

Kirsti Greiff

Sodium reduction in muscle foods

Analytical methods for measuring sodium and changes in the food matrix during sodium reduction in muscle foods.

Thesis for the degree of Philosophiae Doctor

Trondheim, June 2015

Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Biotechnology



NTNU – Trondheim
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of Philosophiae Doctor

Faculty of Natural Sciences and Technology
Department of Biotechnology

© Kirsti Greiff

978-82-326-0974-1 (print)
978-82-326-0975-8 (digital)
1503-8181

Doctoral theses at NTNU, 2015:159

Printed by NTNU Grafisk senter

SUMMARY

Salt (NaCl) is the world's most established food additive, because of its excellent preservative effects, its positive effect on technological and sensory properties, and low cost. This combination of factors has resulted in salt being used at higher levels than necessary in many food products, particularly processed foods. However, a high consumption of sodium is also associated with an increased risk of high blood pressure, which has been found to be a significant contributor to the development of cardiovascular diseases and strokes in humans. In addition, a high salt intake has been linked to increased risk of stomach cancer, renal stones, and decreased bone mineral density. For these reasons, the WHO recommends a decrease in sodium consumption, to a total of 5 g NaCl per person per day (2 g Na per day). To reach the WHO target, the food industry therefore has to reduce the sodium content in their products by 30 -50%. A reduction of this magnitude could lead to challenges with regards to processing, physicochemical properties, yield, sensory attributes, texture, and food safety of the products. Partial replacement of the sodium salt by salt replacers and other ingredients will therefore be necessary.

Given this, the main objectives of the present thesis are 1) to evaluate different analytical methods for measuring sodium in a food matrix, and to evaluate changes in this food matrix as a function of sodium reduction; and 2) to increase the theoretical understanding of the effect of salt and salt replacers on food sensory and physicochemical properties. The first objective is of importance given the high focus on the necessity of salt reduction in food products, and the increased use of salt replacers such as potassium chloride (KCl) in its stead. This makes it necessary to find new rapid techniques for determining the sodium content in products. For the purposes of this study, the following four methods were evaluated: (1) *Impedance spectroscopy*, (2) *LF-NMR T_2 relaxation method (LF-NMR)*, (3) *Computer Vision*, and (4) *sodium ion-selective electrode*. The methods were applied to food matrixes containing different amounts of salt (NaCl), and the salt replacers KCl and magnesium chloride (MgCl₂). The model products consisted of minced fish prepared from hake (*Merluccius paradoxus/capensis*) and haddock (*Melanogrammus aeglefinus*). These products had different amounts of pure salts and mixtures of salts added beforehand, and were analyzed using the different methods above. Knowledge transfer from research in lab-scale to industry requirements for commercialization has been of utmost importance in

the work with this thesis. Cooked ham of pork and fish pudding were therefore prepared with a more complex composition of ingredients in order to investigate the effect of salt reduction on physicochemical and sensory properties of the final products as well.

The work in this thesis has demonstrated that the method (1) *impedance spectroscopy* could be a potential technique for monitoring a_w in low-salt low-fat fish mince, in that it is able to indirectly predict the amounts of salt in the sample. The (2) *LF-NMR* measurements on both raw and cooked fish mince added low levels of salt (0-3%), showed that this method was sensitive to the resultant changes in protein structure of the products due to the salt addition. The results of this thesis furthermore confirmed that the (3) *multimodal machine vision system (Computer Vision)* showed changes in lightness as a function of reduced salt content in cooked ham, and that the (4) *sodium ion selective electrode* is another good method for direct measurement of sodium in low-salt low-fat food products.

The extractability of salt soluble proteins (SSP), were found to increase with higher concentration of salt in the products. The results indicated that Na^+ can partially be replaced with K^+ and Mg^{2+} without changing the solubility of proteins. However the addition of Mg^{2+} should only be used in small amounts, due to the negative effect on protein solubility at 0.55 M MgCl_2 . Freezing and thawing of haddock fillets furthermore decreased the solubility of SSP and influenced the physicochemical properties in cooked fish minces, adding other types of cations than Na^+ did not compensate for these changes.

This work also confirmed that the solubility of proteins affect the quality in the final product. This was demonstrated by both the Water-holding capacity (WHC) and breaking force increasing with higher levels of SSP in the study on fish pudding, for instance. A linear relation between breaking force and WHC was also found. In the model product made of haddock mince, with added water and different amount of pure salts, salt reduction led to an increase in moisture, reduced WHC, increased cooking loss and a decrease in breaking force. The pH also increased with decreasing salt content. Replacing NaCl with pure KCl had no, or only a small, effect on the pH, moisture, breaking force, WHC and cooking loss in the model products. Replacing NaCl with an equal molar concentration of MgCl_2 , however, affected several of the physicochemical properties and in particular WHC, cooking loss and pH.

In light of this, and in summation, a partial replacement of Na⁺ - ions with K⁺ - ions is possible without changing the protein solubility and the physicochemical properties in fish products. A reduction of salt from 1.0 down to 0.6%, without any salt replacers, affected the taste experience more than the physicochemical properties in fish pudding. Based on the investigated factors in this study a 40% sodium reduction is possible in fish pudding using high mineral permeate as a salt replacer. The study on cooked ham, however, showed that only a 25% replacement of Na⁺ - ions with K⁺ - ions was possible without changing the quality in the final product. However, when the sodium was reduced by more than 35% (< 2.1% salt in the product), the salt reduction influenced both the sensory and physicochemical properties negatively.

ACKNOWLEDGEMENT

This work has been carried out at SINTEF Fisheries and Aquaculture (SINTEF), and the Norwegian University of Science and Technology (NTNU), Department of Biotechnology in the period April 2009 to April 2015. The work on this thesis was funded by the National Research Council of Norway, KMB project Low-Salt products, id. 185063/110.

First of all I would like to thank my supervisors **Turid Rustad** (NTNU), **Ida G. Aursand** (SINTEF) and **Marit Aursand** (SINTEF). Professor **Turid Rustad** is deeply thanked for always having the time to help me, for all the valuable support during scientific writing, and believing that I could manage to be a PhD someday. Research manager PhD **Ida G. Aursand** is sincerely thanked for always supporting me, for sharing her knowledge and experience in the field of salting of food and measurement techniques, and for constructive feedback on my scientific writing.

Special thanks and gratitude belong to Research Director at SINTEF Fisheries and Aquaculture, PhD **Marit Aursand** for accepting me as a PhD student, and giving me the opportunity to work as a research scientist at SINTEF. Her good advice, motivation and support have been of great importance to keep up my motivation.

During this period I was lucky to receive an overseas research grant from the Norwegian Research Council for being a guest scientist at the Polytechnic University of Valencia in Spain (three months during the summer 2012). A special thank goes to **Ana Fuentes** and her co-workers at the Department of Food Science and Technology. **Ana** introduced me to the Impedance spectroscopy measurement technique, and gave me useful input on how to plan an experiment for scientific writing of a paper. She was greatly helpful in writing my first accepted paper. I and my family had a great time in Valencia.

Huge thanks goes to all of my co-authors: **Irina Viktorovna Andreetta-Gorelkina**, **Ulf Erikson**, **Ekrem Mismi**, **John Reidar Mathiassen**, **Kjell D. Josefsen**, **Rafael Masot**, **Miguel Alcañiz**, **Margrethe Hersleth**, **Berit Nordvi** and **Charlotte Staurem** for excellent collaboration and valuable discussions. Thank you for contributing with your ideas and knowledge. Thanks to **Marte Schei** for helping me with the experiments and some of the analytical work. I really appreciate it!

I would like to thank my colleagues and friends at SINTEF Fisheries and Aquaculture for inspiring discussions and support during my work with the thesis and for providing a great and friendly working environment. A special thanks to **Inger Beate, Eirin** and **Hanne** for being great company during my thesis writing breaks. Thank you to my office-neighbour **Vera**, for your advice on scientific writing, and tips and tricks on writing in Word.

Thank you to my friends, **Brita** and **Sigfrid**, for patiently listening to my ups and downs.

I wish to thank my parents and my family in law, for always supporting and believing in me. Thank you for your care and support!

Finally, I will thank my lovely children **Sindre, Synne** and **Even** keeping my mind on more important things in life than salt reduction in food. I am greatly thankful to my dear husband, **Anders**, for always listening to me, supporting and encouraging me. I am deeply grateful for that he took care of everything at home during the last months so I could focus on finishing my PhD work. We are a great team, **Anders!**

TABLE OF CONTENTS

1	Introduction	1
2	Objectives	3
3	Background	4
3.1	Salt (NaCl)	4
3.2	Salt and health	4
3.2.1	Negative health effects of sodium consumption	5
3.2.2	Health benefits of reducing the sodium intake	5
3.2.3	Health authorities and industry's perspective	6
3.3	Muscle and salt	7
3.3.1	Structure of muscles	8
3.3.2	Salt and functional properties in muscles	11
3.4	Salt reduction strategies	16
3.4.1	Substitution of sodium by potassium chloride (KCl)	20
3.4.2	Substitution of sodium by magnesium chloride	20
3.4.3	Dairy-based salt replacers	21
3.5	Effect of sodium reduction on taste	21
3.6	Effect of sodium reduction on microbial food safety	22
3.7	Salt reduction in heavily salted fish and meat products	23
4	Methodology	25
4.1	Impedance spectroscopy	25
4.1.1	Sensors	27
4.2	Low-field NMR	28
4.3	Computer Vision	30
4.3.1	The principles of SINTEF Food Scanner	31
4.4	Sensory analysis	33

4.5	Salt determination by sodium selective electrode and chloride titration	33
4.5.1	Salt content (NaCl) of the muscle - Volhard method	34
4.5.2	Sodium ion selective electrode	34
4.6	Protein solubility	35
4.7	Different measurement techniques used for measuring sodium and salt (NaCl), an overview.....	37
5	Results and discussion	39
5.1	Analytical methods for measuring sodium and changes in the food matrix during sodium reduction in muscle foods (Paper I and II).....	40
5.1.1	Evaluation of the impedance spectroscopy method for determination of salt and physicochemical changes in the fish mince (Paper I).....	40
5.1.2	Determination of changes in proton mobility in low-salt fish mince measured by LF-NMR (Paper I and Paper III)	42
5.1.3	Developing a multimodal <i>machine vision system</i> for fast and non-destructive measurements of textural changes in the surface of low-salt ham.	46
5.1.4	Determination of sodium with Sodium ion-selective electrode (ISE)	48
5.2	Understanding of the mechanism of salt and salt replacers in a food matrix.	49
5.2.1	Salt reduction and salt replacers effect on protein solubility. (Paper IV and V)	49
5.2.2	The effect of salt reduction and replacing of sodium with K ⁺ or Mg ²⁺ -ions on physicochemical properties (Paper I and Paper III)	52
5.3	Possibilities for salt reduction in final products (Paper II and V)	55
5.3.1	Sodium reduction in Fish pudding (Paper V).....	55
5.3.2	Development of cooked ham with reduced content of sodium (Paper II)	58
6	Conclusions and future perspectives.....	60
7	References	62
Part II	73

LIST OF PAPERS

- I. Greiff, K., Fuentes, A., Aursand, I. G., Erikson, U., Masot, R., Alcañiz, M., & Barat, J. M. (2014). **Innovative Nondestructive Measurements of Water Activity and the Content of Salts in Low-Salt Hake Minces.** *Journal of Agricultural and Food Chemistry*, 62(12), 2496-2505. doi: 10.1021/jf405527t
- II. Greiff, K., Mathiassen, J. R., Misimi, E., Hersleth, M., & Aursand, I. G. **Gradual Reduction in Sodium Content in Cooked Ham, with Corresponding Change in Sensorial Properties Measured by Sensory Evaluation and a Multimodal Machine Vision System.** *PLOS one*. Submitted
- III. Greiff, K., Aursand, I., Erikson, U., Josefsen, K. D., & Rustad, T. **Sodium reduction in minced fish products by adding Magnesium and Potassium Chloride; effect on physicochemical properties.** *LWT- Food Science and Technology*. Revised version resubmitted.
- IV. Andreetta-Gorelkina, I. V., Greiff, K., Rustad, T., & Aursand, I. G. (2015). **Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins.** *Journal of Aquatic Food Product Technology*. doi: 10.1080/10498850.2013.879241
- V. Greiff, K., Staurem, C. J., Nordvi, B., & Rustad, T. **Novel utilization of Milk-Based Ingredients in salt reduced Fish Pudding.** *LWT- Food Science and Technology* 63 (2015), pp. 92-99. doi:10.1016/j.lwt.2015.03.073

Contributions

The author, Kirsti Greiff (KG), was responsible for planning and performing the experimental work (production trials, lab experiments and analysis), participating in the evaluation of the results and was responsible for writing the manuscript in Paper I, Paper II and Paper III. KG also participated in the planning and performing of the experimental work (production trials and lab experiments), in evaluating of the results and was involved in the writing in paper IV. She furthermore participated in the planning of the experimental work, in the evaluation of the results and was responsible for the writing of paper V. Ida G. Aursand (IGA) participated in planning the experimental work, in the evaluation of the results, and was involved in the manuscript writing in Paper I, Paper, II, Paper III and Paper IV. IGA was also responsible for processing the LF-NMR data in Paper I and Paper III. Ana Fuentes participated in the planning and evaluation of the work and contributed to writing the manuscript in paper I. Rafael Masot and Miguel Alcañiz contributed to the experimental setup, analysing the results of the Impedance Spectroscopy measurement and was involved writing the manuscript in Paper I.

Jose Manuel Barat participated in the planning of the experiment in Paper I. Ulf Erikson participated in planning of the experiment and the evaluation of the results in Paper I and Paper III, and was involved in the manuscript writing in Paper I and III. John Reidar Mathiassen contributed to the design of the study and the experimental setup of the Multimodal machine vision system, analysis of the images, and contributed to writing the manuscript in Paper II. Ekrem Misimi contributed to the design of the study and the experimental setup of the Multimodal Machine Vision System and conducted the image acquisition. Margrethe Hersleth managed the Sensory Descriptive Analysis and was involved in the manuscript writing in Paper II. Kjell D. Josefsen participated in planning of the experiment, evaluation of the results and contributed to writing the manuscript in Paper III. Berit Nordvi was involved in the planning of the experiment and in writing the manuscript in Paper V. Turid Rustad was the corresponding author in Paper IV, she was involved in planning of the experiment, evaluation of the results and contributed to the writing in Paper III, Paper IV and Paper V. Irina Victorovna Andretta-Gorelkina participated in the planning of the experiment and responsible for the protein solubility analysis, evaluation of the results and writing in paper IV.

1 INTRODUCTION

Salt (NaCl) has been exceptionally important to humans for thousands of years because of its food preservation properties. The location of salt deposits was particularly relevant in ancient Rome, ancient Egypt and Middle East (Albarracín et al., 2011), where the Egyptians called salt "natron", which means "divine salt". The Latin term "Salarium", furthermore, is derived from salt and refers to the amount of salt that was given to a worker or Roman legionary as payment for his job. History also illustrates how the Vikings (800-1050 A.D.) brought salt to the Nordic countries, and these countries have a large assortment of traditional salty foods today (Christie, 2007).

Salt is furthermore the world's most established food additive, because of its excellent preservative effects, sensory effect, its positive effect on technological properties, and low cost. This combination of factors has resulted in salt being used at higher levels than necessary in many food products, particularly processed foods (Brandsma, 2006). A high consumption of sodium by humans has also been directly associated with a greater likelihood of increased blood pressure, which in turn has been directly related to the development of cardiovascular and renal diseases (He & MacGregor, 2002). For these reasons, national and international bodies have set the recommended targets for sodium consumption at a maximum of 5 g NaCl per pers per day (2 g Na per day), whereas the current average is 8-12 g NaCl per pers per day (Brandsma, 2006; Kilcast & Angus, 2007).

Given the necessity of food producers to reduce salt (NaCl) content in products for human consumption in order to meet the targets of the national and international bodies, the first method of choice is naturally adding less salt. To reach the reduced target of 5 g NaCl per day, the food industry however needs to reduce the salt content in some food products down to critical levels with regards to palatability, texture, processing yield and shelf-life of the product. A partial replacement of salt with other ingredients will therefore be necessary since manufacturers are de facto faced with the dilemma of reducing the salt content of foods without losing the desired and expected quality in the product. While some alternative ingredients can perform a few of the functions of salt, no other ingredient has all the functions offered thereof. Thus, alternative combinations of functional ingredients and/or processing technologies must be developed or optimized. This requires a full understanding of the technological challenges associated with salt

INTRODUCTION

reduction, as well as a better theoretical understanding of the observed mechanisms and roles of salt in food products.

In Norway, 24% of the daily salt intake by humans comes from meat products and 11% comes from fish products (The Norwegian Directorate for Health, 2012). If the meat and fish producers are able to reduce the salt content in these products alone, they will contribute to reaching the targets for salt reduction and provide healthier food for the consumer, and thereby contribute to lower health risks from food consumption by the general population. For this reason, the main focus in the current thesis has been salt reduction in processed lean meat and fish products.

For a deeper understanding of the effects of salt and salt replacers on muscle proteins and physicochemical properties in fish mince, it was efficient to use pure salts and develop model products containing raw minced fish made of hake (*Merluccius paradoxus/capensis*) and haddock (*Melanogrammus aeglefinus*), to which the pure salts were added. Knowledge transfer from research in lab-scale to real-life applicability to industry requirements for commercialization has been of utmost importance in the work with this thesis as well. Cooked ham of pork and fish pudding was prepared with a more complex composition of ingredients order to investigate the effect of salt reduction on physicochemical and sensorial properties of the final products.

The following thesis therefore answers the calls of the national and international bodies that challenge food producers to have a high focus on salt reduction in food products today. Given that sodium is the component associated with the negative health effects in food products, it is important to have analytical methods for direct measurement of sodium in these products, as well as methods that can detect changes in the food matrix in light of sodium reduction in fish or meat products, which is what we aim to develop and present in the following thesis.

Facts

1 g Na corresponds to 2.5 g salt

1 g NaCl contain approximately 0.4 g sodium

100 mmol NaCl corresponds to 5.8 g NaCl

A teaspoon of NaCl (5 ml) weighs approximately 7 g.

It is the sodium component in salt that is associated with the negative health effect.

2 OBJECTIVES

The main objectives of the present thesis are 1) to evaluate different analytical methods for measuring sodium in a food matrix, and to evaluate changes in the food matrix as a function of sodium reduction, and 2) to increase the theoretical understanding of the effect of salt (NaCl), and salt replacers, on food sensory and physicochemical properties.

The specific goals are:

- To evaluate the application of *impedance spectroscopy* and *low field NMR* to measure physicochemical parameters in salted fish products with and without sodium replacement, and to establish a fast and consistent method of measuring sodium and potassium contents in fish products;
- To study the sensory quality of cooked pork ham containing a gradually reduced salt/sodium content, and with a partial replacement of sodium-ions (Na^+) with potassium-ions (K^+). The product quality parameters addressed were physicochemical and sensory properties related to data obtained by sensory descriptive analyzes and application of a multimodal *machine vision system*;
- To investigate the effect of different concentrations of cations (Na^+ , K^+ , Mg^{2+}) on both fresh and frozen raw material, focusing on the physicochemical properties and protein solubility in the haddock muscle and minces;
- To investigate the use of milk minerals as a method of reducing the salt content in cooked fish mince (fish pudding). This was done to investigate the outcome of their addition with regards to the effects it would have on the flavor of the product, as well as on other physical properties thereof, including texture, color, water-holding and protein solubility.
- To improve the knowledge base of salt reduction possibilities in the food industry and evaluate analytical methods those are both appropriate and efficient for industrial use.

3 BACKGROUND

3.1 Salt (NaCl)

Salt (NaCl) is a chemical compound made of sodium and chloride. There are two main sources of salt, namely sea water salt and rock salt. Sea water salt is dried sea water and there are a variety of sea salts available with different physical properties from this source. Variation in salt origin, water source and manufacturing specifications results in sea salts with unique configurations of chemical composition, as well as their grain sizes, crystal shapes and colors. In addition to this, the Na-content in sea-salt also varies, from 335 to 400 $\mu\text{g/g}$ (Vella et al., 2012). Rock salt, on the other hand, is mined from deposits in the earth. The purity of rock salt varies according to soil condition, and purification before use in food production is always necessary. Purified salt contains 99.9% NaCl. In both cases, the crystal structure of NaCl dissolves to become Na^+ -and Cl^- ions in solvent (water) as shown in **Figure 1**.

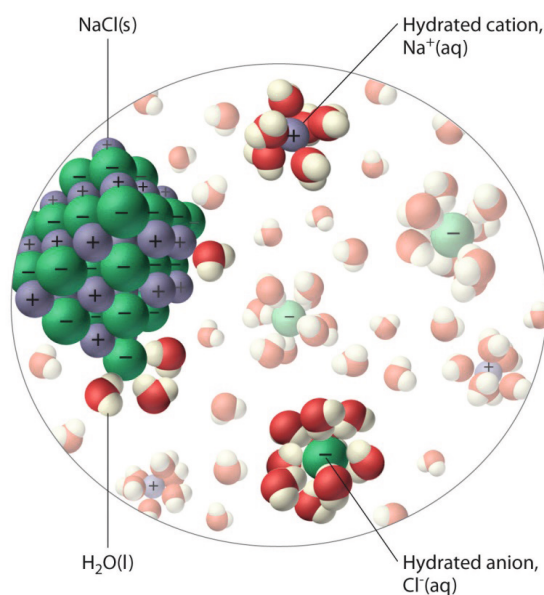


Figure 1. Illustration of NaCl crystal structure and NaCl dissolved in water (2012books.lardbucket.org)

3.2 Salt and health

Epidemiological studies have demonstrated that a high intake of sodium is associated with an increased risk of high blood pressure, which in turn is a significant contributor to the development of cardiovascular disease (CVD) and strokes (Cook et al., 2007; Intersalt, 1988; SACN et al., 2003). In

addition, a high salt intake has been linked to increased risk of stomach cancer, increased risk of renal stones, and decreased bone mineral density (He & MacGregor, 2007) as well as left ventricular hypertrophy (Messerli & Ketelhut, 1993). In the United States it was estimated that the above mentioned diseases caused 395 000 deaths in 2005, that could arguably have been prevented to some extent with a lower salt intake. Therefore, public health and regulatory authorities, including among others the Food Standard Agency (2014), the World Health Organization (WHO, 2012) and The Norwegian Directorate of Health (2014), have published advisory guidelines for daily salt intakes. Depending on the authority, these guidelines advocate a reduction in the average sodium intake by humans to 2.0 or 2.4 g Na per person per day, corresponding to a total intake of 5 or 6 g NaCl per person per day.

