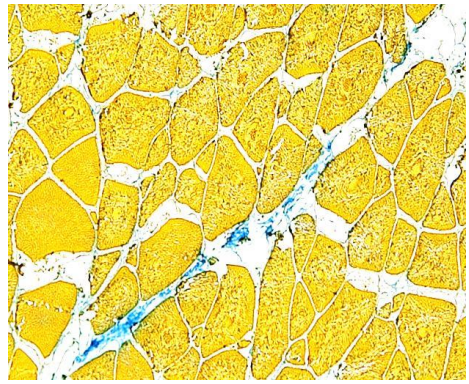
Control

surface

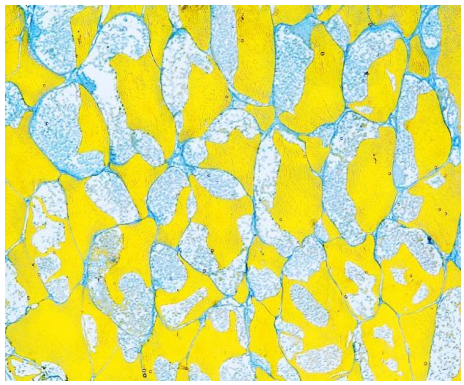
centre

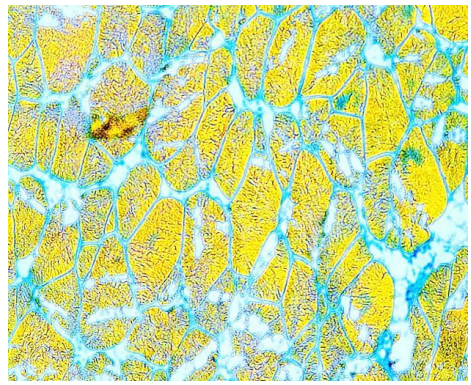
Day1





Day7





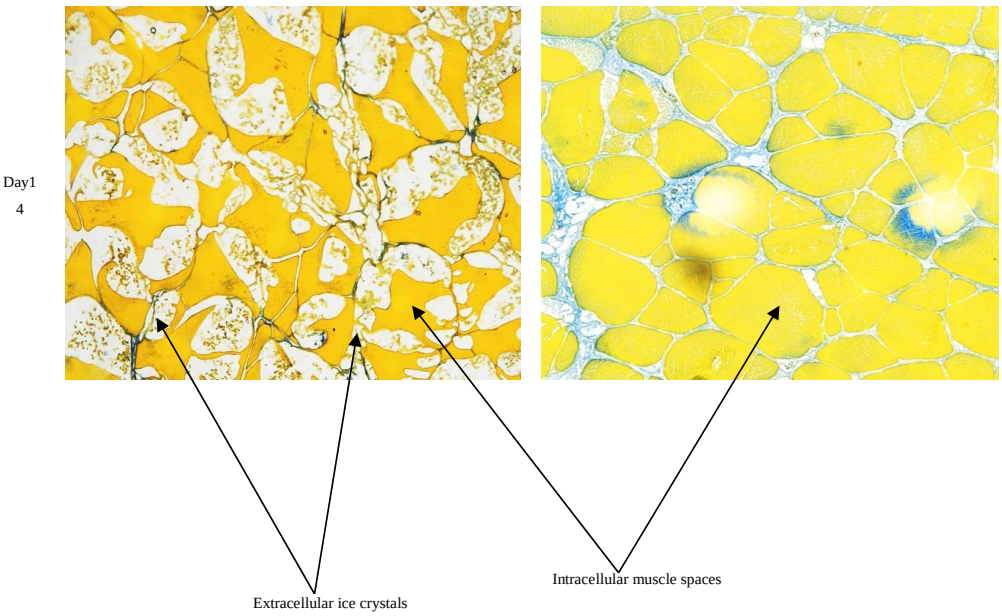
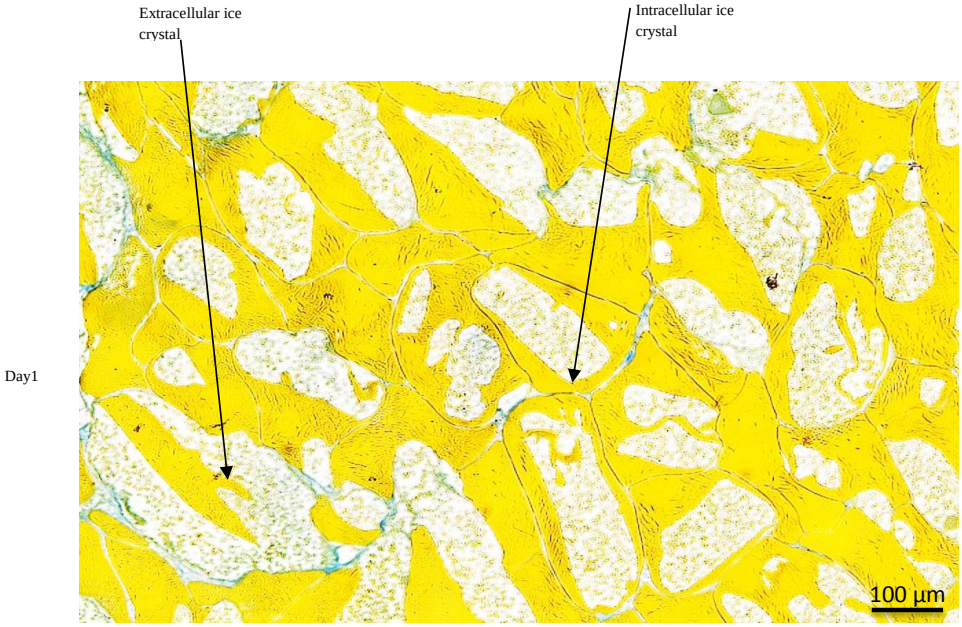
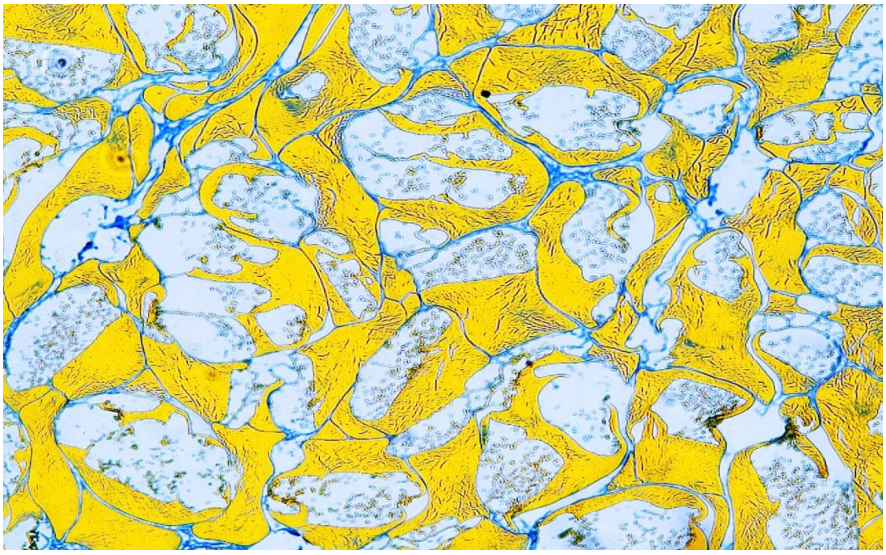


Figure 4: Micrographs of unsuperchilled and superchilled salmon tissues (non-shell frozen samples), show extracellular ice crystals.

It was also interesting to observe the formation of ice crystals in intracellular spaces though the superchilling rate was very low and there was no pre-treatment of the samples Figure 5.