3.2.1 Negative health effects of sodium consumption

The mechanisms whereby salt raises blood pressure, a condition that in turn is a significant contributor to the development of cardiovascular disease (CVD) and strokes, are not fully understood, however. Sodium is required for normal human body functions, but the body only needs around 8–10 mmol (184–230 mg) sodium/day to maintain these functions. Sodium is the principal cation in the extracellular fluid and its main functions in the human body are related to volume maintenance, water balance and maintaining the membrane potential of cells. Most of the ingested sodium is then excreted via kidneys (He & MacGregor, 2007); however, an excessive sodium intake is associated with fluid retention in humans (Gupta et al., 2012).

3.2.2 Health benefits of reducing the sodium intake


According to the WHO, the strategy of reducing the daily salt intake of the population could have a great economic benefit by improving the public health thereof. Several calculations have shown that a stepwise reduction of salt intake by 10-30% could have a major positive effect on health (WHO, 2012). In the United States and United Kingdom (UK), estimates show that a reduction of daily salt intake by 3 g could lead to a reduction of cardiovascular disease by 10% (WHO, 2013). If the target of salt reduction down to 5 g /day is achieved, approximately 35 000 strokes and heart attack deaths could be saved each year in the UK alone (He & MacGregor, 2003). Similar calculations in Denmark have shown that a sodium reduction of 3 g per day could prevent 1000 deaths every year, and 400 000 fewer people with hypertension (The Norwegian Directorate of Health, 2014).

BACKGROUND

3.2.3 Health authorities and industry's perspective

Given the results of these studies, a Norwegian salt reduction action plan was published in 2014 (The Norwegian Directorate of Health, 2014). Several initiatives have been started in order to reach the target for a salt consumption reduction of 30% by 2025. Members of the Norwegian food industry have even submitted a letter of intent, where they claim they will reach the mid-term target of a 15% reduction by 2018 (Business Group Norwegian Food Industry, 2014). In meat and fish products, the Norwegian goal is to reduce the salt content with 15-30% total in the period leading up to 2025. In the UK (Food Standards Agency) and Denmark, however, the food industry has to adhere to government prescribed salt reduction targets for a range of processed foods. Following this lead, a "salt list", which includes salt targets for the Norwegian food industry, will be published in 2015 by the Norwegian Directorate of Health and Social affairs. In order to adhere to consumer interest and information needs, the salt content of a given product will be obligatory on the nutrition declaration at the same time (EU, 2011). This is already the norm in order to obtain the right to use the voluntary Nordic food labeling system of healthy products, the "keyhole". This label on a product is supposed to be a consumer signifier of a healthier product with regards to fat, sugar and salt content. **Table 1** gives an overview of the FSA, DK and "keyhole" targets for salt and sodium in selected meat and fish products.

Table 1. Targets for salt and sodium contents in selected meat and fish products from Food Standard Agency (FSA), Denmark(DK) and products labeled with the health-label "keyhole".

Main product category	Sub-category	Guided target DK ¹ Nov 2013 g salt (mg Na) per 100g	Targets for 2017 FSA ² g salt (mg Na) per 100g	"Keyhole" ³ g salt (mg Na) per 100g 
Meat products	Bacon	2.4 (960)	2.88 (1150)	-
Meat products	Sausages	2.3 (920)	1.5 (600) (average) 1.88 (750) max	2.0 (800) max
Meat products	Meat balls	1.6 (640)	1.35 (540)	1.7 (700) max
Meat products	Cooked ham	3.0 (1.2)	1.63 (650)	2.5 (1000) max
Fish products	Breaded	1.0 (40)	-	1.5 (600) max
Fish products	Marinated or smoked	3.0 (1.2)	-	3.0 (1200) max
Fish products	Canned	1.0 (400)	0.85 (340) (average) 1.5 (600) max	1.5 (600) max

¹ (Danish Saltpartnership, 2012), ² (Food Standard Agency, 2014), ³ (The Ministry of Health and Care Service, 2015)

Given the fact that 77% of human salt intake (**Figure 2**) comes from processed and restaurant foods, it is very important to increase the knowledge of how the food producers and restaurants can reduce the salt content in these types of products. In Norway, meat products are one of the main sources of salt in human diets, and contributes 24% of the daily salt intake, in comparison fish products, that contributes 11% (**Figure 2**). Given that both product from both meat and fish contain more or less the same amount of muscle proteins, it will be useful to study salt reduction in both.

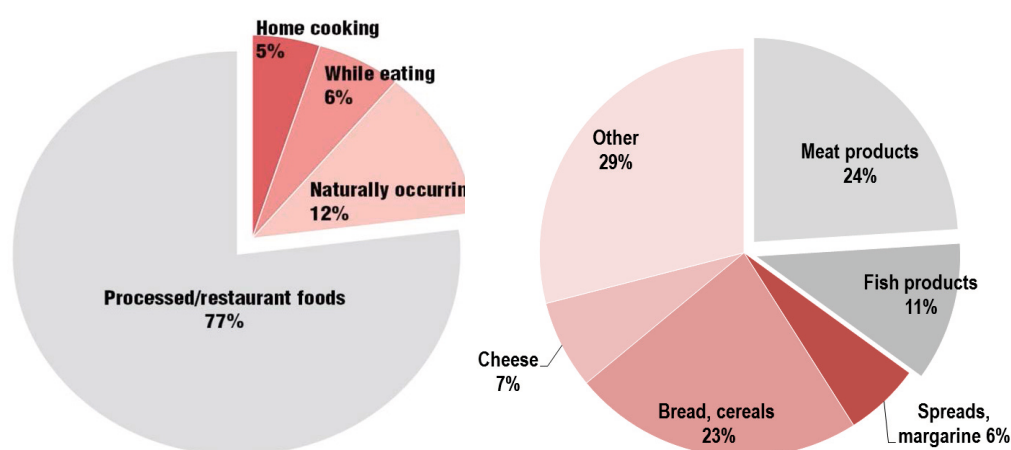


Figure 2. Sources of salt in the diet (Mattes & Donnelly, 1991) (left); and Norwegian salt intake (distribution between food products) (The Norwegian Directorate for Health, 2012)) (right).

3.3 Muscle and salt

Salt has several functions in processed foods. In muscle foods, like meat and fish, the functions performed by salt are to enhance flavor, improve preservation, to increase water-holding capacity in some products, increase the meat binding in tumbled products, and to improve water and fat binding in others. It is well known that proteins in muscles are responsible for the textural properties and water-holding capacity in processed meat and fish products (Foegeding & Lanier, 1996). This section will therefore give an overview of muscle proteins from fish and meat, and describe how salt and salt reduction affects the functional properties in food products derived there from.

BACKGROUND

3.3.1 Structure of muscles

In fish, the edible muscle constitutes 40-68% of its body weight. In comparison, edible muscle content constitutes only 35-41% of the body weight in mammals. The protein content is normally relatively stable, but variations in water and lipid content can be quite large. Examples of the variation in the edible muscle tissue composition of salmon, haddock, hake, and pork are given in **Table 2**.

Table 2.

Table 2. Approximate composition of edible muscle tissue from different fish species and pork.

Species	Name	Chemical composition (%)					Ref
		Water	Protein	Lipid	Ash	Sodium (mg/100g)	
Atlantic salmon	<i>Salmo salar</i>	57-77	15.4-21.8	3.3-21.4	1-5	46-57	1-6
Haddock	<i>Melanogrammus aeglefinus</i>	81	16.6	0.2	-	96	4
Hake	<i>Merluccius merluccius</i>	80	14-17	0.5	1.2	50-143	7
Pork meat	<i>Musclulus longissimus dorsi</i>	50-79	13.6-22.2	1.8-37	1.4	44	1,4

¹Watt and Merrill (1963), ²Murray and Burt (1969), ³Venugopal and Shahidi (1996), ⁴The Norwegian Directorate of Health and University of Oslo (2015), ⁵Norwegian Institute of Nutrition and Seafood Research (2015), ⁶Myhre et al. (2012), ⁷Çükgülmez et al. (2008)

The skeletal muscle is composed of long, narrow, multi-nucleated cells (fibers) that range from a few to some centimeters in length and from 10-100 μm in diameter. **Figure 3** represents a typical arrangement of muscle components in both mammals and birds. The fibers are arranged in parallel fashion to form bundles, and groups of bundles form a muscle. Surrounding the whole muscle is a heavy sheath of connective tissue, called *epimysium* (Foegeding & Lanier, 1996).

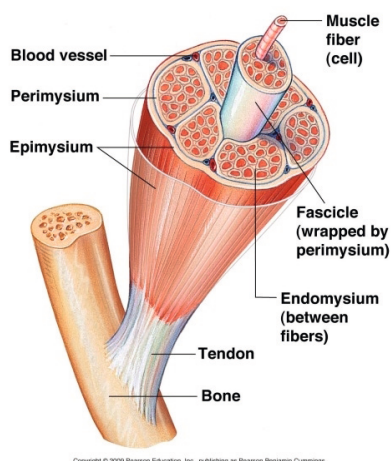


Figure 3. The skeletal muscle of mammals (Copyright 2006 ©.Pearson Education, Inc., publishing as Pearson Benjamin Cummings).

The structural arrangement of fish muscle, however, is markedly different from that of land animals and birds as described above in **Figure 3**. This is because the muscle arrangement is related to the swimming movement of fish. The arrangement of the muscle tissue in a typical bony fish is shown in **Figure 4**. The W-shaped segments are called myotomes. The myotomes are connected to one another by a thin layer of collagenous connective tissue called myosepta (Myocommata) (Foegeding & Lanier, 1996). The muscle fiber in fish muscle is furthermore rarely longer than 2 cm, and 50-200 μm in diameter as compared to that of mammals and birds. The muscle fiber (muscle cell) is located perpendicular to the surface of the myotome. The myotomes are connected to one another by thin layers of collagen connective tissues called myosepta (myocommata).

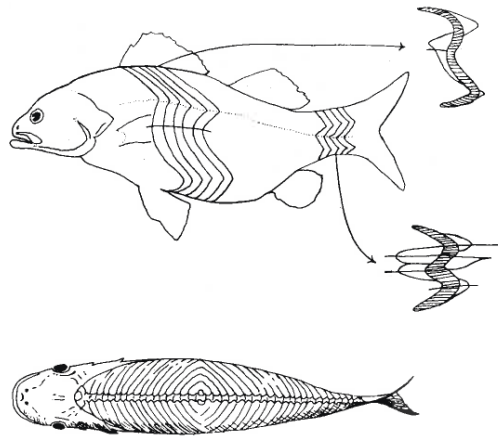


Figure 4. Illustration of the myotome pattern of musculature of bony fish, with detailed lateral views of a single myotome (Foegeding & Lanier, 1996).

BACKGROUND

The following section will mainly focus on the inner component of the muscle fibres (myofibril) in fish and meat muscles, however, since these are arranged in more or less the same way. The main structural component of a muscle fiber is the myofibrils which occupies about 70% of the volume of lean meat (Hamm, 1986). The myofibril contains about 20% protein, with the remainder being water. Myofibrils are the cylindrical bundles of contractile filaments. These individual contractile proteins are called myofilaments, and are composed of thick (myosin) and thin (actin) filaments. They form repeating units along the myofibril, called sarcomeres. **Figure 5** shows the structure of a myofibril.

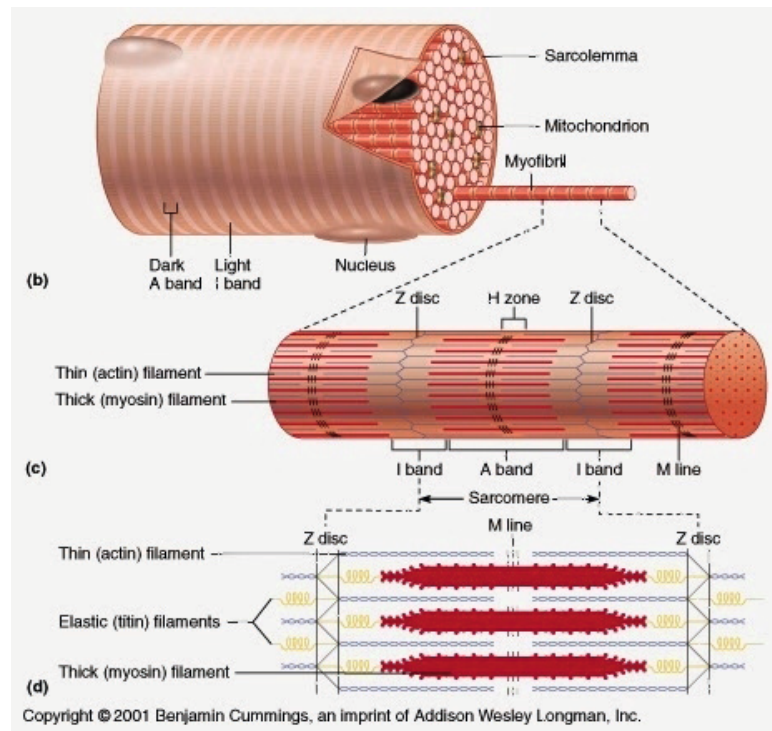


Figure 5. Striated muscle fibril in longitudinal section. The thin filament is the actin and thick filament the myosin. The I-band consists only of thin filaments. The A-band is darker where it consists of overlapping thick and thin filaments and lighter in the H-zone, where it consists solely of thick filaments. The M-line is caused by a bulge in the center of each thick filament, and the pseudo-H-zone is a bare region on either side of the M-line (Tortora & Derrickson, 2006)

3.3.2 Salt and functional properties in muscles

3.3.2.1 Protein solubility and water holding capacity (WHC)

One of the main functions of proteins in processed foods is the solubilisation of the functional myofibrillar proteins discussed above. This solubilisation results in increased hydration and water binding capacity of the product, and improves the binding properties of proteins. The texture improves and influences the physicochemical properties in minces, in particular the gelling ability thereof (Sikorski et al., 1990). The myofibrillar proteins are solubilised in intermediate or high-ionic strength buffers that are referred to as salt-soluble proteins. The solubilised myofibrillar proteins are principally myosin and actin.

Experiments in this thesis have been performed on minced fish muscle and coarsely ground pork muscle. The characteristic structure of the muscle fibres as shown in **Figure 5** are disrupted, which allows a more rapid and complete equilibration of muscle and salt solution. The theoretical aspects of water-holding in meat are discussed by several authors (Huff-Lonergan & Lonergan, 2005; Offer & Trinick, 1983; Puolanne & Halonen, 2010) and the following section gives a brief overview over the subject, since the ability of proteins to entrap water is often associated with juiciness and tenderness of comminuted muscle products.

Water-holding capacity (WHC) is defined as the ability of a food matrix to prevent water release from the three-dimensional structure (Offer & Trinick, 1983). WHC is the sum of the bound, hydrodynamic water and the physically entrapped water. Closely related to WHC is the ability of meat to take up additional water at elevated salt concentrations, and a swelling of myofibril to more than twice their original volume in salt solutions (Offer & Trinick, 1983). The amount of swelling depends on the concentration of NaCl, and the swelling increases when the concentration of salt increases from 0.04 to 0.5 M (approx. 0.2 – 2.9% NaCl), at which point a maximum water uptake is at a final concentration of 0.8 -1 M (4.6-5.8%) NaCl.

When discussing the effects of salt/ions on WHC, different theories are postulated. Hamm (1972) suggested that the chloride ion is more strongly bound to the protein than the sodium ion, and further increases the negative charge of the proteins. **Figure 6** shows the interaction of chloride ions in the protein structure. At pH > 5 the charge of both thick and

BACKGROUND

thin filaments is negative and there is a repulsive force between the filaments tending to enlarge the lattice. Increasing the pH increases the charge on the filaments, causing the lattice to swell.

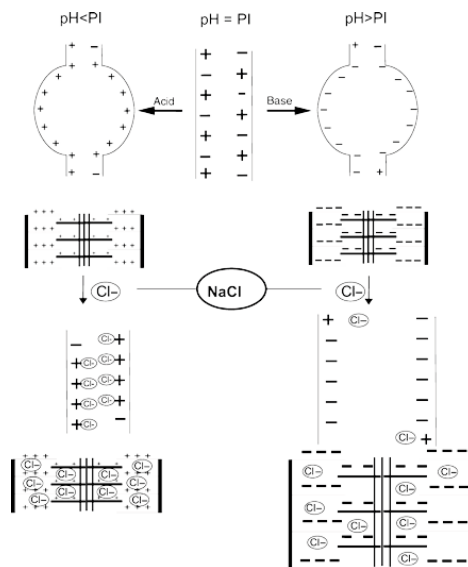


Figure 6. Chloride interaction in protein structure (Girard, 1991).

Another important theory, particularly when dealing with salt reduction and sodium replacement with other ions, discusses the effect of ions on the stabilization of proteins and is explained by the Hofmeister series (Hofmeister, 1888) and exemplified by others (Baldwin, 1996; Lawal, 2006; Puolanne & Halonen, 2010). For anions, the effectiveness to stabilize protein is in the order: $PO_4^{3-} > SO_4^{2-} > H_2PO_4 > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^-$ and for cations $(CH_3)_4N^+ > NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$. Members to the left of the series decrease the solubility of nonpolar molecules ("salting out"). In effect, this means that they strengthen the hydrophobic interactions. Later salts in the series increase the solubility of nonpolar molecules ("salting in") and disturb the ordered structure of water, which means that they in effect weaken the hydrophobic effect (Chaplin, 2014). The highest solubility of proteins is obtained using a salt consisting of a chaotropic anion and a kosmotropic cation, such as NaCl (Puolanne & Halonen, 2010).

The concept of low and high density water explained by Puolanne and Halonen (2010) is also important in understanding the interaction of water with ions. Ions and ionic groups of organic molecules hinder the mobility of water molecules to a greater extent than do any other types of solutes. The strength of water to ion bonds is greater than that of water to water hydrogen bonds, but to a lower degree than that of covalent bonds. The ability of a given ion to alter net structure is closely related to its polarizing power (charge divided by radius) or simply the strength of its electric field (Fennema, 1996). Ions that are small or multivalent organize water, meaning that they interact strongly with the four to six first-layer water molecules, and cause a positive hydration effect. As solutes, they furthermore enforce a hydrogen bond network of neighboring water molecules which makes the water molecules less mobile and more structured than in bulk water (Fennema, 1996). This is called low density (LD) water (Puolanne & Halonen, 2010). Structure-making (kosmotropic, i.e. Na^+ and Mg^{2+}) ions induce LD-water. Large monovalent ions disorder it, producing high density (HD) water (**Figure 7**). The low density water is inert and the viscosity is higher than that of high density water. Chaotropic (structure-breaking) ions (i.e. K^+ and Cl^-) have an opposite, negative hydration effect, however, which means that they weaken the hydrogen bonds of neighboring water molecules, making the water molecules more mobile and less structured than in bulk water.

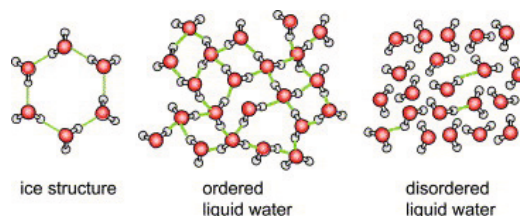


Figure 7. Schematic illustration of water structure as a consequence of the formation of more or less extended networks of hydrogen bonds (Moelbert et al., 2004)

3.3.2.2 Ionic strength and solubility.

The ionic strength of the solution is important for functional properties of proteins. The ionic strength is a measure of the concentrations of ions in the solution and is calculated as shown in equation 1, where c_i is the molar concentration of ions (in M, mol/L), z_i is the charge

BACKGROUND

number of that ion, and the sum is taken over all ions in the solution. In non-ideal solutions, such as in minces, it is often preferable to work with molality b (mol/kg H₂O) instead of molarity c . The total electrolyte concentrations in the solution will affect important properties such as the dissociation or the solubility of the salts (IUPAC, 1997).

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2 \quad \text{Equation 1}$$

At low ionic strength (< 0.5), ions neutralize charges at the surface of the protein (Offer & Trinick, 1983). At constant ion strength, relative effectiveness of various ions on solubility follows the Hofmeister series, as discussed above.

3.3.2.3 Gelation of proteins.

One of the greatest challenges with regard to salt reduction in restructured products is to obtain sufficiently high solubility of the muscle proteins. This is needed to obtain adequate gelation of the products, bind the meat and particles together, obtain desired fat and water binding, and ensure the required texture. A gel is defined as a structure which forms intermediate between a solid and a liquid, consisting of cross-linked chains that create a continuous network in a flowing medium. Gelling takes place in a three-stage process; denaturation of individual protein molecules, aggregation (mainly hydrophobic interactions), and eventually cross-linking of protein aggregates or oligomers which lead to a continuous, viscoelastic network. The ability of the myofibrillar components of muscle tissue to form a strong heat-induced gel requires good dispersion of these proteins and surface reactivity, where the dispersion depends on the swelling and dissolution of the proteins by salt, and use of suitable mixing/blending equipment (Foegeding & Lanier, 1996). Myosin is the most important component of muscle tissue with respect to gel-forming ability. Actin assists in this process by forming F-actomyosin, which in turn interacts with free myosin (Wiskus et al., 1976). In processed meats, the salt-solubilised myofibrillar proteins form a sticky exudate layer on the surface of the meat pieces. The exudate layer forms a matrix of heat-coagulated protein which entraps free water and binds the meat pieces together after cooking (Foegeding & Lanier, 1996). Proteins in several fish species uniquely exhibit gelation of salted minces or batters at temperatures below 40°C, which is attributed to the action of an endogenous Ca²⁺-dependent transglutaminase. The formation of a meat gel involves protein denaturation under specific conditions of heating, where denaturation (i.e. freeze

denaturation) of muscle protein, will result in a weak heat-induced gel (Foegeding & Lanier, 1996).

3.3.2.4 Salt, pH and solubility

At pH values below or above the isoelectric pH, proteins carry a net positive or a net negative charge, respectively. If solubility is plotted against pH, it exhibits a U-shaped curve in most food proteins, where the minimum solubility occurs at the isoelectric pH of proteins (Damodaran, 2008). A decrease in pH in fish flesh and animal meat with increasing additions of salt (NaCl) has frequently been observed (Abass Bakhiet & Khogali, 2011; Leroi & Joffraud, 2000; Puolanne et al., 2001). The effect has been ascribed to an increase in the ionic strength (Leroi & Joffraud, 2000) and interactions between the salt ions and proteins leading to exposure of previously buried charged and/or hydrophilic groups and thus a change in the overall pKa of the proteins (Puolanne et al., 2001). However, (Schwabe, 1967) also found that when neutral salts were added to acidic solutions, pH decreased linearly with increasing salt concentration. The level of decrease depended on the type of salt ions due, among others, to the charge of the ions, their size and hydration number. For the salts relevant to this work, the level of pH decrease with increasing salt concentration was lowest for KCl and highest for MgCl₂.

Addition of salt (NaCl) to meat also decreases the isoelectric point of myosin. As shown in **Figure 8**, the Cl⁻ ions screen the positive charges of the proteins ($-NH_3^+$). As a result, myosin (or actomyosin) within the normal pH range of meat will carry more surface charges and the increased interpeptide electrostatic repulsion enables a stronger protein-water interaction and greater water retention in the meat (Strasburg et al., 2008).

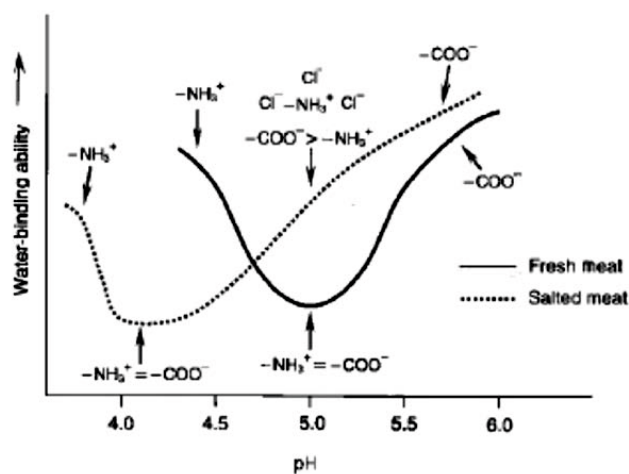


Figure 8. Schematic representation of the relationship between pH and water binding by proteins in fresh and salted meat (Strasburg et al., 2008)

3.4 Salt reduction strategies

Naturally, in order to reduce salt (NaCl) in food products for human consumption, the simplest solution is to add less salt in the formulation. To retain the target of 5 g NaCl per pers per day, the food industry has to reduce the sodium content in processed food upwards of 50%, though. A reduction of this size will lead to not only sensory attributes (less tasty foods), but also challenges regarding processing, physicochemical properties, yield, texture, and, importantly, food safety. Partial replacement of the sodium salt by other ingredients will therefore be necessary, and its implementation and the effects thereof is the focus of this study.

Salt replacers are often defined as components used to compensate for the reduction of salt (NaCl) in the product in question. These replacers can be divided into three groups primarily based on how they affect the *taste* of the products: i) salt replacers: these components have some salty taste (mineral salts: KCl, MgCl₂, MgSO₄, other mineral salts and lactates); ii) salt enhancers: these components enhance the salty taste, but do not taste like salt (glutamate, lysin, 5'-nucleotides and yeast extract); and iii) bitter inhibitors: these components mask bitter and undesirable tastes in the products (sucrose, yeast extract). Another way to classify the salt replacers beyond its effect on the taste of the product is to define them based on how they affect the functionality properties, preservation effect and taste of the products. (1) Mineral salts including sea salt, affect the functional properties, WHC, a_w , and taste. (2)

Lactates and salt of lactates affect the microbiology and taste, whereas (3) organic salt replacers, such as yeast extract, spices, herbs, sodium glutamate (MSG), enzymes, and seaweeds among others, influences the taste (Josefsen et al., 2014).

In addition to these components, there is also a wide variety of ingredients, such as fiber, gum and starches, that can be used as binding agents and therefore negate the reduced gelation effect of salt soluble proteins (Collins, 1997). Phosphate has been shown to be a promising substitute to salt for this purpose, and is used in sodium reduced cooked meat products to enhance WHC and improve the cooking yield thereof (Nayak et al., 1998; Ruusunen et al., 2005). This is because the addition of phosphate slightly increases the pH and moves it away from the isoelectric point of the proteins (about pH 5.5), where the gelling ability is optimum near pH 6.0-6.4 for muscle of homotherms (Foegeding & Lanier, 1996). They therefore increase WHC in fresh and cured meat products by increasing the ionic strength, which frees negatively charged sites on meat proteins so that the proteins can bind more water, since the functionality of phosphates acts in synergy with salt (Kilcast & Angus, 2007). There is a variety of phosphate compounds on the market that have the ability to affect the WHC in meat and fish products in different ways. In this regard, it is important to choose a phosphate optimized for the raw material used (fish or meat) and the expected effect it will have on the final product.

Given that this is a current health problem, and the recommendations from health organizations were provided some years ago already, a number of salt replacers already exist on the market and they are often sold in mixtures of many different components. Josefsen et al. (2014), CTAC (2009) and Leatherhead Food Research (2012) give brief overviews of commercial salt replacers and will not be listed here. Salt replacers used in experiments presented in this thesis are potassium chloride (KCl), magnesium chloride (MgCl₂) and dairy-based salt replacers, and will be explained in detail below.

A summary of salt reduction and the effects thereof on physicochemical properties, taste and shelf-life in low-salt fish and meat products is shown in **Table 3**.

BACKGROUND

Table 3. Summary of salt reduction and the effects thereof on physicochemical properties, taste and shelf-life in low-salt fish products.

Low-salt fish				
Product	Salt reduction/ replacement	Whole muscle/ mince	Physicochemical properties/ shelf-life/taste	Ref
Low-salt food	Low sodium mixtures	Fish, meat, food	Patents aimed to develop mixtures with low sodium content	Review by Toldrá & Barat (2012)
Cod, ready-to-eat	Desalting water with NaCl/KCl	Whole muscle	Physicochemical properties/Shelf life/taste	Aliño et al. (2011)
Smoked sea bass	Salting 100% NaCl, 50% NaCl-50% KCl, packaging. 926-1279 mg Na/100g in salted and smoked fish	Sea bass fillets	Chemical, microbial and sensory attributes	Fuentes et al. (2011); Fuentes et al., (2012)
Fish gel	NaCl (0-1%), KCl (0-1%), CaCl ₂ (0-1%), iota-carrageenan, kappa-carrageenan, sodium alginate.	Blue whiting muscle gel	Texture, color, WHC	Montero & Pérez-Mateos (2002)
Surimi	NaCl (0, 1, 2, 3 g/100g), equal molar concentrations of KCl	Alaska pollock surimi	Protein endothermic transitions, rheological properties, texture	Tahergorabi et al. (2012)
Cod	Fresh and frozen, fillet injection (0, 50, 150 and 250 g NaCl/L), additional 25 g sodium bicarbonate/L (NaHCO ₃)	Whole muscle, fillet	Yield, liquid retention during storage, flavor, texture	Åsli & Mørkøre (2012)
Cod	Brine salting in NaCl (10 – 50 g/L) or KCl (13 – 64 g/L) solution	Whole muscle, fillet	Water uptake, drip loss and retention of low molecular components	Larsen & Elvevoll (2008)
Low-salt foods	Slow and gradual reduction, replacement of Na ⁺ with K ⁺ , ammonium, calcium, and lithium and by anion such as phosphate and glutamates.		Taste, sensory profile	Morley (2012)
Smoked salmon	NaCl or NaCl + KCl in a 2:1 ratio. Dry salting or by injection	Whole muscle	Sensory quantitative descriptive analysis. Consumer test	Almli & Hersleth, (2013)

Table 3 continued. Summary of salt reduction and the effect on physicochemical properties, taste and shelf-life in low-salt meat products.

Product	Salt reduction/ replacement	Low-salt meat		Ref
		Whole muscle/ mince	Physicochemical properties/ shelf-life/taste	
Frankfurters sausage	Replacement of flake salt with naturally brewed soy sauce, natural flavor enhancer, and KCl.	Emulsion of beef and pork	Consumer sensory and quality impact, color, pH, emulsion stability, yield	McGough et al., (2012a); McGough et al. (2012b)
Frankfurter sausage	Gradually reduction of NaCl (1.6-1.1% NaCl), modified tapioca starch, wheat bran, sodium citrate, fat	Emulsion of beef and pork	Physical properties and sensory attributes	Ruusunen et al. (2003b)
Frankfurter sausage	Gradually reduction of salt (2.5, 2.0, 1.5% NaCl), preblended, non preblended meat	Emulsion of beef and pork	Emulsion stability, color, Kramer shear and palatability	Hand et al. (1987)
Frankfurter sausage	Salt (1.5-2.5% NaCl), sodium triphosphate, kappa-carrageenan, isolated soya protein	Emulsion of beef and pork	Physicochemical properties, sensory attributes, texture	He & Sebranek (1996)
Bologna sausage	Tree levels of salt, Sodium citrate, carboxymethyl cellulose, carrageenan, fat	Lean pork and pork back fat	Chemical and physical composition, sensory attributes	Ruusunen et al. (2003a)
Cooked sausage	Post-rigor pH, phosphate, salt (0.5, 1.0, 1.5, 2.0, 2.5% NaCl)	Beef and pork	WHC, firmness, sensory attributes	Puolanne et al. (2001)
Cooked ham	Salt replacer (Ocean's Flavor – OF45, OF60) and flavor enhancer (Fonterra™)	Restructured ham of pork	Functional and sensory properties	Pietrasik and Gaudette (2014)
Cooked ham	Tumble salting with NaCl brine (0, 11.2, 16.5 or 22.0% NaCl)	Cubes of pork (<i>semimembranosus</i>)	Salt contents effect on the adhesion between pieces	Bombrun et al. (2014)
Cooked ham	Gradually reduced NaCl content (1.1, 1.4, 1.7, 2.0, 2.3 and 2.6% NaCl)	Coarsely ground lean ham	Sensory saltiness	Ruusunen et al. (2001)
Cooked ham	KCl, tumbling time, tumbling speed	Coarsely ground lean pork	Color, shrinkage, yield, WHC, sensory attributes	Lin et al. (1991)
Cooked ham	Gradually reduced NaCl content (1.30, 0.74, 0.18% NaCl)	Coarsely ground pork	Chemical- and microbial properties, sensory attributes	Aaslyng et al. (2014)
Meat patties	Gradually reduced NaCl content (300, 450 and 600 mg Na/100g), phosphate and meat content	Coarsely ground beef and pork meat	Sensory attributes, cooking loss, firmness	Ruusunen et al. (2005)
Meat batter	NaCl, 0.05% CaCl ₂ , MgCl ₂ or ZnCl ₂ , with or without 0.4% sodium tripolyphosphate, fat	Beef	pH, yield, texture, gel ultrastructure	Nayak et al. (1998)

3.4.1 Substitution of sodium by potassium chloride (KCl)

As shown in **Table 3**, KCl is used in several studies on sodium reduction. The partial substitution of NaCl by KCl has been shown to be one of the best alternatives for reducing sodium content (Aliño et al., 2011; Fuentes et al., 2011; Toldrá & Barat, 2012). This is among others because KCl has similar antimicrobial effect on pathogenic bacteria as sodium chloride (Leatherhead Food Research, 2012). The differences in behavior of NaCl and KCl have been discussed by Puolanne and Halonen (2010), where it was found that NaCl is formed from a kosmotropic cation (water- structure maker) and a chaotropic anion (water-structure breaker), whereas in KCl, both the cation and anion are chaotropic. This means that these ions will affect the cell protein structure in a different manner. Replacement of NaCl by more than 50% of KCl will have a negative influence on the flavor intensity and produce bitter, metallic and chemical taste in the products (Desmond, 2006), which will limit the use of this cation. A higher potassium intake, as would be the result of a substitution of salt with KCl, has been shown to reduce blood pressure and the risk of stroke (He & MacGregor, 2001). However due to the increased use of KCl as a salt replacer, the health effects of increased potassium intake have been evaluated by the international health authorities, and concerns are raised about the possible vulnerability of certain population sub-groups (including those with Type I diabetes, chronic renal insufficiency, end stage renal disease, severe heart failure and adrenal insufficiency) (EFSA, 2005; FSAI, 2005; Geleijnse et al., 2007; Norwegian Scientific Committee for Food Safety, 2014).

3.4.2 Substitution of sodium by magnesium chloride

From a health point of view, Mg^{2+} is supposed to be a good alternative as a Na^+ replacement (Barat et al., 2012). Anyhow, a large replacement of Na^+ with Mg^{2+} in fish and meat products is not possible, as $MgCl_2$ may generate an off-flavor (Lawless et al., 2003). Additionally, several authors have reported that when NaCl is partially replaced with $MgCl_2$ this will affect the enzyme activity, protein matrix and texture of the product as well (Andreetta-Gorelkina et al., 2015; Barat et al., 2012; Martínez-Alvarez & Gómez-Guillén, 2013). This is because the presence of magnesium brines tended to hinder the general penetrations of chloride into the muscle, thus negatively affecting the WHC and water-extractable proteins (Barat et al., 2012). Magnesium salts is already used in low concentrations in commercial "low-sodium"

salts on the market, though (Barat et al., 2012), and it is important to increase the knowledge regarding usage of Mg^{2+} in food products.

3.4.3 Dairy-based salt replacers

Whey permeates and milk-based products with favourable combinations of milk minerals and lactose can be used as natural ingredients in meat and fish products and can also work as salt replacers. A range of processes can be used in order to separate liquids or remove different substances such as water and proteins from these products (Page et al., 2004). The food industry employs various milk-based ingredients in form of powders such as whey, whey permeate, lactose, skimmed milk and whole milk, which contributes to textural properties, water-holding and desirable milk tastes, including sweet and umami flavors thereof. The latter is important, because in a product with a high percentage of milk and/or cream ingredients, such as fish pudding, the flavor of milk and umami are desired by the consumer. The lactose in the milk-based ingredients contributes to a browning Maillard reaction as well, which again is desirable in products such as fish puddings (BeMiller & Whistler, 1996). Challenges connected to the use of high level of whey permeate and milk-based permeate are mainly connected to their contribution of flavors that might be unwanted in some foods. Whey and milk based permeates typically contain a minimum of 59% lactose, maximum 10% protein and 27% ash, where the ash contains mineral salts such as calcium, phosphate, magnesium and potassium, which therefore function as salt enhancers, while the non-protein compounds may affect the flavor (like those required for fish puddings)(Leatherhead Food Research, 2012).

3.5 Effect of sodium reduction on taste

Given the intense focus on salt reduction in food, extensive work has been carried out on the salt (NaCl) itself, and the desire to maintain the salty taste at lower levels of salt additives. The relationship between the size and shape of salt crystals or size and location (inside or coating the food) (Shepherd et al., 1989) have been investigated. However, in coarsely ground and minced muscle products, salt is not found in crystal form and the optimization of saltiness has to be achieved through microstructure engineering. Sodium chloride contributes to saltiness and the overall flavor and suppresses bitterness as well (Breslin & Beauchamp, 1995). This is done by the Na^+ ions stimulating the taste buds while

the Cl⁻ ions give the salty taste (Murphy et al., 1981). According to Mattes (1997), the flavor release when consuming foods are greatly depended of the nature of the food matrix as well. A step-wise salt reduction may however result in people getting used to less salty food, but as discussed in chapter 3, there will be critical limits due to the functionality of proteins that go beyond the sensory stimulation of the consumer.

3.6 Effect of sodium reduction on microbial food safety

Salt has been exceptionally important to humans for thousands of years because of its food preservation properties. During storage of fresh food, and especially food with high water content, microbial growth will occur and either cause spoilage or food poisoning. Working with salt reduction in food products, it is very important to keep the shelf-life of the product, and to prevent growth of undesirable microorganisms during this time, a role often played by salt (Kilcast & Angus, 2007). The preservative effect of salt is due to its ability to reduce the water activity (a_w) in the product. Increased addition of salt decreases the a_w , and by measuring the a_w it is possible to predict the risk for growth of microorganisms in the product. It is the a_w and not the moisture content that determines the lower limit of available water for microbial growth. Salt replacers i.e. KCl may negatively affect the a_w , however, since K⁺ is a larger ion than Na⁺, and replacing NaCl with an equal amount of KCl could therefore lead to a lower number of dissolved ions (colligative units) per volume and thus in fact an *increase* in a_w of the product.

Naturally, each microorganism will have a minimum a_w , under which it is unable to grow. Limiting the a_w value will depend on the type of solute used, however. Food-born microorganisms such as *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*, have their minimum a_w for growth at 0.92-0.95, 0.83, 0.93-0.95, and 0.92, respectively (Kilcast & Angus, 2007). Decreasing salt content will increase a_w , and thus increase the potential for growth of these pathogens and spoilages. It is clear that NaCl therefore plays a critical role in controlling microbial growth, particularly in refrigerated ready-to-eat foods (hams and sausages), refrigerated ready-to-cook foods (bacon, fresh sausage, meat patties) and ready-to-eat, shelf stable food products (dry and semi-dry sausages, dry cured ham, smoked fish) (Taormina, 2010). However, the preservation effect of a_w in food product is only one of many factors that affects the shelf-life and safety of the

product. The levels of a_w in low-salt products is often too high (> 0.96) to represent the only hurdle for unwanted spoilage- and food-born microorganisms, and it is necessary to combine this with other preservations factors such as lowering the pH, adding preservatives, using modified atmospheres, as well as heating and chilled storage for controlling the growth of microorganisms (Kilcast & Angus, 2007).

3.7 Salt reduction in heavily salted fish and meat products

When salt solution or dry salt are used as salting agents, two main simultaneous flows are usually generated; water loss and salt uptake (Gallart-Jornet et al., 2007). These methods are used for the salting of heavily salted products ($> 4\%$ NaCl), which is higher than in the products studied in the present thesis. Regardless, these products contribute to the consumption of salty foods, and it is important to have an overview over different salting techniques and strategies for salt reduction used for these types of products as well. *Kench-salting* is an old method used for heavily salted lean fish. The cod fish is filleted or butterflied, split and piled into stacks in alternating layers of fish and salt. The fish meat absorbs the salt while liquid diffusion from the muscle is allowed to drain away. The same method is used for production of dry-cured ham. In *pickle salting* a similar procedure is performed, but in closed vats. The liquid diffusion from the muscle during salting therefore forms a saturated brine solution (25% NaCl) as the salt dissolves (Barat et al., 2003). The pickle salting method is used for fatty fish such as herring and for long time preservation. Another salting method used both for salting of fish and meat products is *brine salting*, where the concentration of salt is lower than saturated ($<25\%$ NaCl), the fish or meat product are treated in the brine for shorter time. With this method it is easier to control the salting-in process, increase the weight yield and control the quality.

The following salting methods are optimized for the purpose of increasing yield, reducing the salting time and enhancing production control. *Injection-salting* with different types of brines (Casiraghi et al., 2007), *vacuum pulse application* on brine salting (Fito & Pastor, 1994) and *vacuum tumbling* are methods used for this purpose. Optimizing the salting methods can also lead to a more even salt distribution in the product and less salt used in the production step. Several studies have been done on heavily salted fish and meat products to reduce the sodium content in the end product. **Table 4** gives an overview of salt reduction in different types of heavy salted products.

BACKGROUND

Table 4. Summary of salt reduction and the effect on physicochemical properties, taste and shelf-life in heavy salted fish and meat products.

Product	Salt reduction/replacement	Whole muscle/ mince	Physicochemical properties/ shelf-life/taste	Ref
Heavy salted fish				
Cod	Brine salting, pH (6.5 and 8.5), NaCl, KCl, CaCl ₂ and/or MgCl ₂	Whole muscle, fillet	WHC, protein extractability, dry matter, ion content and hardness.	Martínez-Alvarez et al. (2005)
Cod	Brine salting, pH, NaCl, KCl, CaCl ₂ and/or MgCl ₂	Whole muscle, fillet	Water loss, salt uptake, entry of chloride, WHC, water extractable protein (WEP) and hardness	Martínez-Alvarez and Gómez-Guillén (2013)
Heavy salted meat				
Dry-cured products	Partial replacement of sodium chloride with KCl, CaCl ₂ , MgCl ₂ ,	Pork loin, pork ham, fermented sausage	Sensory effect and other	A review by Barat et al. (2012)
Dry-cured ham	KCl and potassium lactate instead of NaCl	Whole muscle, ham	Food-borne pathogens; <i>Listeria monocytogenes</i> and <i>Salmonella</i>	Stollewerk et al. (2012)
Dry-cured ham	Reduction of NaCl (5.5-4% NaCl as per cent edible meat)	Italian style dry-cured ham	Sensory properties, proteolysis	Benedini et al. (2012)
Dry-cured bacon	Partial substitution of NaCl with KCl	Pork	Proteolysis and sensory properties	Wu et al. (2014)

4 METHODOLOGY

The aim of the following section is to provide an overview of the principles of the main methods used in this thesis; (1) Impedance spectroscopy, (2) LF-NMR, (3) Computer Vision, (4) descriptive sensory analysis, (5) salt measurement with sodium selective electrode and chloride titration, and protein solubility. The analytical methods used were developed by others and applied on food matrixes containing different amount of salt, sodium and salt replacers. The high focus on salt reduction and increasing use of salt replacers such as potassium chloride makes it necessary to find new rapid techniques for determining the sodium content directly. There will be several ingredients in addition to NaCl, which contribute to sodium in the final product (i.e. sodium phosphates, sodium lactate and the raw material) and the addition of salt replacers such as KCl will also affect the results. Measuring the chloride content may give an under- or over-calculation of the total sodium content in the food product.

4.1 Impedance spectroscopy

The measurement of electrical conductivity has been broadly applied for the evaluation of the total water content in fish. The relationship between sodium chloride content and impedance measurements have been demonstrated by many authors (Chanet et al., 1999; Guerrero et al., 2004; Masot et al., 2010; Rizo et al., 2013). The module and phase of the impedance can vary significantly according to the charges present (free ions), types of microstructure and electrolytes, as well as texture, geometry, and the electrodes used (Masot et al., 2010).

The first method used in this study is therefore the Impedance spectroscopy. Impedance spectroscopy allows for the analysis of the properties of the material and system by applying alternate electric signals of different frequencies (voltage and current) to them and measuring the corresponding electric output signals (current and voltage) (Bard & Faulkner, 2001; Macdonald & Barsoukov, 2005). The ratio of the signal voltage to the signal current is called impedance and it is frequency dependent. A low-cost, flexible, light, non-destructive measurement system was developed by the Institute of Molecular Recognition and Technological Development at the Polytechnic University of Valencia (UPV) (Masot et al.,

2010). This impedance spectroscopy measurement system applies an electric sweep between 1 Hz and 1 MHz (**Figure 9**).



Figure 9 Impedance measurement in salt solution with a double electrode (left) and impedance Measurement equipment (right).