Day 7

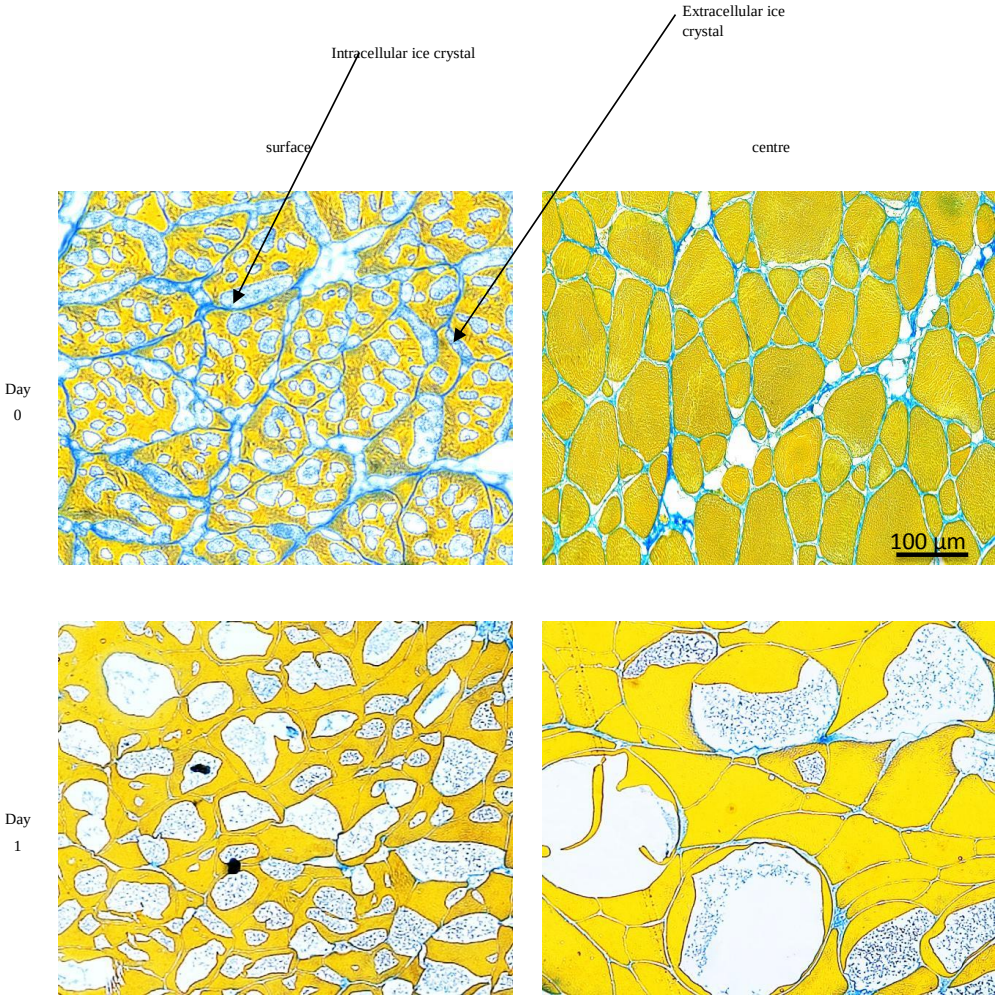


Day 14

Figure 5: superchilled salmon tissues (non-shell frozen samples) show intracellular and extracellular ice crystals.

In shell frozen samples, the ice crystals were analysed on days 0, 1 and 14. The statistic results were not performed in this study because similar studies (Kaale et al., 2013b; 2013c; 2013d) with the same conditions (-30℃, 227 W/m^2.K and 2.1 min) have been carried out, and the development of ice crystals was studied intensely. Figure 6 clearly shows that the ice crystals location in shell frozen samples was different from that in non-shell frozen samples. On day 0 (initial surface freezing), the ice crystals at the surface were smaller, and finely distributed inside and outside the cells. However, this advantage was reduced during superchilled storage due to the rapid increase of ice crystal sizes

(recrystallization) Figure 6. In a similar study, Kaale et al. (2013a); (2013b); (2013c) reported that, the ice crystal size was 4 times larger after only one day of superchilled storage, compared to the size of the ice crystals during the superchilling process (day 0). This is due to the temperature fluctuation between the superchilling process -30℃ and superchilling storage temperature -1.7 ± 0.3℃. The influence of the storage temperature is well known, Martino and Zaritzky (cited by Blond and Meste, 2004) reported that the mean ice crystal diameter, which was 10 µm in beef muscle frozen at -40℃, becomes equal to 40 µm after a 150 h storage at -5℃. Syamaladevi et al. (2012) reported significant increase of ice crystal sizes during storage of salmon due to temperature fluctuation.



Day 14

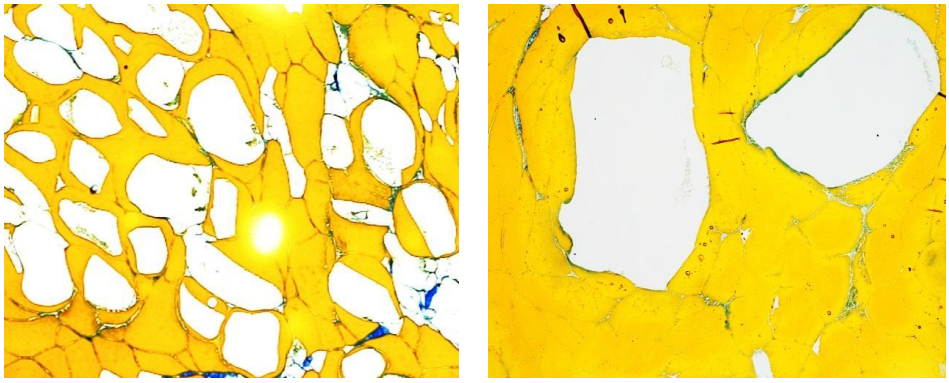


Figure 6: superchilled salmon tissues (shell frozen samples) show intracellular and extracellular ice crystals.

Prior to temperature equalisation, ice crystal growth progresses from the surface to the centre of the superchilled food products. No ice crystals were formed at the centre during the superchilling process (on day 0) Figure 6. Kaale et al. (2013b); (2013c); (2013d) reported that the ice crystals at the centre of the superchilled salmon were 3 times larger than those at the surface. This was due to the slow superchilling rate and thermal behaviour within the superchilled sample. Details on ice crystal development during superchilling process and storage are explained elsewhere (Kaale et al., 2013b; 2013c; 2013d)

3.3. Liquid loss (LL) and drip loss during storage of shell and non-shell frozen samples

Quality changes have been studied with the focus on water holding capacity and drip loss. The results showed that liquid loss (LL) decreased with storage time (i.e. the increasing of water holding capacity with storage time) both for the shell and non-shell frozen samples Figure 7. However, LL and drip loss in shell frozen samples were analysed only on days 7 and 14 of storage because a similar study (Kaale et al., 2014) with similar conditions (-30℃, 227 W/m2.K and 2.1 min) has been carried out, and LL was studied intensely. There was no significant difference $p<0.05$ in LL between shell and non-shell frozen samples at 7 and 14 days of storage. There was significant difference of LL on day 1 in non-shell samples, and no significant differences were observed between 7 and 21 days of storage. The increase in WHC (decrease in LL) with storage time has been observed by Kristensen and Purslow (2001) in pork, Olsson et al. (2003) in halibut muscle, Duy et al. (2007) during the superchilling of arctic charr (*Salvelinus alpinus*) fillets and Kaale et al.(2014) during the superchilled storage of Atlantic salmon. Duy et al. (2007) claimed that, the decreases in LL (increases in WHC) may partly have been caused by a higher ratio of the loosely bound water which was released as drip loss with time and proteolytic activity in the muscle during storage.

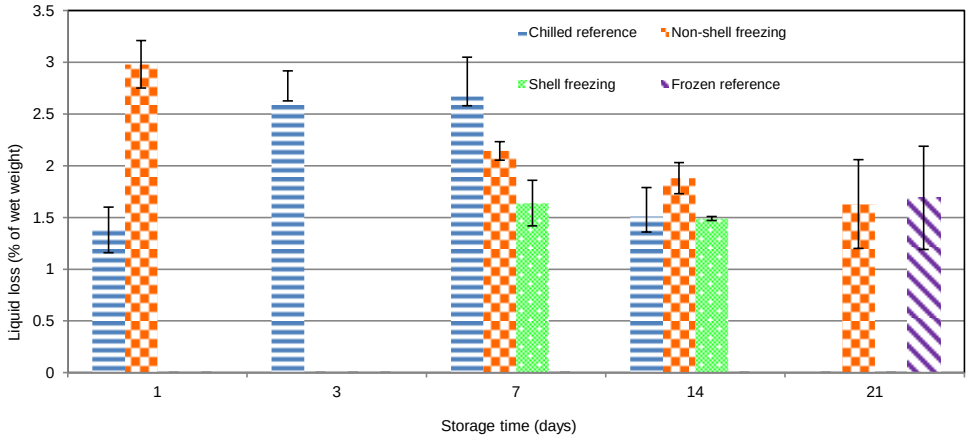


Figure 7: Changes in liquid loss of salmon fillets during superchilled storage.

WHC plays an important role in the partial freezing of food products because it relates to the quality of the final superchilled product. Since LL is highly related to structural changes in the muscle (Erikson et al., 2011; Olsson et al., 2003), the results from our study indicated that there was no significant change in the protein network that occurred during the storage time. In our previous study Kaale et al. (2014) there was also no significant difference between 1 and 14 days of storage however, the significant decreased of LL was observed on day 21 of storage.

In the chilled samples, the LL was increasing from day 1 to day 3, $p<0.05$ and there was no significant difference between 3 to 7 days of storage. However, the LL decreased significantly ($p<0.05$) after 14 days of storage compared to day 7. The initial increase and subsequent decrease in LL has also been reported by Olsson et al. (2003); Kristensen and Purslow (2001) and in our previous study Kaale et al. (2014). It has been suggested that the increase of the WHC is due to reduced water content described as the ‘‘leaking out’’ effect (Olsson et al., 2003).