It consists of a software application that runs on a computer, along with the accompanying equipment as well as an electrode. Using the software application, the user establishes the frequencies and the amplitude of the sinusoidal voltage signals. For each one of the frequencies the electronic equipment generates the corresponding sinusoidal voltage waveform and applies it to the electrode (**Figure 10**). The current (i) and voltage (v) signals at the electrode are then sampled and the collected data are sent to the PC where a Fourier analysis (DFT) is performed to determine their amplitude and phase. The module $|Z|$ and the phase (φ) of the impedance are then calculated using Equation 2, where $v(t)$ is the voltage signal, $i(t)$ the current signal, f the frequency of the signals, and Δt is the time interval between the zero crossing of the voltage and current signals.

$$Z = |Z|e^{j\varphi} \begin{cases} |Z| = \frac{|v(t)|}{|i(t)|} & \text{Module} \\ \varphi = 2\pi f\Delta t & \text{Phase} \end{cases} \quad \text{Equation 2}$$

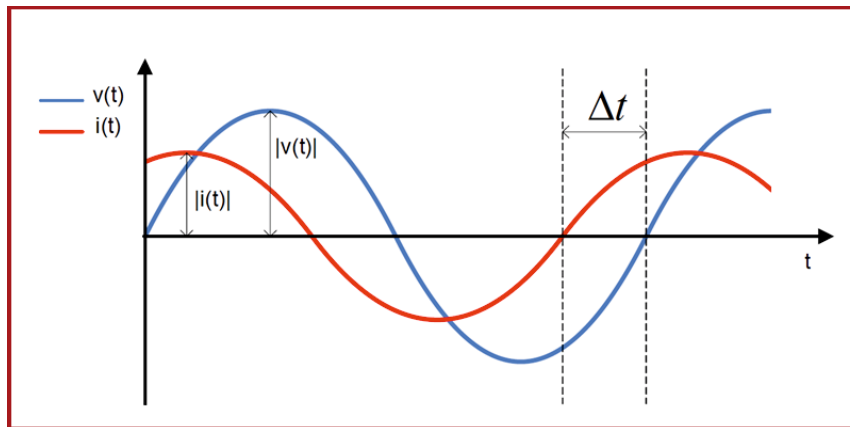


Figure 10. Impedance measurement, scheme of registered signal: $v(t)$, voltage signal; $i(t)$, current signal, Δt , time interval between the zero crossing of the voltage and current signals.

4.1.1 Sensors

The electrical response depends on the type of the electrode used (**Figure 11**). These are: 1) Needle electrode - this consist of a hollow needle with an internal isolated wire, so that a two- electrode system is configured. The external part of the needle is made of stainless steel and acts as the other electrode. The internal wire is also made of stainless steel and acts as the inner electrode; 2) Arrowhead (AH): This tool was designed using a thick-film technology, which uses high-resolution screen printing methods to deposit pastes or inks of different electrical characteristics (conductive, resistive and dielectric) on an insulating substrate, in order to form an electronic circuit. This electrode is designed with a pointed end to help it to penetrate through the sample; and 3) Double electrode (DE): This final tool is composed of two stainless steel needles that are 1.5 cm long and 1 mm in diameter, separated by a distance of 1 cm in non-conductive frame. This design keeps the separation between both needles constant during measurement.



Figure 11 Sensor; needle electrode (left), arrowhead electrode (middle) and double electrode (right).

4.2 Low-field NMR

Low-Field Nuclear Magnetic Resonance (NMR) is a fast and powerful method that can be used for non-invasive determination of water and fat content as well as to study water and fat mobility in e.g. muscle foods. The technique is less time consuming and less costly than conventional chemical-physical analytical methods.

The basis of NMR is that nucleic exhibit magnetic properties such as a nuclear spin that can be utilized to yield chemical information. In LF NMR (often also called time-domain NMR), an electromagnetic pulse excites protons in the sample, and the relaxation of protons towards equilibrium is measured. This magnetic resonance signal is very rich in measurable characteristics, including intensity, frequency of oscillation and rate of the recovery/decay. These characteristic reflect the nature of a population of atoms, the structure of their environment and the way in which the atoms interacts with this environment. Small differences in the precessing frequency in the ensemble of nuclei will cause loss of the transversal coherence, a process called spin-spin relaxation or transversal relaxation with its characteristic time T_2 . A larger T_2 generally means greater mobility.

In food, the NMR proton signal basically originates from small molecules like water and fat. Different tissue water populations can be studied because protons in different environments exhibit different T_2 relaxation properties. For example, tissue swelling after addition of salt leads to a more open microstructure causing higher proton relaxation. The relaxation time for bulk water is long, typically 2 to 2.5 s. Interactions between water molecules and macromolecules (proteins) take place in the muscle tissues of the food product. As a result of this, relaxation times are reduced. At least two relaxation components are usually

reported in fish muscle, often referred to as T_{21} with relaxation times in the range of 40–60 ms and T_{22} with relaxation times in the range of 150–400 ms (Erikson et al., 2012).

Low-field NMR has been applied in several studies on food products. LF- NMR has been used for determination of water holding capacity in meat (Bertram et al., 2001; Tornberg et al., 1993). The method may be applied to monitor changes in proton relaxation behavior as a result of a salting process (Aursand et al., 2010; Aursand, Gallart-Jornet, et al., 2008; Erikson et al., 2004; Gudjónsdóttir et al., 2011).

The most widely used pulse experiment for determination of T_2 is the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The measurements in this thesis were performed using a Bruker minispec mq 20 (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz. The instrument was equipped with a 10 mm temperature-variable probe. A built-in heating element was connected to the temperature control unit (BVT3000, Bruker Optik GmbH). **Figure 12** shows the low-field NMR bench top instrument used in the work with this thesis.



Figure 12. Picture of the low-field NMR bench top instrument.

In order to extract information from the relaxation curves several methods are available, each with their own strengths and weaknesses as reviewed by Erikson et al. (2012). The methods may be classified into single sample algorithms and multivariate data analysis. In the work of this thesis the NMR transverse relaxation data were analyzed using two different calculations methods: (1) biexponential analysis of T_2 relaxation data was performed by fitting of the following equation to the experimental CPMG curves, similar to that reported by Erikson et al. (2004) and Lambelet et al. (1995).

$$\text{Signal} = A_{21}e^{-t/T_{21}} + A_{22}e^{-t/T_{22}} \text{ Equation 3}$$

T_{21} and T_{22} are the relaxation time component, and A_{21} and A_{22} are the corresponding amplitudes; and (2) the multivariate data analysis Principal Components Analysis (PCA) was performed for all raw relaxation (CPMG) curves. These curves were normalized by setting the sampled echo to a value of 100 and thereafter scaling the rest of the echo train. PCA has frequently been applied to multivariate data (Jolliffe, 1986; S. Wold et al., 1987). Typically, in PCA projects a multi-dimensional data set onto a new coordinate base formed by the orthogonal directions with data maximum variance. The eigenvectors of the data matrix are called principal components and they are uncorrelated between each other. The principal components (PCs) are ordered so that PC1 displays the greatest amount of variance, followed by the next greatest PC2 and so forth. The main features of PCA are the coordinates of the data in the new base (scores plot) and the contribution to each component of the sensors (loads plot).

4.3 Computer Vision

Process optimization and higher levels of automation in the food industry contributes to the growing needs for efficient production methods. *Computer vision* is a technology used to automate visual inspection and measurement tasks using digital cameras and image analysis techniques. The computer uses image data to perform pre-defined measurement tasks and to draw conclusions based on these data (Lind & Murhed, 2012). Meat products are normally classified based on features that are suitable to analyze with computer vision systems, such as color, fat distribution, texture and morphology (USDA Agricultural Marketing Service, 2012). Several studies on computer vision data representing appearance, texture attributes, tenderness and classification such as; prediction of lamb tenderness (Chandraratne et al., 2006), characterizing and classifying of pre-sliced pork and Turkey hams (Iqbal et al., 2010), prediction of beef eating quality (Jackman et al., 2008), analysis and classification of sliced pork, turkey and chicken hams (Mendoza et al., 2009) and characterization of the texture appearance of pre-sliced pork .

In fish products, computer vision has been applied purposely to determinate color attributes of fish muscle, as affected by irradiation (Yagiz et al., 2010), high pressure and cooking (Yagiz et al., 2009). Other applications in the fish industry have been regarded to automated

grading of whole Atlantic salmon according to their quality grade (Misimi et al., 2008; Misimi et al., 2006), automated weight-based sorting (Balaban, Chombeau, et al., 2010; Balaban, Ünal Şengör, et al., 2010; Mathiassen et al., 2011) and in automated slaughter lines for Atlantic salmon (Bondø et al., 2011). Due to this knowledge, computer vision is an interesting alternative to human expert grading and might be an efficient tool for on-line measurements of surface (textural) changes in low-salt cooked ham.

4.3.1 The principles of SINTEF Food Scanner

The computer vision has to be tailor-made when it comes to the product in which information one require. The SINTEF Food Scanner is tailor-made for the sorting and grading of whole Atlantic Salmon in the fish industry and has been further developed for the measuring of changes on the surface of food such as cooked ham (**Figure 13**).

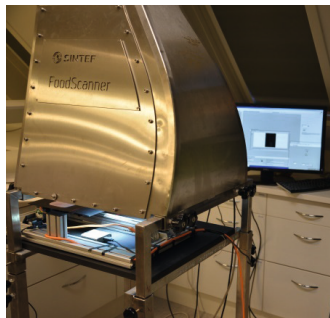


Figure 13. SINTEF Food Scanner measuring cooked ham.

The imaging system used in this thesis consisted of a ColorRanger multimodal line-scan camera, two high-intensity white LED linear array lights, with polarizers on the LED arrays and in front of the camera lens. Additionally, a nematic liquid crystal industrial-grade polarization rotator was placed in front of the polarizer on the camera lens. The polarization rotator is controlled by the image acquisition PC and enables the effective rotation of the polarizer on the lens by means of an electric signal. The purpose of rotating the polarizer direction on the lens polarizer relative to the polarizer on the LEDs, was to be able to capture light reflected from the object for two polarization states.

To separate the subsurface from the surface image, two images – I_{\parallel} LED camera polarizers oriented parallel to each other, and I_{\perp} with the polarizers oriented perpendicular to each

other can be acquired. Image I_{\parallel} will image both the subsurface and the surface components, whereas I_{\perp} will image the subsurface components only, and hence the difference $I_{\parallel} - I_{\perp}$ between the two images will image only the surface components of the light. This principle of light interactions as a function of polarization state is illustrated in images in **Figure 14**.

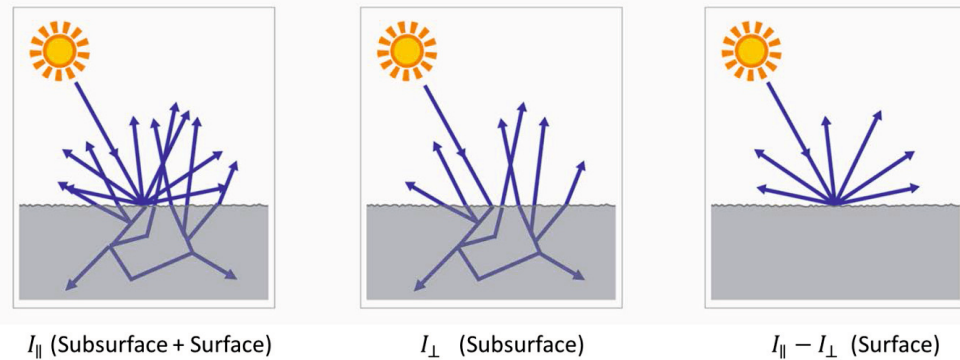


Figure 14. Illustration of the components of light interacting with the imaged raw material using parallel polarizers (left), crossed polarizers (middle) and the difference between the two (right).

As shown in **Figure 15**, subsurface imaging revealed the bulk color of the food product near the surface, whereas surface imaging revealed the surface roughness, shininess and other effects such as "mother-of-pearl" appearance.

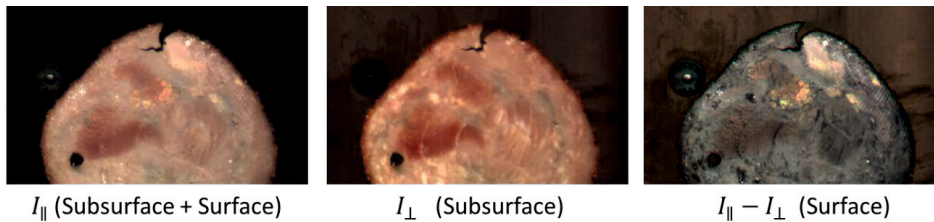


Figure 15. Images of a slice of ham using parallel polarizers (left), crossed polarizers (middle) and the difference between the two (right).

Computer vision generates an enormous amount of data, and a Lab VIEW program (National Instruments, USA) (**Figure 16**) is used to extract imaging features. The extracted imaging features are simply the mean of the red, green and blue (r,g,b) values, as acquired with the ColorRanger (SICK IVP, Sweden), over the entire ham slice and for both polarizer orientations.

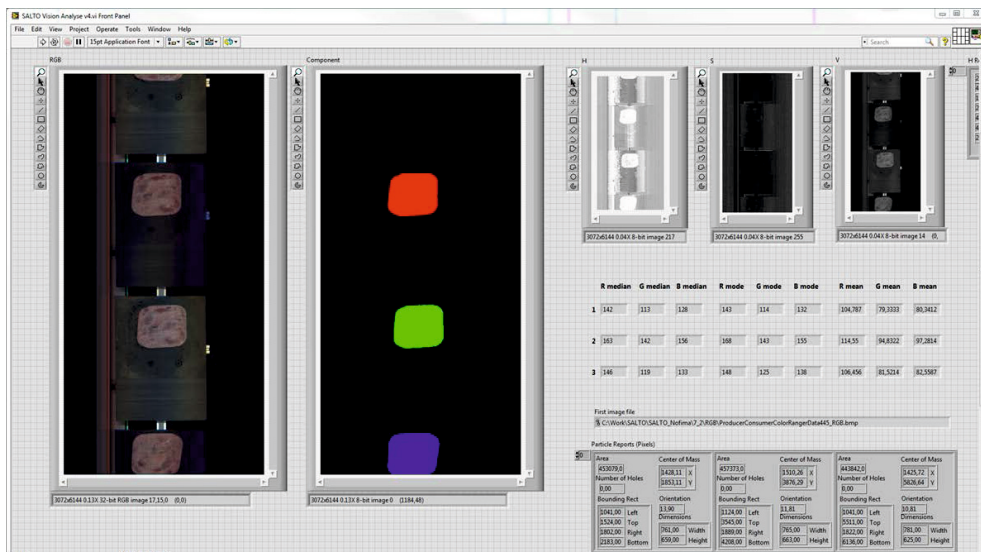


Figure 16. A screenshot of the Lab VIEW program used for analyzing of the images.

4.4 Sensory analysis

Sensory analysis is the most appropriate approach to fully describe the sensory perception of foods and descriptive profiling has been a popular sensory technique for cognitive descriptions of products in many years. These techniques give a complete sensory description of products and make it possible to identify underlying ingredients and process variables (Lawless & Heymann, 2010). This method is illustrative and relatively easy to apply, but can require a panel that is trained in evaluating the product. For measurement of flavor, no instruments which exist today can replace the human senses and describe the sensory perception better. Sensory analysis, therefore, involves measuring properties like colour, appearance, texture, smell and taste by use of the human senses. They are crucial for understanding the relation between food properties and human liking and buying behavior. Sensory analysis can be applied in areas like mapping and monitoring of the industry, quality controls, product development and research (Berg & Dyrnes, 1997).

4.5 Salt determination by sodium selective electrode and chloride titration

The Volhard method is an often used method for analyzing salt (NaCl) content in food (AOAC, 2008). This method is based on chloride titration and calculation of the salt (NaCl) content in the product. As the focus is on reducing the sodium content, it is important to be able to measure the correct amount of sodium already in the food product.

4.5.1 Salt content (NaCl) of the muscle - Volhard method

Chloride content in food measured by the Volhard method (AOAC, 2008) has to be measured in an extract of the sample. The principle of analyzing salt by the Volhard method is end-point titration of chloride with silver nitrate (AgNO_3). The titration includes three chemical reactions: **1:** $\text{AgNO}_3 + \text{NaCl} \rightarrow \text{AgCl} + \text{Na}^+ + \text{NO}_3^-$, where the excess of silver nitrate (AgNO_3) titrates with ammonium thiocyanate (NH_4SCN) **2:** $\text{AgNO}_3 + \text{NH}_4\text{SCN} \rightarrow \text{AgSCN} + \text{NH}_4^+ + \text{NO}_3^-$, and a ferric indicator is added for determination of the end point, where a fair red-brownish color appeared **3:** $\text{FeNH}_4(\text{SO}_4)_2 + \text{NH}_4\text{SCN} \rightarrow \text{Fe}(\text{SCN})_3 \times \text{Fe}(\text{SCN})_2^+$. The salt content is calculated as percentage of the sample.

4.5.2 Sodium ion selective electrode

Measurement of sodium content in food measured by a sodium selective electrode has to be done in an extract of the sample, in this work a Dual Star™ pH/ISE Meter (Thermo Fisher Scientific, Waltham, MA, USA) with a Na-selective electrode (Ross® Sodium Ion Selective Electrode, Thermo Fisher Scientific, Waltham, MA USA) where used. **Figure 17** shows the sample preparation and the pH/ISE Meter. The Na-selective electrode method used in this work was a modification of the Kivikari (1996) method. In these studies the direct calibration method was used, whereas Kivikari, used the known addition method. A calibration curve was made by using three standards of analytical-grade NaCl from (Merck KGaA, Darmstadt, Germany) and Sodium ionic strength adjustor (Thermo Fisher Scientific, Waltham, MA, USA) was added to all solutions to ensure that samples and standards had similar ionic strength.



Figure 17. Sample preparation of cooked ham for measuring of sodium content with sodium selective electrode and a Dual Star™ pH/ISE Meter.

The principle of the operation of sodium measurement is the potential developed at a sodium selective membrane which is measured through the use of two internal electrochemical cells. The membrane potential is added in series, to the potential of the sensing reference cell, and their sum is measured against the potential of the second (reference cell). Since the potentials of the two internal references cells remain constant, changes in potential are due to changes in sodium concentrations (Thermo Scientific, 2008). The measured potential corresponding to the level of sodium ion in solution is described by the Nernst equation ($E = E_0 + S \log A$ Equation 4),

$$E = E_0 + S \log A \text{ Equation 4}$$

Where the E = measured electrode potential, E_0 = constant potential largely dependent of the reference electrode, A = sodium ion activity or "effective concentration of sodium" and S = electrode slope

The Dual Star™ pH/ISE Meter gives the amount of sodium in the extract, and the sodium in the sample can be calculated as mg Na⁺/100 g or % Na⁺ of the sample.

4.6 Protein solubility

Solubilisation of the functional myofibrillar proteins is important for the properties and quality in processed foods. The amounts of proteins extracted from the raw materials have been found to be related to other functional properties such as WHC and texture properties (Jafarpour & Gorczyca, 2012). The solubility properties in the raw material can therefore be related to the properties of the final product.

METHODOLOGY

The method used to extract water- and salt-soluble proteins in this work was a modification of (Anderson & Ravesi, 1968; Licciardello et al., 1982) as described by (Hultmann & Rustad, 2002). The Bis Tris buffer was chosen because a phosphate buffer cannot be used in combination with $MgCl_2$, due to precipitation of $MgPO_4$. The protein solubility was analyzed in the fish mince before cooking. The type of buffer, Bis Tris and phosphate, ionic strength and type of salt in the buffer was examined, in order to select the most appropriate conditions for determination of influence of salts on the extractability of proteins. The amount of soluble proteins was determined by the BioRad protein assay (Bradford, 1976) using bovine serum albumin as a standard (0.1-1.0 mg/ml).

4.7 Different measurement techniques used for measuring sodium and salt (NaCl), an overview.

This section summarizes different measuring techniques used for measuring sodium and salt (NaCl) in food, and changes in the food matrix during salting (Table 5).

Table 5. Overview over analytical methods used for measuring sodium and salt (NaCl) in food.

Direct measurement of sodium (Na ⁺)			
Method	Application	Type of food	Ref
Atomic emission spectrophotometry with inductively coupled plasma (ICP-AES)		Low-salt infant formula	AOAC,(2008)
Flame atomic absorption spectrophotometry (FAAS)	Ground state metals absorb light at specific wavelengts.	Low-salt infant formula Milk and milk products Wheat brain (food containing> 1500 mg Na/kg)	AOAC, (2008); EN, (2008);ISO,(1996)
Ion-selective electrode	Potentiometry, Na-measurement	Low-salt Milk, bread, cheese, wine, butter and processed meats.	Ehling et al.,(2010); Kivikari,(1996) Paper I-V
Ion chromatography	Ion-exchange chromatography allows the separation of ions based on their affinity to the ion exchanger.	Low-salt fish mince	Greiff et al., (2014) Paper I
Atomic Absorption Spectroscopy	Wet digestion, using a microwave oven technique	(broccoli, carrot, bread, saithe fillet, pork, and cheese	Julshamn et al., (2005)
Indirect estimation of sodium			
Method	Application	Type of food	Ref
Chloride analyzer stoichiometrically	Coulometric end-point titration by the use of a sensing electrode	Low-salt salmon	Åsli & Mørkøre, (2012)
Volhart	Potentiometric titration method for chloride ions, indirect estimation av sodium concentration	Dry-cured ham	AOAC,(2008)
Volhart	Potentiometric titration method for chloride ions, indirect estimation av sodium concentration	Cod	AOAC,(2008)
Chloride analyzer	Potentiometric titration	Dry-cured ham	ISO, (1996); Pérez-Olmos et al. (1997); Santos-Garcés et al., (2010)

METHODOLOGY

Table 5. continued. Overview over analytical methods used for measuring sodium and salt (NaCl) in food.

Prediction of NaCl and changes during salting			
Method	Application	Type of food	Ref
Impedance Spectroscopy, needle electrode	Prediction of NaCl concentration	Minced pork loin	Masot et al., (2010)
Impedance Spectroscopy, double electrode	Predicting salt content and a_w	Low-salt fish mince	Greiff et al., (2014) Paper I
Impedance Spectroscopy, needle electrode	NaCl content, moisture and a_w during salting-smoking process.	Smoked Salmon, 1.67-3.62 g NaCl/100g	Rizo et al., (2013)
Computer tomography	Monitoring salting in pork	Pork	Fulladosa, Santos-Garcés, Picouet, et al., (2010); Fulladosa, Santos-Garcés, Picout, et al., (2010)
Computer tomography	Predicting salt content	Dry-cured ham	Vestergaard et al., (2005)
Computer tomography	Prediction water activity, salt and water	Dry-cured ham, ground pork	Håseth et al., (2007); Håseth et al., (2012)
LF NMR	Changes during brine salting	Salmon	Aursand et al. (2010); Aursand, Veliyulin, et al. (2008)
LF NMR	Changes during brine salting	Cod and Salmon	Aursand, Gallart-Jornet, et al. (2008)
LF NMR	Changes during salting and desalting	Cod	Erikson et al., (2004)
LF NMR	Changes during pre-salting, dry salting and rehydration	Cod	Gudjónsdóttir et al. (2011)
LF NMR	Changes during brine injection, brining and freezing.	Wild and farmed cod	Gudjónsdóttir et al., (2010)
Near infrared imaging (NIR)	Water, fat and protein	Dry-salted coalfish (bacalao)	J.P. Wold et al., (2006)
Fourier Transforming Infrared Microspectroscopy-FTIR	Protein structural changes	Beef	Perisic et al., (2011)
SPRITE ^{23}Na MRI (single-point ramped imaging with T_1 enhancement)	Quantitative ^{23}Na Magnetic Resonance Imaging	Model products of muscle food	Veliyulin et al., (2009)
^{23}Na MR imaging	Spin-echo ^{23}Na MRI MRI measure total free sodium (protein-bound sodium is not MRI visible) "see" and quantify how sodium is distributed in the sample	Heavily salted cod and salmon	Aursand et al. (2010); Gallart-Jornet et al. (2007); Veliyulin and Aursand (2007)

5 RESULTS AND DISCUSSION

The final product quality in minced fish and meat products depend on many different factors, for instance, the quality and composition of the raw material, processing parameters, type and content of salt, and other ingredients added. These factors will affect the product quality alone or in interaction with each other. The research activities performed in this work are divided into two main objectives: i) to evaluate different **measurement techniques** for measuring sodium directly in a food matrix, and indirectly by analyzing the changes in the food matrix as a function of sodium reduction in muscle foods; and ii) to study the effect of sodium reduction on **quality parameters** in muscle food, as presented in **Figure 18**.

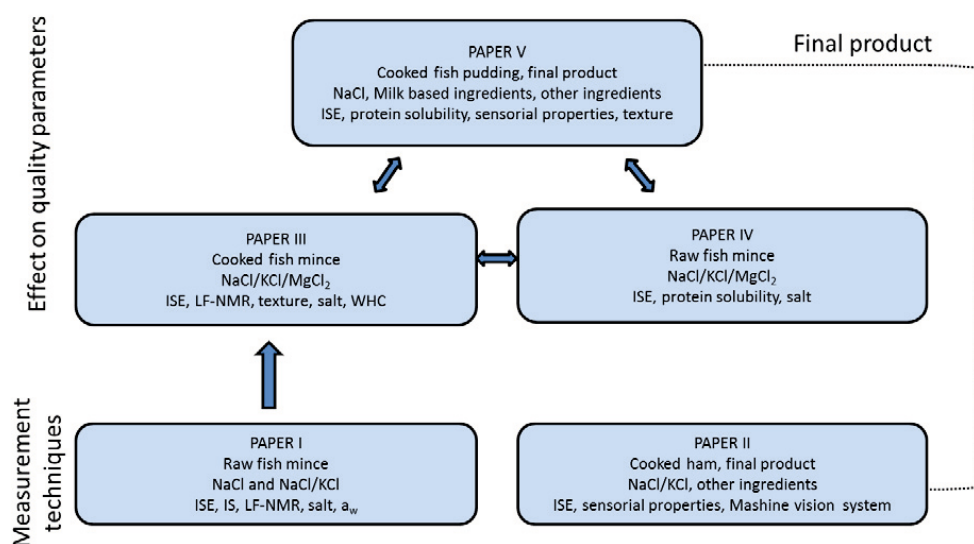


Figure 18. A schematic overview and connection between the work carried out in this thesis.

For a deeper understanding of the effect of salt and salt replacers on properties of muscle proteins and on physicochemical properties in fish mince, it was efficient to use model products containing raw hake mince, and both pure salts and mixtures (**Paper I**) as well as fresh and frozen haddock, with water and different types of pure salts added (**Paper III** and **Paper IV**). In **Paper III** the fish minces were added different types of salt, and were cooked in order to understand the effect of different types of salt on physicochemical properties in heat treated fish mince. In the work with this thesis, the knowledge transfer from research in lab-scale to industry requirements for commercialization has been important. The cooked

ham (**Paper II**) and fish pudding (**Paper V**) had a more complex composition of ingredients and were produced in a small-scale industry production, in order to investigate the effect of salt reduction on physicochemical and sensory properties in the final products. The main results of this thesis can be found in the papers enclosed in Part 2. In this chapter, a short summary and discussion of the main results is given. The relevance for the fish and meat industry is also discussed.

5.1 Analytical methods for measuring sodium and changes in the food matrix during sodium reduction in muscle foods (Paper I and II).

The goal of this part of the work was to evaluate different analytical methods for measuring sodium and changes in the food matrix during sodium reduction in fish or meat model products.

5.1.1 Evaluation of the impedance spectroscopy method for determination of salt and physicochemical changes in the fish mince (Paper I)

The electrical impedance spectroscopy (IS) has been used in several studies to monitor quality changes in meat and fish products. A relationship between sensory properties and electrical IS in dry cured ham was studied by Guerrero et al. (2004). Chanet et al. (1999) used the IS method to estimate the water and lipid content in minced pork, and Masot et al. (2010) showed a correlated relationship between pure sodium chloride content in minced pork meat and impedance data. Parameters such as a_w and salt content have important implications for product shelf- life and consumer safety. In this regard, the development of rapid, accurate, and non- destructive methods for monitoring these parameters, independent of the sodium replacement, is of industrial interest. In addition, it is important to have accurate methods for determination of sodium content in foods. In the work with this thesis, it was wanted to investigate the possibility of using IS for the purpose of measuring the sodium and potassium content in low-salt products containing small amounts of sodium and potassium. Furthermore, the application of IS to monitoring physicochemical parameters in salted fish products with, and without sodium replacement was evaluated (**Paper I**).

In Paper I, salt solutions and raw hake minces were analyzed. Both the solutions and minces were added the same amount of different salts (NaCl and NaCl/KCl) and contents (0.0-3.0g/100g sample). The results from the experiment is described in **Paper I**, and show a stronger correlation between IS data and salt content in the fish samples than in the solutions. As discussed in chapter 3.4, the conformational changes due to the effect of salt on the muscle proteins, could be responsible for the different behaviour in the IS as were observed between the fish mince and the solutions. The IS measurement was able to differentiate between different salt contents down to 0.5%. At some salt contents, the IS method also distinguished between types of cations (Na^+ or Na^+/K^+) in the fish mince.

As shown in **Paper I**, a PLS regression was applied to the IS data, and models for a_w , gram solutes (salts) (g/100g) and gram solutes/100g liquid phase showed very good behavior with R^2 values close to, or higher than 0.9. However, the results obtained for Na^+ (mg/100g), K^+ (mg/100g) and NaCl (g/100g) verified that the proposed technique is not able to discriminate between the different types of salt. The results obtained from the PLS confirmed the potential of IS with double needle electrode for monitoring a_w in low - salt low – fat fish minces. These results agree with other studies on the use of IS in the characterization of commercial cod and smoked salmon, in which the best predictions were obtained on a_w (Karásková et al., 2011; Rizo et al., 2013).

To summarize, the impedance spectroscopy measurement could separate between different salt contents down to 0.5%. The results obtained from the PLS confirmed the potential of IS with double needle electrode for monitoring a_w in low-salt low –fat fish minces. However, further work is needed to calibrate the technique for measuring different types of food matrixes (i.e. animal species, high or low fat) to ensure that the IS method gives the correct a_w in the sample. At some salt contents, the IS method also distinguished between types of cations (Na^+ or Na^+/K^+) in the fish mince. Compared to NaCl minces, replacing 50% of the NaCl in the mixture with KCl, gave too small changes in the physicochemical properties in the mince for IS method to be able to detect it.

For the fish and meat processing industry, at-line and non-destructive monitoring of a_w can be an interesting technique for process control and predicting of shelf-life. The IS method is fast, and no sample preparations is needed.

5.1.2 Determination of changes in proton mobility in low-salt fish mince measured by LF-NMR (**Paper I and Paper III**)

The LF-NMR method has been used in many studies to monitor changes in the muscle tissue during fish salting processes. As described in 4.2, tissue swelling after addition of salt leads to a more open microstructure causing higher proton relaxation. The interpretation of T_2 relaxation data have been controversial, but it is now becoming more accepted that the observed changes in relaxation behavior is primarily due to chemical and diffusive proton exchange between water molecules and biopolymers (e.g. proteins) (Belton, 2011a; Belton, 2011b; Halle, 2004, 2006). A number of studies have nevertheless shown that these processes are linked to the morphology of the sample which in turn can be affected by for example processing, such as salting (Erikson et al., 2012). T_2 relaxation is sensitive to protein unfolding (denaturation) (Lambelet et al., 1995). Several studies have been carried out where LF NMR T_2 relaxation analysis has been used to monitor changes in water distribution including: salting and frozen thawed raw material (Aursand, Veliyulin, et al., 2008); comparison of salting of lean and fatty fish (Aursand, Gallart-Jornet, et al., 2008); pre-salting methods of cod (Gudjónsdóttir et al., 2011); and the state of water in the muscle during processing (Gudjonsdottir et al., 2010). However, these previous studies have dealt with high-salt tissues. The goal of the present work with was to explore the method further as a potential tool for low-salt application as well. Specifically, the application of low field NMR to evaluate physicochemical parameters in low-salted fish minces with different types and amounts of salt was studied.

The LF-NMR T_2 relaxation method was used to study the relaxation behavior in raw hake mince added different salts (NaCl and NaCl:KC and contents (0.0-3.0 g/100g salted mince) (**Paper I**). In **Paper III** the method was used to study the relaxation behavior in cooked haddock mince added different salts (NaCl, KCl and $MgCl_2$) and contents (0.4-3.2 g/100 g salted mince), and prepared with fresh and frozen raw material.

For **raw hake mince** the T_{21} proton relaxation times, found by biexponential fitting, was 54 ms. The samples were stored at -18°C for 86 days before LF-NMR measurements were performed. The T_{21} values were in the same range as for frozen hake stored 18 weeks in a walk-in freezer at -20°C , 50.7 ± 0.8 ms, respectively (Sánchez-Alonso et al., 2014). The LF

NMR T_2 relaxation data for low-salt hake minces (**Paper I**) showed a clear separation between raw mince without added salt and the minces added 0.5% salt. The T_{21} relaxation times increased when salt was added (**Figure 19**).

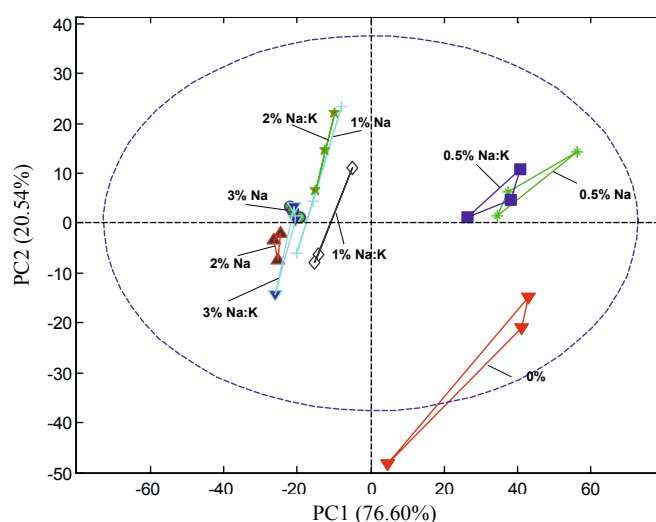


Figure 19. PCA score plot of LF ^1H NMR T_2 relaxation data obtained from hake mince with salt content (NaCl (Na) or NaCl/KCl (Na:K) 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 g/100 g salted mince, respectively) (**Paper I**).

These results are in agreement with the results from cooked haddock mince (**Paper III**), where the T_{21} proton relaxation times increased with increasing NaCl content (from 0.4 to 1.0%, or 0.07 - 0.17 mol pr kg mince). In the **cooked haddock mince** (**Paper III**) prepared from fresh raw material the proton relaxation times were slightly higher; 64-93 ms (T_{21}) and 402-598 ms (T_{22}), compared to those reported in Erikson et al. (2012); 40-60 ms (T_{21}) and 150-400 ms (T_{22}). These results can be explained by the changes in the protein matrix due to the added salt, water and heat denaturation of the proteins.

The method was unable to distinguish between hake minces where pure NaCl was added, and minces where a mixture of NaCl/KCl (50/50 w/w %) had been added (**Figure 19**). On the other hand, in cooked haddock mince where pure salts were added, a PCA score plot indicated that the addition of pure NaCl had more influence on LF ^1H NMR T_2 relaxation data than samples where the equal amount of KCl and MgCl_2 were added (**Figure 20**). One explanation for the differences between samples containing pure NaCl and pure KCl can be

RESULTS AND DISCUSSION

that Na^+ and K^+ affect the stabilization of the proteins according to the Hofmeister series (Hofmeister, 1888) as discussed by Puolanne and Halonen (2010).

A clear separation between MgCl_2 and the other samples (NaCl and KCl) is shown in **Figure 20**. An increased addition of MgCl_2 to the mince seems to have a different effect on the proton relaxation than that of the case where there was an increased addition of NaCl . Some of the explanation might be related to the ability of divalent cation (Mg^{2+}) to form cross-bridges between peptide chains in the muscle structure, (Aliño et al., 2009) and thus on proton relaxation. Mg^{2+} is highly electronegative who leading to a strong binding to polar groups of the proteins, and strengthening protein interactions (Xiong & Brekke, 1991).

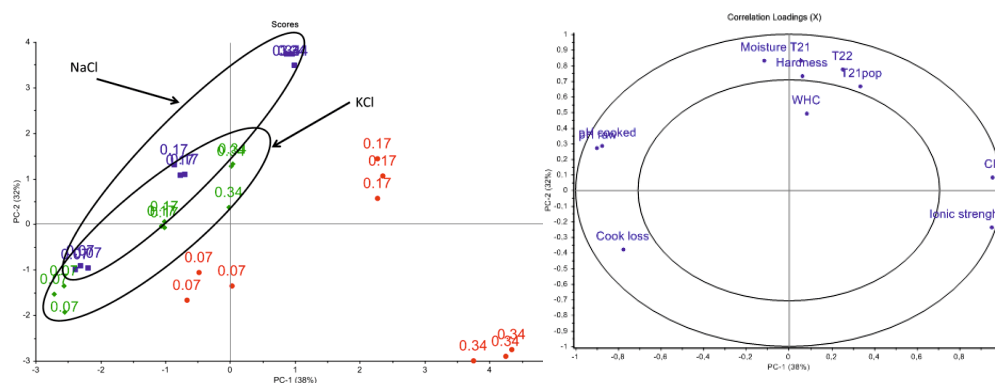


Figure 20. PCA score and correlation loadings plot of physicochemical results and LF ^1H NMR T_2 relaxation data obtained from fresh haddock mince with salt content (Na =blue, K =green and Mg =red, 0.07, 0.17 and 0.34 mol per kg mince).

When comparing the LF-NMR T_2 relaxation data in a PCA score plot for salted fish mince prepared from **fresh and frozen** haddock fillets (**Figure 21**), a clear separation in proton relaxation between fresh and frozen raw material is shown.

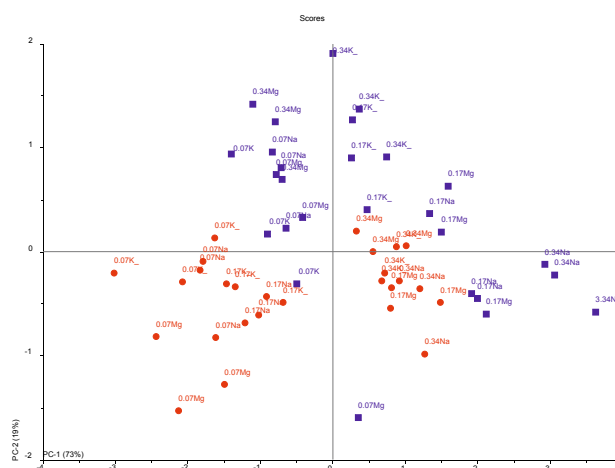


Figure 21. PCA score plot of LF ^1H NMR T_2 relaxation data obtained from fresh (blue) and frozen (red) haddock mince added Na (NaCl), K (KCl) and Mg (MgCl_2).

These results indicate that the storage of the raw material (fresh or frozen) has a bigger influence on the tissue microstructure/proton relaxation than the type and concentration of cations added to the mince.

The results from the presented work, show that the LF ^1H NMR T_2 relaxation method is sensitive to changes in the protein structure as an effect of additions of small amounts of salt. The LF ^1H NMR T_2 relaxation method can be a suitable technique to study changes in the protein structure in low-salt products. The LF-NMR T_2 relaxation method is less time consuming and less costly than conventional physicochemical analytical methods, which is also beneficial.

5.1.3 Developing a multimodal *machine vision system* for fast and non-destructive measurements of textural changes in the surface of low-salt ham.

The classification of ham qualities (pork and turkey) is possible based on color and textural features extracted from digital color images. Logistic regression, used on these features, could to a large degree explain consumer responses for visually-based sensory attributes (Iqbal et al., 2010). The qualities of pork ham could be distinguished based on visual texture characteristics extracted from fractal analysis of digital color images (Valous et al., 2009). Results demonstrated that the fractal-based features are able to quantify quality-specific visual texture characteristics. In this thesis a novel dual-polarization *multimodal machine vision system* was explored further. The goal of the experiment in **Paper II** was to investigate its potential as a tool for objectively studying changes in color and texture in the surface of salt reduced cooked ham. The instrumental set up is fully described in **Paper II**.

The images of cooked ham prepared with decreasing addition of salt using SINTEF Food Scanner are shown in **Figure 22**.

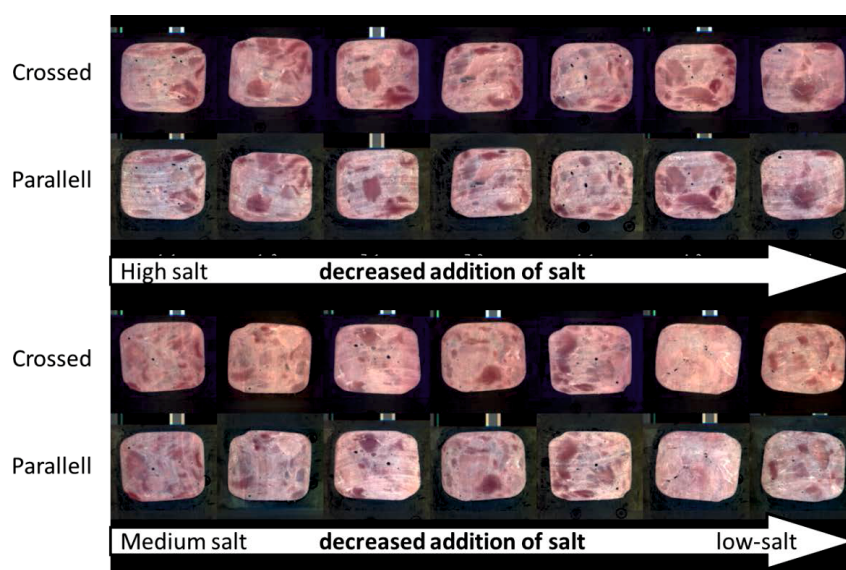


Figure 22. Cooked ham prepared with decreasing addition of salt (from left to right) is imaged using SINTEF Food Scanner. The top images show the photo from perpendicular camera polarization (crossed). The lower images show the photo from parallel camera polarization.

The sensory evaluation of the cooked ham indicated an increase in whiteness and decrease in color-hue with decreasing salt content. While the intensity of the subsurface

backscattered reflected blue light b_{\perp} (lightness) shows a clear increase as the salt content is reduced (**Figure 23**). A reduction in surface shininess, as measured by $b_{\parallel}-b_{\perp}$, using imaging, was only weakly linked to reduction in salt content, where no changes were seen in the shininess in the sensory evaluation.

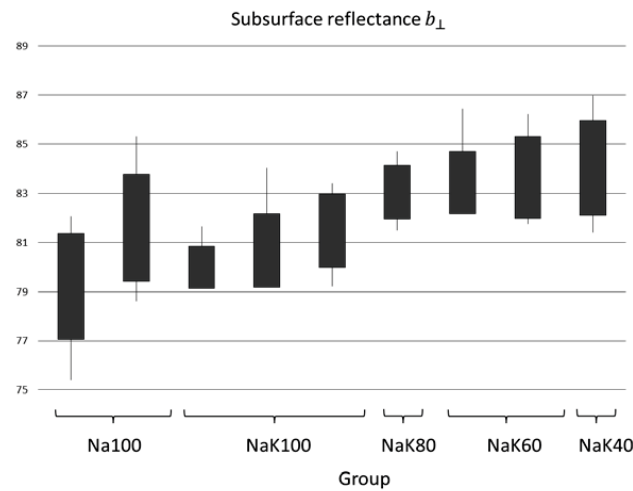


Figure 23. Mean subsurface reflectance b_{\perp} for the hams with varying salt content. (Na100 corresponds to cooked ham containing 3.4% salt, NaK100: 2.7% salt, NaK80: 2.3%, NaK60: 2.1% salt and NaK40: 1.7% salt)

Because of the challenges in the imaging setup in the main experiment, a supplementary experiment with improvements was performed. Results from the supplementary experiment showed that imaging with two polarization orientations can provide images that potentially can quantify subtle changes in visual appearance. However, we argue that there is a great potential in dual-polarization imaging if more advanced features such as those described in the literature (Iqbal et al., 2010; Mendoza et al., 2009) are applied.

A multimodal machine vision system would be of interest for the meat processing industry with the purpose for an automated visual inspection of the final product. Automatic segmentation between the dark and light portions of the mean values both for dark and light muscles would help improve the quantification of the visual appearance characteristics. This will simulate the way that human sensory evaluators do with their combined vision and decision process.

RESULTS AND DISCUSSION

5.1.4 Determination of sodium with Sodium ion-selective electrode (ISE)

Ion-selective electrode (ISE) has earlier been used for determination of sodium in low-salt products (**Table 6**). However, it was wanted to use this method for controlling the sodium content in the model products produced, and to compare the ISE method with the novel non-destructive methods applied in this thesis. The sodium selective electrode method was used in all papers presented in this thesis (**Paper I-V**), and the sample preparation is described in detail in **Paper I**.

In the work with experiments done in **Paper III** the results obtained from Na^+ and Cl^- measurements were used to compare the results. This was done on the basis of that chloride titration is an often used method for calculating NaCl in foods (**Table 5**). Both the measured Na^+ content adapted from the ISE method, and measured chloride content adapted from the chloride titration, showed good correlation between added and measured salt (**Figure 24**).