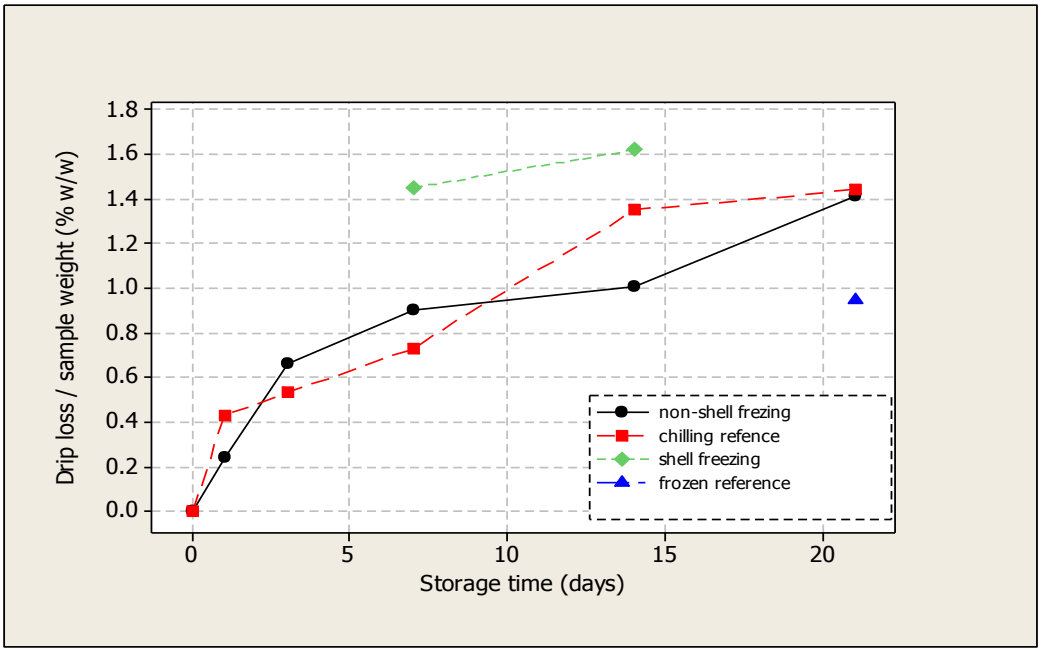


Figure 8: Linear plot showing drip loss vs. storage time.

Drip loss is an important meat quality aspect which is perceived by consumers as juiciness, appearance and colour of product (Kragten and Bee, 2010). Figure 8 shows the drip loss for the shell and non-shell frozen samples during superchilled storage, chilled and frozen references during storage. The drip loss in shell frozen samples was significantly higher than in non-shell frozen, chilled and frozen samples. The drip losses were 1.62 and 1.41% (as maximum values) in shell and non-shell frozen samples, respectively. It has, however, been reported that the values between 1-2% of drip loss cannot be regarded as high (Duun and Turid 2008; Einen et al., 2002) and cannot be considered as a major problem in superchilled salmon. There was also no significant difference between non-shell frozen and chilled samples. These results are different from those reported by Kaale et al. (2014) which observed that the drip loss between 1 and 14 days of storage was significantly lower in superchilled samples (shell frozen samples) compared to chilled and frozen samples. The drip loss directly quantifies the deterioration of appearance and further facilitates surface microbial growth (Duun, 2008). The changes of drip loss observed in this study compared to the previous one might be due to the good quality of the material. Blond and Meste (2004) reported that the raw material quality is important, and this quality must be preserved during processing and storage. However, the age of the materials for both studies was not known. Nevertheless, since the drip loss was between 1-2%, the results from our study indicated that no significant change in the texture had occurred during superchilled storage.

4. Conclusion

There was no significant difference $p<0.05$ in LL between shell and non-shell frozen samples during superchilled storage (sampling days for both methods).There was a significant difference of LL in day 1 for the non-shell samples, and no significant differences were observed between 7 and 21 days of storage. There was a significant difference $p<0.05$ in drip loss between shell frozen samples (1.62% as maximum) and non-shell frozen samples (1.41 as maximum). It is however, well known that the values between 1-2% of drip loss cannot regarded as high.

Nevertheless, since most of the ice crystals were formed in extracellular spaces in non-shell frozen samples the cells in the surface layer might have been destroyed. The samples in this study were taken randomly from the surface to the centre and therefore it was not possible to detect the destructive effect in this layer. In the future it is necessary to study quality parameters separately at different locations (surface and centre) within the superchilled product in order to see if the formation of extracellular ice at the surface layer has an effect on the quality of the final superchilled food. It is also necessary to perform biochemical and microbiological analyses in both methods in order to compare their capabilities of storing foods in relation to shelf life and the quality of the final superchilled food. If non-shell freezing method will give positive impact on the quality of the final superchilled food, this might be a good alternative of doing/performing superchilling because is cheaper than shell freezing method. Non-shell freezing does not need initial surface freezing (i.e. no need of freezer), will only need a flexible and effective storage facility.

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