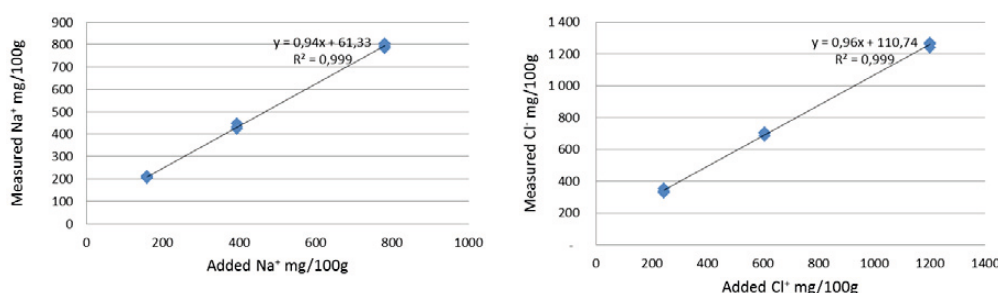


Figure 24 Comparison of added vs. measured Na^+ with ISE (left), and added vs. measured Cl^- from chloride titration (right).

In **Paper I** a comparison between sodium concentrations in the different minces as determined by the ISE and by ion chromatography was showed. Good correlation was observed between the sodium content determined by the ion selective electrode method and ion chromatography. This was confirmed by a simple regression carried out by the data obtained by both methodologies ($y=1.066x+7.961$, $R^2=0.967$).

These results confirm that ISE is a suitable method for direct measurement of sodium in lean fish products. In novel research on the effect of NaCl in food matrixes, both Na^+ and Cl^- are of

interest. ISE and chloride titration have been shown to be appropriate methods. For the food industry, the recommendation is to measure the Na⁺ by use of the ISE method. From these results the total NaCl content will be calculated, in accordance with the food regulations (EU, 2011). The ISE method is fast and easy to use, the equipment is relatively cheap and the method gives the sodium content in the sample, which is important with regard to the focus on sodium.

5.2 Understanding of the mechanism of salt and salt replacers in a food matrix.

5.2.1 Salt reduction and salt replacers effect on protein solubility. (Paper IV and V)

In the manufacture of minced fish products such as fish pudding, the functional properties of the muscle proteins including water holding capacity and gelling properties are important when it comes to product quality (Martínez-Alvarez & Gómez-Guillén, 2005). In the work with salt reduction and replacement of sodium with other salts, it is of interest to investigate how these changes affect the protein solubility. In light of this, raw haddock minces were added different salts (NaCl, KCl and MgCl₂), with varying contents (0.4-3.2%) and prepared prepared from either fresh or frozen raw material (**Paper IV**). In the work with more complex matrixes such as fish pudding, it is of interest to investigate the effect of ions from other ingredients, such as milk minerals, on protein solubility (**Paper V**).

Pure salt and haddock mince. The protein solubility was analyzed in the fish mince before cooking. The type of buffer (Bis Tris and phosphate), ionic strength and type of salt in the buffer on the solubility properties of the proteins was studied. Results from this study show that the amount of extracted proteins depends not only on the salt added to the raw material, but also on the salt in the extraction buffer. These results are further discussed in **Paper IV**.

The extractability of SSP increased with increasing concentration of salt (from 0.11 to 0.55 M) when extracted with 0.6 M salt corresponding to the salt in the mince. Addition of 0.55 M NaCl gave highest extractability of SSP (**Figure 25**).

RESULTS AND DISCUSSION

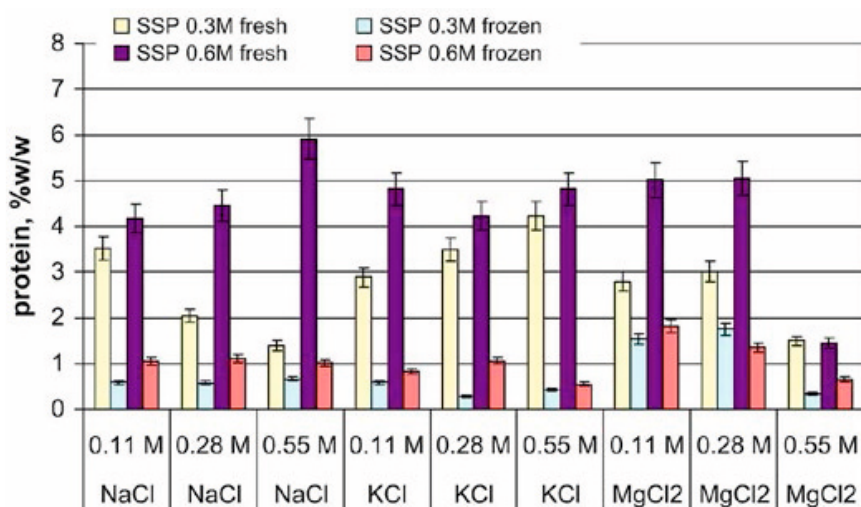


Figure 25. Extractability of salt soluble proteins in salted minces made from fresh and frozen haddock. Extraction was done in 0.3 M and 0.6 M salt corresponding to the salt in the mince. Results are given in %w/w of muscle in the sample. Values are given as mean (n=2) ± uncertainty of the method (7.5%).

These results can be explained by the protein stabilization effectiveness of cations, in accordance with the Hoffmester series, where the greatest solubility of proteins is obtained using a salt consisting of a chaotropic anion and a kosmotropic cation, such as NaCl (Puolanne & Halonen, 2010).

To summarize, extractability of SSP increased with increasing concentration of salt where NaCl gave highest extractability. Results from salted fresh haddock minces showed slight differences in the protein solubility with regard to the types of salt (NaCl, KCl and MgCl₂) at low concentrations (0.11 and 0.28 M). The addition of higher amounts of MgCl₂ (0.55 M) gave a decreased protein solubility. These results indicated that Na⁺ can partially be replaced with K⁺ and Mg²⁺ without changing the solubility of proteins. However the addition of Mg²⁺ is suggested only in small amounts, because of the protein solubility.

The extractability of SSP was significantly lower in minces prepared from frozen raw material compared to mince prepared from fresh raw material as well. For frozen raw materials the highest extractability was found in mince added 0.11 M MgCl₂ (in 0.6 M MgCl₂).

In the work presented in **Paper IV**, the ionic strength corresponding to the mass of mince without added water was calculated. In the further work (**Paper III**), the author found that it was more accurate to calculate the ionic strength corresponding to the mass of the whole

mince (fish and water). This is the reason why the estimated ionic strength in **Paper IV** and **Paper III** do not match, even though the addition of salt is the same.

Milk minerals and fish pudding. The fish puddings contained haddock fillets, skimmed milk, potato flour and salt (NaCl), in addition to two different permeate powders; low mineral whey permeate (LM) with high lactose and low mineral content, and high mineral milk permeate (HM) with low lactose and high mineral content. The salt concentrations in the minces (0.5-1.0 g/100 g mince) were based on the sodium content in all the added ingredients (total sodium content x 2.54). **Figure 26** shows the amount of extractable proteins in the minces. The amount of SSP were higher in the minces with 1.0 g salt/100 g (HS), showing that the extractability of SSP increased with increasing ionic strength. Both LM and HM showed an increase in SSP compared with the control at 1.0 g salt/100 g (and at 0.5 g salt/100 g for HM). Less water - soluble proteins (WSP) being extracted from minces with 1.0 g salt/100 g could be due to a greater amount of dissolved SSP in the mince, potentially trapping the WSP, or to a more swollen network.

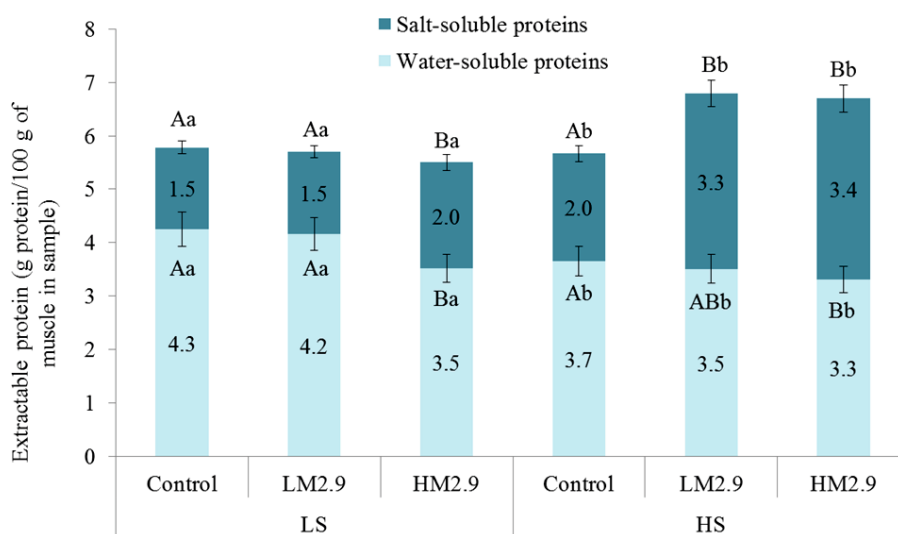


Figure 26. Extractable SSP and WSP in fish mince (n=2) extracted in 0.05 mol/l phosphate buffer (pH 7.0) without (WSP) and with 0.6 mol/l KCl (SSP). The results are expressed as g protein/100 g of muscle in sample \pm the uncertainty of the method (7.5 %). LS=low salt (0.5 g/100 g), HS=high salt (1.0 g/100 g), LM=low mineral permeate (2.9 g/100 g) and HM=high mineral permeate (2.9 g/100 g). Significant difference between groups, with respect to salt level and permeate powder fraction within the salt levels, are indicated with lower-case and upper-case letters, respectively.

The results from the study on fish pudding showed a relationship between the amount of extractable proteins and water holding capacity (WHC), demonstrating increased WHC with increasing SSP ($p=0.028$) and decreasing WSP ($p=0.000$). The breaking force was also correlated with the amount of extractable proteins, with increased breaking force with increasing SSP ($p=0.010$) and decreasing WSP ($p=0.001$). This is in accordance with earlier studies (Jafarpour & Gorczyca, 2012). The strong correlation between WSP and both WHC and breaking force might be explained by the salt levels leading to a greater amount of dissolved SSP in the mince, potentially retaining more WSP. If a greater amount of WSP is trapped in the gel matrix, these proteins could lead to increasing amounts of bound water due to osmotic forces, leading to an increased WHC.

5.2.2 The effect of salt reduction and replacing of sodium with K^+ or Mg^{2+} -ions on physicochemical properties (**Paper I and Paper III**)

How different types and concentrations of cations affected the physicochemical properties was studied in the work presented in **Paper I** and **Paper III**. Pure fish mince made of raw hake added different salts (NaCl and NaCl:KCl) and contents (0.0-3.0 g/100g salted mince) (**Paper I**) and a model product of cooked haddock mince added different salts (NaCl, KCl and $MgCl_2$), contents (0.4-3.2 g/100 g salted mince) and prepared with fresh and frozen raw material (**Paper III**) were used.

The addition of 3 w/w % salt to hake mince led to a reduction in **moisture** content from 80 % in mince without added salt to 78% for mince added NaCl/KCl (50/50 w/w %) and 78% added NaCl (**Paper I**). Similar results were obtained in cooked haddock mince where the moisture decreased due to increasing salt content. Replacing NaCl with KCl had no effect on the moisture content. The moisture in cooked haddock mince added 0.34 mol $MgCl_2/kg$ mince (corresponding to 3.2% salt), had significantly lower moisture content than all the other minces (**Paper III**).

The **WHC** in cooked haddock mince (**Paper III**) tended to decrease slightly with decreasing content of different salts, however. Only the mince added 0.07 mol $MgCl_2/kg$ (corresponding to 0.7% salt) had lower WHC than the rest of the minces added NaCl and KCl (**Paper III**). Neither ionic strength nor pH appears to explain these results. It may be related to the

ability of divalent Mg^{2+} -ion to form cross-bridges between peptide chains (Aliño et al., 2009), and this effect of Mg^{2+} is masked by the increased ionic strength when the addition of $MgCl_2$ is increasing. Although the addition of KCl and NaCl gave similar WHC, minces added NaCl had slightly higher WHC than the corresponding minces added KCl. These results correlate with the results in **Paper IV**, where an addition of 3.0 g NaCl/ 100 g in raw mince had higher extractability of salt soluble proteins (SSP) than the KCl mince. This is possibly because NaCl can lead to more muscle swelling than KCl due to cation effects in accordance with the Hofmeister series, as discussed in section 5.2.1.

Freezing of haddock fillets before preparing the mince resulted in reduced WHC. The WHC in minces with added NaCl decreased significantly compared to the WHC in minces with similar NaCl contents prepared from fresh raw material. This effect was much less pronounced in minces added KCl, and not present in minces added $MgCl_2$ (**Paper III**).

The **cooking loss (Paper III)** in cooked haddock mince decreased with increasing addition of salt. The cooking loss decreased with increasing concentrations of chloride ions and increasing ionic strength. Anyhow, the extractability of SSP in Paper IV cannot explain these results. Use of frozen raw material (haddock fillets) increased the cooking loss compared to fresh raw material. These results, on the other hand, may be related to the dramatic drop in SSP in the raw mince prepared from frozen fillets, as shown in Paper IV.

The **water activity (a_w)** decreased from 0.992 (hake mince without salt) to 0.974 for mince added 3% NaCl/KCl (50/50 w/w%) and 0.969 added 3% NaCl (Paper I). This can be explained by the increased mineral content. As expected, slightly higher a_w were found in the NaCl:KCl hake minces than in minces containing pure NaCl. a_w decreased with increasing number of colligative units dissolved per volume. As K^+ is a larger ion than Na^+ , replacing NaCl with equal amount by weight of KCl lead to a lower number of dissolved ions (colligative units) per volume and thus an increase in a_w of the product.

The **pH** measured in raw hake was 6.97. The pH was reduced when adding salt. The decrease was more pronounced in NaCl minces (decrease to 6.76) than in NaCl/KCl minces (decrease to 6.81) (**Paper I**). In the cooked haddock mince, pH decreased significantly in 0.07 mol $MgCl_2$ /kg mince (corresponding to 0.7 w/w %), compared to raw haddock mince without

RESULTS AND DISCUSSION

added salt, pH 6.09 and pH 6.52, respectively (**Paper III**). pH decreased with increasing salt content for both cooked haddock minces added KCl, NaCl and MgCl₂. The decrease was most pronounced for minces added MgCl₂ (**Paper III**).

Texture/breaking force. The breaking force in cooked haddock mince increased with increasing NaCl content. This result can be explained by the increased extractability of SSP in raw NaCl minces, shown in **Paper IV**. Cooked minces added 0.34 mol MgCl₂/kg mince was softer than minces added the same amount of KCl and NaCl. The reduced breaking force in the cooked minces added 0.34 mol MgCl₂/kg mince can be correlated to the low ability of MgCl₂ to solubilize salt soluble proteins as shown in **Paper IV**.

A PCA was performed on the physicochemical data and the LF-NMR T₂ relaxation data obtained from the experiment with cooked haddock mince (**Paper III**), shown in **Figure 27**.

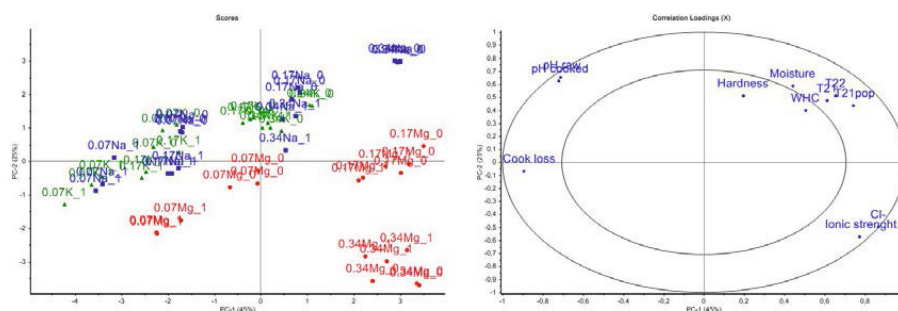


Figure 27. PCA score (left) and correlation loading (right) plot of physicochemical results and LF ¹H NMR T₂ relaxation data obtained from both fresh (0) and frozen (-1) haddock mince added Na⁺ (blue squares), K⁺ (green triangles) and Mg²⁺ (red circle) cations.

The present work reveals new information about KCl and MgCl₂ used as salt replacers. A reduction of salt led to an increase in moisture, reduced WHC, increased cooking loss and decrease in breaking force in the model products. The pH also increased with decreasing salt content. Replacing NaCl with pure KCl had no, or only a small, effect on the pH, moisture, breaking force, WHC and cooking loss in the model products, as shown in **Figure 27**. However, replacing NaCl with an equal molar concentration of MgCl₂ affected several of the physicochemical properties and in particular WHC, cooking loss and pH. The results show that if MgCl₂ is used as a sodium replacer in commercial fish products, it should be used in controlled conditions to ensure the desired effect on the physicochemical properties and not least the taste (sensory properties were not analyzed in this study). Frozen raw material

affects the physicochemical properties as well, and adding other types of cations than Na^+ , did not compensate for the changes.

For the food industry these results demonstrate that replacing NaCl with KCl is a good alternative with regard to the physicochemical properties in the product. It will nevertheless not be appropriate to replace 100% NaCl with KCl because of sensory properties in the final product, and because of the bitter and metallic taste from too much KCl. From a research perspective, further studies of the effect of Mg^{2+} -ions on the protein matrix is of interest for a better understanding of the effect on the myofibrillar proteins and to optimize the amount of Mg^{2+} -ions used as salt replacer in muscle foods.

5.3 Possibilities for salt reduction in final products (Paper II and V)

This part of the work will discuss the possibilities of sodium reduction in final end products, where the content of sodium from other ingredients than pure salt (NaCl) has to be taken in consideration. Milk mineral was used as a salt replacer in fish pudding (**Paper V**), and Na^+ was partly replaced by K^+ in cooked ham (**Paper II**) for the purposes of this study.

5.3.1 Sodium reduction in Fish pudding (Paper V)

In the work with sodium reduction in foods it is of interest to investigate different types of salt replacers which can be labelled as a "natural ingredients". Whey and milk based permeates contain high amounts of minerals such as Ca^{2+} , Na^+ and K^+ and due to this it would be valuable to increase the knowledge of using whey permeate as a salt replacer. In **Paper V**, sodium reduction in fish pudding was therefore studied. The salt contents were in the range of 1.0 to 0.6 %.

No differences in cooking loss were observed in these studies. The cooking losses in the different fish puddings were low (around 6 g/100 g), compared to the cooking loss observed in **Paper III** where the cooking loss in fish mince added 0.4% salt and water was more than 20 g/100 g. These results indicate that the addition of other ingredients than salt, such as milk proteins and potato starch, influences the cooking loss. Reduction of the salt content from 1.0 to 0.6% did not have any effect on WHC either. However, both types of milk mineral tested showed an improvement in WHC at all three salt levels. A significant linear

RESULTS AND DISCUSSION

relation was found between breaking force and WHC ($p=0.003$, $R^2=0.75$), which reinforces the findings from the protein solubility results that showed a relation between breaking force, WHC and soluble proteins.

The descriptive quantitative sensory analysis (DA) of fish puddings showed no changes in the texture attributes due to the reduction of salt in the control samples (1.0, 0.8, and 0.6% without addition of milk minerals). **Figure 28** shows a selection of the DA results; all the results from the DA are shown in **Paper V**. The addition of milk minerals increased the texture attributes firmness, cohesiveness, chewiness and elasticity, whereas the addition of HM had the highest influence on these attributes. One explanation for the textural changes in the puddings containing HM might be the high mineral content, a.o. K^+ ions. Textural changes have been observed for salt substitution with K^+ ions in previous studies on salt replacement in smoked salmon (Almli & Hersleth, 2013).

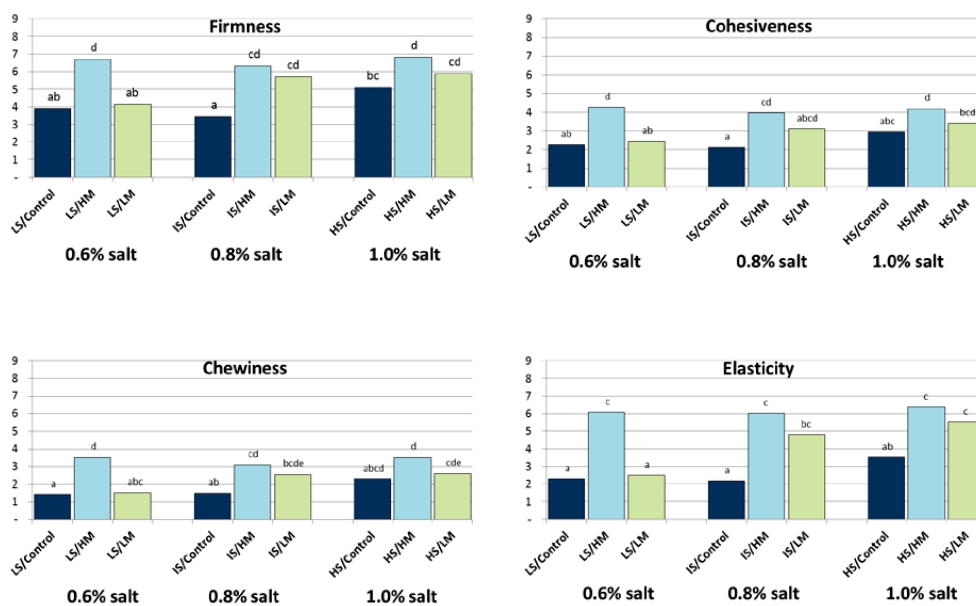


Figure 28. Descriptive sensory profile (firmness, cohesiveness, chewiness, elasticity) of 9 samples of fish pudding, control (blue), added LM= low mineral permeate (2.8 g/100 g) (green) and added HM= high mineral permeate (2.2 g/100 g) (light blue). ^{a-e} Different upper-case letters indicate significant differences ($p < 0.005$) between different groups. Means that do not share a common letter are significantly different.

The intensity of the salty taste decreased due to decreased salt content in the fish pudding (Figure 29), however. The addition of milk mineral increased the salty taste, though, whereas the addition of HM clearly dominated the salty taste.

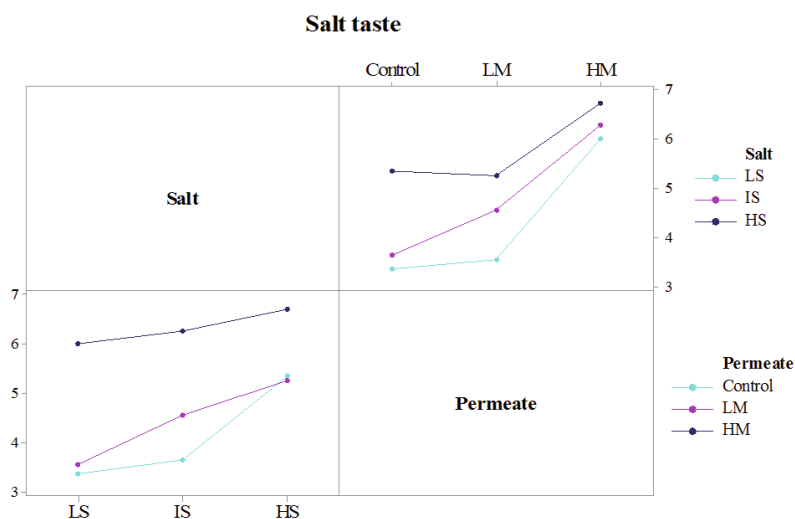


Figure 29. Interaction plot of salty taste where increased score indicates increasing salt intensity of the puddings. The left plot shows the effect of increasing salt level on perceived saltiness and the right plot shows the effect of permeate addition on perceived saltiness. Salt level is indicated by LS=low salt (0.6 g salt/100 g), IS=intermediate salt (0.8 g/100 g) and HS=high salt (1.0 g/100 g), and permeate addition with LM=low mineral permeate (2.9 g/100 g) and HM=high mineral permeate (2.2 g/100 g).

To summarize, this work shows that salt reduction without any salt replacers in fish pudding from 1.0 down to 0.6 % will affect the taste more than the cooking loss, WHC and texture attributes measured with DA. The addition of low mineral permeate (LM) improved the textural and water-holding properties of puddings at salt concentrations down to 0.8 % salt, while it did not affect the salty taste. Additions of high mineral permeate (HM) contributed to change in the textural and water-holding properties, and also increased salty taste. For the food industry, this result shows that sodium/salt reduction is possible in fish pudding. Based on investigated factors, a 40% sodium reduction is possible in fish pudding using high mineral permeate as a salt replacer.

5.3.2 Development of cooked ham with reduced content of sodium (**Paper II**)

This part of the work was conducted in close collaboration with the meat industry. The main goal for the industry was to develop a cooked ham with reduced content of sodium without changing the quality of the final products. The cooked ham was prepared with the same ingredients as in a commercial cooked ham (lean pork meat, phosphate, gelatin, carrageenan, Na-lactate, maltodextrine, nitrite and water). The total salt content was gradually decreased from 3.4 down to 1.4 % salt. The reference ham contained only NaCl (3.1%), whereas in the other cooked hams KCl was used as a salt replacer. In these hams, the mole ratio of sodium (Na^+) and potassium (K^+) was kept constant ($\text{Na}^+:\text{K}^+= 3:1$).

The result from this work shows that a replacement of 25% Na^+ ions by K^+ ions is possible without changing the WHC, moisture, pH, expressed moisture and the sensory profile attributes. This corresponds to a salt reduction from 3.4 to 2.7 % (total sodium content $\times 2.5$). A further reduction down to 1.7-1.4 % salt led to a decrease in WHC and increase in expressible moisture. **Figure 30** shows a selection of the DA results; all the results from the DA on cooked ham are shown in **Paper II**. The salt reduction had the highest influence on the sensory attributes salty taste. After taste, tenderness, hardness and color hue was most affected. The intensity of salty taste decreased with decreasing salt content, naturally. However, the salt content had to be 1.7 % salt and lower before the differences was significant. The reduced saltiness can be explained by the reduced content of Cl^- ions in the cooked ham, since Cl^- ions have an effect on the receptor cells and consequently on the perception of salt taste (Murphy et al., 1981). The salt content had to be reduced down to 1.4% before the changes were significant for after taste. A reduction of total salt content down to 1.4%, gave a more tender and less hardness in cooked ham. These results were as expected and in accordance with other studies. Ruusunen and others (2003) found that added salt increased the firmness in Bologna type sausages added 1.10, 1.35 to 1.60% salt, respectively and Sofos (1983) found that a reduction in salt content of more than 20% (<2.0% NaCl) in Frankfurters resulted in softer and less firm texture.

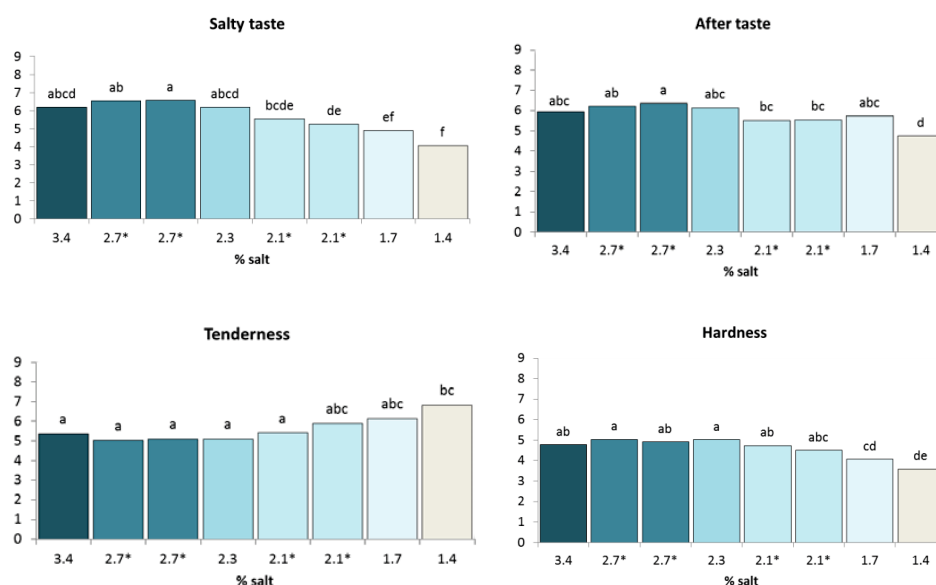


Figure 30. Descriptive sensory profile (salty taste, after taste, tenderness and hardness) of 8 samples of cooked ham, reference ham containing 3.4% salt to the left (dark blue) (* duplicate batches). ^{a-f} Different upper-case letters indicate significant differences ($p < 0.005$) between samples. Means that do not share a common letter are significantly different.

To summarize, the study on cooked ham showed that salt reduction influence both the sensory and physicochemical properties negatively. However, a 25% replacement of Na^+ ions with K^+ ions was possible without changing the quality in the final product (corresponding to 3.4 – 2.6% salt).

For the food industry these results show that sodium/salt reduction is possible in cooked ham. For the cooked ham in this experiment the results show that the critical limit for sodium reduction is above 35% (2.1% salt and lower). The industry partner in this work has already applied these results to further develop a low-salt cooked ham, and the product is now available on the market for Norwegian consumers. In further work with salt reduction on cooked ham it is suggested to work with other salt replacers and improvement of the production process to keep the textural properties, salty taste and to bind water.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

In this work, different analytical methods for measuring sodium and changes in a food matrix as a function of sodium reduction has been evaluated. The results showed that impedance spectroscopy could be a potential technique for monitoring a_w in low-salt low-fat fish minces and to indirectly predict the amounts of salt in the sample. However, it is suggested to prepare a larger number of samples with different concentrations of pure salts in order to distinguish between different types of cations in the sample. Further studies have to be done to optimize the electrode and the IS equipment used to measure sodium and other cations indirectly in the sample.

The LF-NMR measurements on both raw and cooked fish mince with 0-3 % salt added showed that this method is sensitive to changes in the protein structure as an effect of addition of small amounts of salt. The LF-NMR results indicate that storage of the raw material (fresh or frozen) has larger influence on the tissue microstructure/proton relaxation than the type and concentration of cations added to the mince. The results in these studies showed that the LF-NMR method is a suitable tool in future research on sodium reduction in foods and may increase the understanding and explanation of the water/protein dynamics.

The multimodal machine vision system showed changes in lightness as a function of reduced salt content in cooked ham. However, the multimodal machine vision system needs to be further developed to measure small changes in surface texture obtained in cooked ham with gradually decreased sodium content.

The results in this thesis confirm that sodium ion selective electrode is a good method for direct measurement of sodium in low-salt low-fat food products.

Furthermore, the work in this thesis demonstrates the effect of sodium reduction and replacement of sodium with other cations (K^+ and Mg^{2+}) on protein solubility and physicochemical properties:

- The extractability of salt soluble proteins (SSP) increased with increasing concentrations of salt where NaCl gave the highest extractability;
- Low concentrations (0.11 and 0.28 M) of KCl and $MgCl_2$ changed the extractability of SSP slightly;

- The addition of high amounts (0.55 M) of MgCl_2 decreased the extractability of SSP in fresh mince;
- The extractability of SSP was significantly lower in minces prepared from frozen raw material compared to mince prepared from fresh raw material;
- Salt reduction led to an increase in moisture, reduced WHC, increased cooking loss and a decrease in breaking force in the model product made of haddock mince added water and different amounts of pure salts;
- The pH increased with decreasing salt content;
- Replacing NaCl with pure KCl had no or only a small effect on the pH, moisture, breaking force, WHC and cooking loss in the model products;
- Replacing NaCl with an equal molar concentration of MgCl_2 affected several of the physicochemical properties and in particular WHC, cooking loss and pH;
- Both the WHC and breaking force increased with increasing SSP in the study on fish pudding; and
- A linear relation between breaking force and WHC was found in the study on fish pudding.

To conclude, a partial replacement of Na^+ - ions with K^+ - ions is possible without changing the protein solubility and the physicochemical properties in fish products. A reduction of salt from 1.0 down to 0.6 % without any salt replacers affected the taste more than physicochemical properties in fish pudding. Based on the factors investigated in this study, a 40% sodium reduction is therefore possible in fish pudding using high mineral permeate as a salt replacer. The study on cooked ham showed that a 25% replacement of Na^+ - ions with K^+ - ions was possible without changing the quality in the final product. However, when the sodium was reduced with more than 35% (< 2.1% salt in the ham), the salt reduction had a negative influence both on the sensory and physicochemical properties.

Based on the results from this work, it is suggested to consider KCl in further development of low-salt products in order to be able to reach the target of 30-50% reduction of sodium in muscle foods in the future. From a research perspective, further studies of the effect of Mg^{2+} -ions on the protein matrix is of interest for a better understanding of the effect on the myofibrillar proteins and to optimize the amount of Mg^{2+} -ions used as salt replacer in muscle foods.

7 REFERENCES

- Aaslyng, M. D., Vestergaard, C., & Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, *96*(1), 47-55. doi: <http://dx.doi.org/10.1016/j.meatsci.2013.06.004>
- Abass Bakhiet, H. H., & Khogali, F. A. E. (2011). Effect of different salt concentrations on chemical composition of the fish *hydrocynus spp.* *Online Journal of Animal and Feed Research*, *1*(6), 461-464.
- Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. *International Journal of Food Science & Technology*, *46*(7), 1329-1336. doi: [10.1111/j.1365-2621.2010.02492.x](https://doi.org/10.1111/j.1365-2621.2010.02492.x)
- Aliño, M., Fuentes, A., Fernández-Segovia, I., & Barat, J. M. (2011). Development of a low-sodium ready-to-eat desalted cod. *Journal of Food Engineering*, *107*(3-4), 304-310. doi: [10.1016/j.jfoodeng.2011.07.012](https://doi.org/10.1016/j.jfoodeng.2011.07.012)
- Almli, V., & Hersleth, M. (2013). Salt replacement and injection salting in smoked salmon evaluated from descriptive and hedonic sensory perspectives. *Aquaculture International*, *21*(5), 1091-1108. doi: [10.1007/s10499-012-9615-4](https://doi.org/10.1007/s10499-012-9615-4)
- Anderson, M. L., & Ravesi, E. M. (1968). Relation between Protein Extractability and Free Fatty Acid Production in Cod Muscle Aged in Ice. *Journal of the Fisheries Research Board of Canada*, *25*(10), 2059-2069. doi: [10.1139/f68-183](https://doi.org/10.1139/f68-183)
- Andreetta-Gorelkina, I. V., Greiff, K., Rustad, T., & Aursand, I. G. (2015). Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins. *Journal of Aquatic Food Product Technology*. doi: [10.1080/10498850.2013.879241](https://doi.org/10.1080/10498850.2013.879241)
- AOAC. (2008). Salt (Chlorine as Sodium Chloride) in Seafood, method 937.09. In AOAC INTERNATIONAL (Ed.), (18th ed.). Gaithersburg, MD: Official Methods of Analysis.
- Aursand, I. G., Erikson, U., & Veliyulin, E. (2010). Water properties and salt uptake in Atlantic salmon fillets as affected by ante-mortem stress, rigor mortis, and brine salting: A low-field 1H NMR and 1H/23Na MRI study. *Food Chemistry*, *120*(2), 482-489. doi: <http://dx.doi.org/10.1016/j.foodchem.2009.10.041>
- Aursand, I. G., Gallart-Jornet, L., Erikson, U., Axelson, D. E., & Rustad, T. (2008). Water Distribution in Brine Salted Cod (*Gadus morhua*) and Salmon (*Salmo salar*): A Low-Field 1H NMR Study. *Journal of Agricultural and Food Chemistry*, *56*(15), 6252-6260. doi: [10.1021/jf800369n](https://doi.org/10.1021/jf800369n)
- Aursand, I. G., Veliyulin, E., Böcker, U., Ofstad, R., Rustad, T., & Erikson, U. (2008). Water and Salt Distribution in Atlantic Salmon (*Salmo salar*) Studied by Low-Field 1H NMR, 1H and 23Na MRI and Light Microscopy: Effects of Raw Material Quality and Brine Salting. *Journal of Agricultural and Food Chemistry*, *57*(1), 46-54. doi: [10.1021/jf802158u](https://doi.org/10.1021/jf802158u)
- Balaban, M. O., Chombeau, M., Cırbán, D., & Gümüş, B. (2010). Prediction of the Weight of Alaskan Pollock Using Image Analysis. *Journal of Food Science*, *75*(8), E552-E556. doi: [10.1111/j.1750-3841.2010.01813.x](https://doi.org/10.1111/j.1750-3841.2010.01813.x)
- Balaban, M. O., Ünal Şengör, G. F., Soriano, M. G., & Ruiz, E. G. (2010). Using Image Analysis to Predict the Weight of Alaskan Salmon of Different Species. *Journal of Food Science*, *75*(3), E157-E162. doi: [10.1111/j.1750-3841.2010.01522.x](https://doi.org/10.1111/j.1750-3841.2010.01522.x)
- Baldwin, R. L. (1996). How Hofmeister ion interaction affect protein stability. *Biophysical Journal*, *71*(4), 2056-2063.

- Barat, J. M., Pérez-Esteve, E., Aritoy, M.-C., & Toldra, F. (2012). Partial replacement of sodium in meat and fish products by using magnesium salts. A review. *Plant Soil*. doi: 10.1007/s11104-012-1461-7
- Barat, J. M., Rodríguez-Barona, S., Andrés, A., & Fito, P. (2003). Cod salting manufacturing analysis. *Food Research International*, 36(5), 447-453. doi: 10.1016/s0963-9969(02)00178-3
- Bard, A. J., & Faulkner, L. R. (2001). *Electrochemical Methods: Fundamentals and Applications*: John Wiley & Sons, Inc.
- Belton, P. (2011a). Spectroscopic Approaches to the Understanding of Water in Foods. *Food Reviews International*, 27(2), 170-191. doi: 10.1080/87559129.2010.535234
- Belton, P. S. (2011b). NMR studies of hydration in low water content biopolymer systems. *Magnetic Resonance in Chemistry*, 49, S127-S132. doi: 10.1002/mrc.2848
- BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In F. O. R (Ed.), *Food Chemistry* (3 ed ed.): Marcer Dekker, Inc.
- Benedini, R., Parolari, G., Toscani, T., & Virgili, R. (2012). Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Science*, 90(2), 431-437. doi: 10.1016/j.meatsci.2011.09.001
- Berg, E. W., & Dyrnes, U. (1997). *Sensorisk analyse*. Oslo.
- Bertram, H. C., Andersen, H. J., & Karlsson, A. H. (2001). Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat Science*, 57(2), 125-132. doi: http://dx.doi.org/10.1016/S0309-1740(00)00080-2
- Bombrun, L., Gatellier, P., Carlier, M., & Kondjoyan, A. (2014). The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. *Meat Science*, 96(1), 5-13. doi: http://dx.doi.org/10.1016/j.meatsci.2013.06.029
- Bondø, M. S., Mathiassen, J. R., Vebeustad, P. A., Misimi, E., Bar, E. M. S., Toldnes, B., & Østvik, S. O. (2011). An automated salmonid slaughter line using machine vision. *Industrial Robot: An International Journal*, 38(4), 399-405. doi: doi:10.1108/01439911111132085
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. doi: http://dx.doi.org/10.1016/0003-2697(76)90527-3
- Brandsma, I. (2006). Reducing sodium a European perspective. *Food Technology*, 60(3), 24-29.
- Breslin, P. A. S., & Beauchamp, G. K. (1995). Suppression of Bitterness by Sodium: Variation Among Bitter Taste Stimuli. *Chemical Senses*, 20(6), 609-623. doi: 10.1093/chemse/20.6.609
- Business Group Norwegian Food Industry. (2014). Joint effort to reduce salt intake with 15% in 2018.
- Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Am. J. Physiology*, 94, 630-638.
- Casiraghi, E., Alamprese, C., & Pompei, C. (2007). Cooked ham classification on the basis of brine injection level and pork breeding country. *LWT - Food Science and Technology*, 40(1), 164-169. doi: http://dx.doi.org/10.1016/j.lwt.2005.07.007
- Chandraratne, M. R., Samarasinghe, S., Kulasiri, D., & Bickerstaffe, R. (2006). Prediction of lamb tenderness using image surface texture features. *Journal of Food Engineering*, 77(3), 492-499. doi: 10.1016/j.jfoodeng.2005.06.063

REFERENCES

- Chanet, M., Rivière, C., & Eynard, P. (1999). Electric impedance spectrometry for the control of manufacturing process of comminuted meat products. *Journal of Food Engineering*, 42(3), 153-159. doi: 10.1016/s0260-8774(99)00113-2
- Chaplin, M. (2014), Hofmeiser Series. Water Structure and Science. from http://www1.lsbu.ac.uk/water/kosmotropes_chaotropes.html. Retrieved 21.03.2015.
- Christie, H. (2007). *Fenalaar. The story of a Norwegian delicacy and speciality* (A. Christie & Animalia Fagsenteret for kjøtt Eds.): Kolon Forlag.
- Collins, J. E. (1997). Reducing salt (sodium) levels in process meat poultry and fish products,. In A. M. P. T. R. Dutson (Ed.), *Advances in meat research. Production and processing of healthy meat, poultry and fish products*, (Vol. 11). London: Blackie Academic & Professional.
- Cook, N. R., Cutler, J. A., Obarzanek, E., Buring, J. E., Rexrode, K. M., Kumanyika, S. K., . . . Whelton, P. K. (2007). Long term effects of dietary sodium reduction on cardiovascular disease outcomes: Observational follow-up of the trials of hypertension prevention (TOHP). *British Medical Journal*, 334(7599), 885-888.
- CTAC. (2009). Reformulation of products to reduce sodium: Salt Reduction guide for the Food Industry. Conseil de la transformation agroalimentaire et des produits de consommation (CTAC). from <http://www.foodtechcanada.ca/siteimages/Salt%20reduction%20guide%20for%20the%20food%20industry.pdf>
- Çükgülmez, A., Çelik, M., Ersoy, B., Yanar, Y., & Sangün, L. (2008). Seasonal variations in proximate and fatty acid compositions of two commercially important fish, hake (*Merluccius merluccius*) and lizardfish (*saurida undosquamis*), from the northeastern mediterranean sea. *Journal of Muscle Foods*, 19(4), 352-361. doi: 10.1111/j.1745-4573.2008.00119.x
- Damodaran, S. (2008). Amino Acids, Peptides, and Proteins. In O. R. Fennema, S. Damodaran, & K. L. Parkin (Eds.), *Fennema's Food Chemistry* (4th Ed), pp. 217-329). Florida: CRC Press.
- Danish Saltpartnership. (2012). Indicative reduction of salt in food, Saltlist. Retrieved 16.02, 2015
- Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, 74(1), 188-196.
- EFSA. (2005). Opinion of the Scientific Panel of Dietetic Products, Nutrition and Allergies on request from the Commission related to the Tolerable Upper Intake Level of Potassium. *The EFSA Journal*, 193, 1-19. doi: <http://www.efsa.europa.eu/en/efsajournal/doc/193.pdf>
- Ehling, S., Tefera, S., Earl, R., & Cole, S. (2010). Comparison of Analytical Methods to Determine Sodium Content of Low-Sodium Foods. *Journal of AOAC International*, 93(2), 628-637.
- EN. (2008). EN 15505:2008: Foodstuff-Determination of trace elements-Determination of Sodium and Magnesium by Flame Atomic Absorption Spectrometry (AAS) after Microwave Digestion.
- Erikson, U., Standal, I. B., Aursand, I. G., Veliyulin, E., & Aursand, M. (2012). Use of NMR in fish processing optimization: a review of recent progress. *Magnetic Resonance in Chemistry*, 50(7), 471-480. doi: 10.1002/mrc.3825
- Erikson, U., Veliyulin, E., Singstad, T. E., & Aursand, M. (2004). Salting and desalting of fresh and frozen-thawed cod (*Gadus morhua*) fillets: A comparative study using ²³Na NMR, ²³Na MRI, low-field ¹H NMR, and physicochemical analytical methods. *Journal of Food Science*, 69(3).
- EU. (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the council of 25 October 2011. In Official Journal of the European Union L304/18 22.11.2011.
- Fennema, O. R. (1996). Kap 2. Water and Ice. In O. R. Fennema (Ed.), *Food Chemistry* (3rd. ed.): University of Wisconsin, Marcel Dekker, Inc. New York.

- Fito, P., & Pastor, R. (1994). Non-diffusional mechanisms occurring during vacuum osmotic dehydration. *Journal of Food Engineering*, 21(4), 513-519. doi: [http://dx.doi.org/10.1016/0260-8774\(94\)90070-1](http://dx.doi.org/10.1016/0260-8774(94)90070-1)
- Foegeding, E. A., & Lanier, T. C. (1996). Characteristics of Edible Muscle Tissues. In O. R. Fennema (Ed.), *Food Chemistry* (3rd ed.): Marcel Dekker, Inc.
- Food Standard Agency. (2014). 2017 UK salt reduction targets. <http://www.food.gov.uk/scotland/scotnut/salt/saltreduction>. Retrieved 16-02, 2015
- FSAI. (2005). Food Safety Authority of Ireland, Salt and health: review of the scientific evidence and recommendations for public policy in Ireland.
- Fuentes, A., Fernández-Segovia, I., Barat, J. M., & Serra, J. A. (2011). Influence of sodium replacement and packaging on quality and shelf life of smoked sea bass (*Dicentrarchus labrax* L.). *LWT - Food Science and Technology*, 44(4), 917-923. doi: 10.1016/j.lwt.2010.11.030
- Fuentes, A., Fernández-Segovia, I., Serra, J. A., & Barat, J. M. (2012). Effect of partial sodium replacement on physicochemical parameters of smoked sea bass during storage. *Food Science and Technology International*, 18(3), 207-217. doi: 10.1177/1082013211415156
- Fulladosa, E., Santos-Garcés, E., Picouet, P., & Gou, P. (2010). Prediction of salt and water content in dry-cured hams by computed tomography. *Journal of Food Engineering*, 96(1), 80-85. doi: <http://dx.doi.org/10.1016/j.jfoodeng.2009.06.044>
- Fulladosa, E., Santos-Garcés, E., Picout, P., & Gou, P. (2010). Salt and water content prediction by computed tomography in dry-cured hams. *Journal of Food Engineering*, 96(1), 80-85.
- Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., & Fito, P. (2007). A comparative study of brine salting of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*). *Journal of Food Engineering*, 79(1), 261-270. doi: 10.1016/j.jfoodeng.2006.01.053
- Geleijnse, J. M., Witterman, J. C. M., Stijnen, T., Kloos, M. W., Hofman, A., & Grobbee, D. E. (2007). Sodium and potassium intake and risk of cardiovascular events and all-causes mortality: The Rotterdam study. *European Journal of Epidemiology*, 22(11), 763-777. doi: 10.1007/s10654-007-9186-2
- Girard, J. P. (1991). *Technologie de la Carne y los Productos Cárnicos* (pp. 295). Zaragoza: Acribia.
- Greiff, K., Fuentes, A., Aursand, I. G., Erikson, U., Masot, R., Alcañiz, M., & Barat, J. M. (2014). Innovative Nondestructive Measurements of Water Activity and the Content of Salts in Low-Salt Hake Minces. *Journal of Agricultural and Food Chemistry*, 62(12), 2496-2505. doi: 10.1021/jf405527t
- Gudjónsdóttir, M., Arason, S., & Rustad, T. (2011). The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydrated cod – A low-field NMR study. *Journal of Food Engineering*, 104(1), 23-29. doi: 10.1016/j.jfoodeng.2010.11.022
- Gudjónsdóttir, M., Gunnlaugsson, V. N., Finnbogadóttir, G. A., Sveinsdóttir, K., Magnússon, H., Arason, S., & Rustad, T. (2010). Process Control of Lightly Salted Wild and Farmed Atlantic Cod (*Gadus morhua*) by Brine Injection, Brining, and Freezing-A Low Field NMR Study. *Journal of Food Science*, 75(8), E527-E536. doi: 10.1111/j.1750-3841.2010.01808.x
- Guerrero, L., Gobantes, I., Oliver, M. À., Arnau, J., Dolors Guàrdia, M., Elvira, J., . . . Monfort, J. M. (2004). Green hams electrical impedance spectroscopy (EIS) measures and pastiness prediction of dry cured hams. *Meat Science*, 66(2), 289-294. doi: 10.1016/s0309-1740(03)00101-3
- Gupta, D., Georgiopoulou, V. V., Kalogeropoulos, A. P., Dunbar, S. B., Reilly, C. M., Sands, J. M., . . . Butler, J. (2012). Dietary sodium intake in heart failure. *Circulation*, 126(4), 479-485.

REFERENCES

- Halle, B. (2004). Protein hydration dynamics in solution: a critical survey. *Philos Trans R Soc Lond B Biol Sci*, 359(1448), 1207-1223; discussion 1223-1204, 1323-1208. doi: 10.1098/rstb.2004.1499
- Halle, B. (2006). Molecular theory of field-dependent proton spin-lattice relaxation in tissue. *Magnetic Resonance in Medicine*, 56(1), 60-72. doi: 10.1002/mrm.20919
- Hamm, R. (1972). *Kolloidchemie des Fleisches*: Parey, Berlin.
- Hamm, R. (1986). Functional Properties of the Myofibrillar System and Their Measurements. In P. J. Bechtel (Ed.), *Muscle as Food* (pp. 135-199). Orlando, USA: Academic Press.
- Hand, L. W., Hollingsworth, C. A., Calkins, C. R., & Mandigo, R. W. (1987). Effects of Preblending, Reduced Fat and Salt Levels on Frankfurter Characteristics. *Journal of Food Science*, 52(5), 1149-1151. doi: 10.1111/j.1365-2621.1987.tb14030.x
- He, F. J., & MacGregor, G. A. (2001). Fortnightly review: beneficial effects of potassium. *BMJ*, 323, 497-501.
- He, F. J., & MacGregor, G. A. (2002). Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens*, 16(11), 761-770. doi: 10.1038/sj.jhh.1001459
- He, F. J., & MacGregor, G. A. (2003). How far should salt intake be reduced? *Hypertension*, 42, 1093-1099.
- He, F. J., & MacGregor, G. A. (2007). Dietary salt, high blood pressure and other harmful effects on health. In D. Kilcast & F. Angus (Eds.), *Reducing salt in foods. Practical strategies* Cambridge, England: Woodhead Publ. Ltd.
- He, Y., & Sebranek, J. G. (1996). Frankfurters with Lean Finely Textured Tissue as Affected by Ingredients. *Journal of Food Science*, 61(6), 1275-1280. doi: 10.1111/j.1365-2621.1996.tb10978.x
- Hofmeister, F. (1888). Zur Lehre von der Wirkung der Salze. Zweite Mitteilung. *Archiv für Experimentelle Pathologie und Pharmakologie*, 24, 247-260.
- Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, 71(1), 194-204. doi: 10.1016/j.meatsci.2005.04.022
- Hultmann, L., & Rustad, T. (2002). Textural Changes During Iced Storage of Salmon (*Salmo salar*) and Cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology*, 11(3-4), 105-123. doi: 10.1300/J030v11n03_09
- Håseth, T. T., Egelanddal, B., Bjerke, F., & Sørheim, O. (2007). Computed tomography for quantitative determination of sodium chloride in ground pork and dry-cured hams. *Journal of Food Science*, 72(8).
- Håseth, T. T., Sørheim, O., Høy, M., & Egelanddal, B. (2012). Use of computed tomography to study raw ham properties and predict salt content and distribution during dry-cured ham production. *Meat Science*, 90(3), 858-864. doi: <http://dx.doi.org/10.1016/j.meatsci.2011.11.029>
- Intersalt. (1988). An international study of electrolyte excretion and blood pressure. *Intersalt Cooperative Research Group Brit. Med.J.*, 279, 319-328.
- Iqbal, A., Valous, N. A., Mendoza, F., Sun, D.-W., & Allen, P. (2010). Classification of pre-sliced pork and Turkey ham qualities based on image colour and textural features and their relationships with consumer responses. *Meat Science*, 84(3), 455-465. doi: 10.1016/j.meatsci.2009.09.016

- ISO. (1996). ISO 1841:2:1996 Meat and meat products - Determination of chloride content - Part 2: Potentiometric method.
- IUPAC. (1997). Compendium of Chemical Terminology 2nd ed. *The Gold Book. Online corrected version: (2006-) ionic strength*. from <http://goldbook.iupac.org/I03180.html>
- Jackman, P., Sun, D. W., Du, C. J., Allen, P., & Downey, G. (2008). Prediction of beef eating quality from colour, marbling and wavelet texture features. *Meat Science*, *80*(4), 1273-1281.
- Jafarpour, A., & Gorczyca, E. M. (2012). Contribution of Sarcoplasmic Proteins to Myofibrillar Proteins Gelation. *Journal of Food Science*, *77*(2), R73-R81. doi: 10.1111/j.1750-3841.2011.02521.x
- Jolliffe, I. T. (1986). *Principal Component Analysis*. New York.
- Josefsen, K. D., Greiff, K., Carvajal, A., & Erikson, U. G. (2014). Commercial salt replacers- a brief overview. <http://www.sintef.no/Publikasjonssok/Publikasjon/?pubid=SINTEF+A26584>: SINTEF Materials and chemistry.
- Julshamn, K., Lea, P., & Norli, H. S. (2005). Determination of sodium in foods by flame atomic absorption spectrometry after microwave digestion: NMKL interlaboratory study. *J AOAC Int*, *88*(4), 1212-1216.
- Karášková, P., Fuentes, A., Fernández-Segovia, I., Alcañiz, M., Masot, R., & Barat, J. M. (2011). Development of a low-cost non-destructive system for measuring moisture and salt content in smoked fish products. *Procedia Food Science*, *1*(0), 1195-1201. doi: 10.1016/j.profoo.2011.09.178
- Kilcast, D., & Angus, F. (Eds.). (2007). *Reducing salt in foods, practical strategies*. UK: CRC Press, Cambridge.
- Kivikari, R. (1996). Analysis of sodium in meat products using an Na-selective electrode. (In Finnish). Proc. of Meat Day Seminar 1996, Nr, 536. In D. o. M. T. University of Helsinki, Reports from Meat Technology (Ed.), (pp. 64-66).
- Lambelet, P., Renevey, F., Kaabi, C., & Raemy, A. (1995). Low-Field Nuclear Magnetic Resonance Relaxation Study of Stored or Processed Cod. *Journal of Agricultural and Food Chemistry*, *43*(6), 1462-1466. doi: 10.1021/jf00054a009
- Larsen, R., & Elvevoll, E. O. (2008). Water uptake, drip losses and retention of free amino acids and minerals in cod (*Gadus morhua*) fillet immersed in NaCl or KCl. *Food Chemistry*, *107*(1), 369-376. doi: <http://dx.doi.org/10.1016/j.foodchem.2007.08.031>
- Lawal, O. S. (2006). Kosmotropes and chaotropes as they affect functionality of a protein isolate. *Food Chemistry*, *95*(1), 101-107. doi: <http://dx.doi.org/10.1016/j.foodchem.2004.12.041>
- Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food - principles and practices (2nd ed.)*. New York: Springer.
- Lawless, H. T., Rapacki, F., Horne, J., & Hayes, A. (2003). The taste of calcium and magnesium salts and anionic modifications. *Food Quality and Preference*, *14*(4), 319-325. doi: [http://dx.doi.org/10.1016/S0950-3293\(02\)00128-3](http://dx.doi.org/10.1016/S0950-3293(02)00128-3)
- Leatherhead Food Research. (2012). Evaluation of Technological Approaches to Salt Reduction. In Food and Drink Federation (FDF) and British Retail Consumption (BRC) (Ed.). http://www.brc.org.uk/downloads/Leatherhead_Salt_Research.pdf: Leatherhead Food Research, UK.
- Leroi, F., & Joffraud, J. J. (2000). Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *Journal of Food Protection*, *63*(9), 1222-1227.

REFERENCES

- Licciardello, J. J., Ravesi, E. M., Lundstrom, R. C., Wilhelm, K. A., Correia, F. F., & Allsup, M. G. (1982). Time- Temperature tolerance and physical –chemical quality tests for frozen red hake. *Journal of Food Quality*, 5(3), 215-234. doi: 10.1111/j.1745-4557.1982.tb00745.x
- Lin, G. C., Mittal, G. S., & Barbut, S. (1991). Optimization of tumbling and KCl substitution in low sodium restructured hams. *Journal of Muscle Foods*, 2(2), 71-91. doi: 10.1111/j.1745-4573.1991.tb00444.x
- Lind, R., & Murhed, A. (2012). Computer vision in food processing: an overview. In D.-W. Sun (Ed.), *Computer vision technology in the food and beverage industries*. Food Science, Technology and Nutrition: Number 238: Woodhead Publishing.
- Macdonald, J. R., & Barsoukov, E. (2005). *Impedance Spectroscopy: Theory, Experiment, and Applications*, (2nd ed.): John Wiley & Sons, Inc.
- Martínez-Alvarez, O., Borderías, A. J., & Gómez-Guillén, M. C. (2005). Sodium replacement in the cod (*Gadus morhua*) muscle salting process. *Food Chemistry*, 93(1), 125-133. doi: 10.1016/j.foodchem.2004.10.014
- Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2005). The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*). *Food Chemistry*, 92(1), 71-77. doi: 10.1016/j.foodchem.2004.04.049
- Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2013). Influence of mono- and divalent salts on water loss and properties of dry salted cod fillets. *LWT - Food Science and Technology*, 53(2), 387-394. doi: <http://dx.doi.org/10.1016/j.lwt.2013.04.013>
- Masot, R., Alcañiz, M., Fuentes, A., Schmidt, F. C., Barat, J. M., Gil, L., . . . Soto, J. (2010). Design of a low-cost non-destructive system for punctual measurements of salt levels in food products using impedance spectroscopy. *Sensors and Actuators A: Physical*, 158(2), 217-223. doi: 10.1016/j.sna.2010.01.010
- Mathiassen, J. R., Misimi, E., Toldnes, B., Bondø, M., & Østvik, S. O. (2011). High-Speed Weight Estimation of Whole Herring (*Clupea harengus*) Using 3D Machine Vision. *Journal of Food Science*, 76(6), E458-E464. doi: 10.1111/j.1750-3841.2011.02226.x
- Mattes, R. D. (1997). The taste for salt in humans. *The American Journal of Clinical Nutrition*, 65(2 Suppl), 692s-697s.
- Mattes, R. D., & Donnelly, D. (1991). Relative contributions of dietary sodium sources. *Journal of the American College of Nutrition*, 10(4), 383-393.
- McGough, M. M., Sato, T., Rankin, S. A., & Sindelar, J. J. (2012a). Reducing sodium levels in frankfurters using a natural flavor enhancer. *Meat Science*, 91(2), 185-194. doi: 10.1016/j.meatsci.2012.01.018
- McGough, M. M., Sato, T., Rankin, S. A., & Sindelar, J. J. (2012b). Reducing sodium levels in frankfurters using naturally brewed soy sauce. *Meat Science*, 91(1), 69-78. doi: 10.1016/j.meatsci.2011.12.008
- Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear times. *Rev. Sci. Instrum*, 29, 688-691.
- Mendoza, F., Valous, N. A., Allen, P., Kenny, T. A., Ward, P., & Sun, D.-W. (2009). Analysis and classification of commercial ham slice images using directional fractal dimension features. *Meat Science*, 81(2), 313-320. doi: 10.1016/j.meatsci.2008.08.009
- Messerli, F. H., & Ketelhut, R. (1993). Left ventricular hypertrophy: A pressure-independent cardiovascular risk factor. *Journal of Cardiovascular Pharmacology*, 22(SUPPL. 1).

- Misimi, E., Erikson, U., & Skavhaug, A. (2008). Quality grading of Atlantic salmon (*Salmo salar*) by computer vision. *Journal of Food Science*, *73*(5), E211-E217.
- Misimi, E., Mathiassen, J. R., & Erikson, U. (2006). *Computer vision-based sorting of Atlantic salmon (Salmo salar) according to size and shape*. Paper presented at the International Conference on Computer Vision Theory and Applications - VISAPP (1), Setubal, Portugal.
- Moelbert, S., Normand, B., & De Los Rios, P. (2004). Kosmotropes and chaotropes: Modelling preferential exclusion, binding and aggregate stability. *Biophysical Chemistry*, *112*(1), 45-57.
- Montero, P., & Pérez-Mateos, M. (2002). Effects of Na⁺, K⁺ and Ca²⁺ on gels formed from fish mince containing a carrageenan or alginate. *Food Hydrocolloids*, *16*(4), 375-385. doi: 10.1016/S0268-005X(01)00110-2
- Morley, W. (2012). Novel strategies for reducing sodium. *Food Technology*, *66*(1), 53-62.
- Murphy, C., Cardello, A. V., & Brand, J. G. (1981). Tastes of fifteen halide salts following water and NaCl: Anion and cation effects. *Physiology & Behavior*, *26*(6), 1083-1095. doi: [http://dx.doi.org/10.1016/0031-9384\(81\)90213-4](http://dx.doi.org/10.1016/0031-9384(81)90213-4)
- Murray, J., & Burt, J. R. (1969). *The composition of fish*. Torry Advisory Notes 38: Torry Research Station, Aberdeen
- Myhre, J. B., Borgejordet, Å., Nordbotten, A., Løken, E. B., & Fagerli, R. A. (2012). Nutritional composition of selectet wild and farmed raw fish. The Norwegian Food Safety Authority and Norwegian Directorate of Health.
- Nayak, R., Kenney, P. B., Slider, S., Head, M. K., & Killefer, J. (1998). Cook Yield, Texture and Gel Ultrastructure of Model Beef Batters as Affected by Low Levels of Calcium, Magnesium and Zinc Chloride. *Journal of Food Science*, *63*(6), 945-950. doi: 10.1111/j.1365-2621.1998.tb15829.x
- Norwegian Institute of Nutrition and Seafood Research. (2015). Seafood data. www.nifes.no Retrieved 12.02.2015
- Norwegian Scientific Committee for Food Safety. (2014). Benefit and risk assessment of increasing potassium by replacement of sodium chloride with potassium chloride in industrial food production (Vol. 12-008). <http://www.english.vkm.no/dav/772a8d83c8.pdf>.
- Offer, G., & Trinick, J. (1983). On the Mechanism of Water Holding in Meat: The Swelling and Shrinking of Myofibrils. *Meat Science*, *8*, 245-281. doi: 0309-1740/83/0008-0245/\$03.00
- Page, J., Meyer, D., Haines, B., & Lagrange, V. (2004). *Reference manual for U.S. whey and lactose products*. US Dairy Export Council Retrieved from http://usdec.files.cms-plus.com/PDFs/2008ReferenceManuals/Whey_Lactose_Reference_Manual_Complete2_Optimized.pdf.
- Pérez-Olmos, R., Herrero, R., Lima, J. L. F. C., & Montenegro, M. C. B. S. M. (1997). Sequential potentiometric determination of chloride and nitrate in meat products. *Food Chemistry*, *59*(2), 305-311. doi: [http://dx.doi.org/10.1016/S0308-8146\(96\)00263-4](http://dx.doi.org/10.1016/S0308-8146(96)00263-4)
- Perisic, N., Afseth, N. K., Ofstad, R., & Kohler, A. (2011). Monitoring Protein Structural Changes and Hydration in Bovine Meat Tissue Due to Salt Substitutes by Fourier Transform Infrared (FTIR) Microspectroscopy. *Journal of Agricultural and Food Chemistry*, *59*(18), 10052-10061. doi: 10.1021/jf201578b
- Pietrasik, Z., & Gaudette, N. J. (2014). The impact of salt replacers and flavor enhancer on the processing characteristics and consumer acceptance of restructured cooked hams. *Meat Science*, *96*(3), 1165-1170. doi: <http://dx.doi.org/10.1016/j.meatsci.2013.11.005>

REFERENCES

- Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, 86(1), 151-165. doi: 10.1016/j.meatsci.2010.04.038
- Puolanne, E., Ruusunen, M. H., & Vainionpää, J. I. (2001). Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. *Meat Science*, 58(1), 1-7.
- Rizo, A., Fuentes, A., Fernández-Segovia, I., Masot, R., Alcañiz, M., & Barat, J. M. (2013). Development of a new salmon salting–smoking method and process monitoring by impedance spectroscopy. *LWT - Food Science and Technology*, 51(1), 218-224. doi: <http://dx.doi.org/10.1016/j.lwt.2012.09.025>
- Ruusunen, M., Särkkä-Tirkkonen, M., & Puolanne, E. (2001). Saltiness of coarsely ground cooked ham with reduced salt content. *Agricultural and food science in Finland*, 10, 27-31.
- Ruusunen, M., Vainionpää, J., Lyly, M., Lähteenmäki, L., Niemistö, M., Ahvenainen, R., & Puolanne, E. (2005). Reducing the sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. *Meat Science*, 69(1), 53-60.
- Ruusunen, M., Vainionpää, J., Puolanne, E., Lyly, M., Lähteenmäki, L., Niemistö, M., & Ahvenainen, R. (2003a). Effect of sodium citrate, carboxymethyl cellulose and carrageenan levels on quality characteristics of low-salt and low-fat bologna type sausages. *Meat Science*, 64(4), 371-381.
- Ruusunen, M., Vainionpää, J., Puolanne, E., Lyly, M., Lähteenmäki, L., Niemistö, M., & Ahvenainen, R. (2003b). Physical and sensory properties of low-salt phosphate-free frankfurters composed with various ingredients. *Meat Science*, 63(1), 9-16.
- SACN, Salt and Health, & Scientific Advisory Committee on Nutrition. (2003): The Stationary Office, Norwich, UK.
- Sánchez-Alonso, I., Moreno, P., & Careche, M. (2014). Low field nuclear magnetic resonance (LF-NMR) relaxometry in hake (*Merluccius merluccius*, L.) muscle after different freezing and storage conditions. *Food Chemistry*, 153(0), 250-257. doi: <http://dx.doi.org/10.1016/j.foodchem.2013.12.060>
- Santos-Garcés, E., Gou, P., Garcia-Gil, N., Arnau, J., & Fulladosa, E. (2010). Non-destructive analysis of aw, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, 101(2), 187-192. doi: <http://dx.doi.org/10.1016/j.jfoodeng.2010.06.027>
- Schwabe, K. (1967). Azidität konzentrierter elektrolytlösungen. *Electrochimica Acta*, 12(1), 67-93. doi: [http://dx.doi.org/10.1016/0013-4686\(67\)85007-2](http://dx.doi.org/10.1016/0013-4686(67)85007-2)
- Shepherd, R., Wharf, S. G., & Farleigh, C. A. (1989). The effect of a surface coating of table salt of varying grain size on perceived saltiness and liking for paté. *International Journal of Food Science & Technology*, 24(3), 333-340. doi: 10.1111/j.1365-2621.1989.tb00652.x
- Sikorski, Z. E., Kolakowska, A., & Pan, B. S. (1990). *Seafood: Resources, nutritional composition, and preservation* (Vol. 2 edn). Florida.
- Stollewerk, K., Jofré, A., Comaposada, J., Arnau, J., & Garriga, M. (2012). The effect of NaCl-free processing and high pressure on the fate of *Listeria monocytogenes* and *Salmonella* on sliced smoked dry-cured ham. *Meat Science*, 90(2), 472-477. doi: 10.1016/j.meatsci.2011.09.009
- Strasburg, G., Xiong, Y. L., & Chiang, W. (2008). Physiology and chemistry of edible muscle tissues. In S. Damodaran, Parkin, K. & Fennema, O. R. (Ed.), *Fennema's food chemistry*. Boca Raton: Taylor & Francis.

- Tahergorabi, R., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2012). Salt substitution in surimi seafood and its effects on instrumental quality attributes. *LWT - Food Science and Technology*, *48*(2), 175-181. doi: <http://dx.doi.org/10.1016/j.lwt.2012.03.004>
- Taormina, P. J. (2010). Implications of Salt and Sodium Reduction on Microbial Food Safety. *Critical Reviews in Food Science and Nutrition*, *50*(3), 209-227. doi: 10.1080/10408391003626207
- The Norwegian Ministry of Health and Care Service. (2015). Regulation on voluntary labeling foodstuff with Keyhole., LOV-2003-12-19-124-§10, FOR-2003-12-19-1790. <https://lovdata.no/dokument/SF/forskrift/2015-02-18-139>
- The Norwegian Directorate for Health. (2012). Norkost 3. En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11. . In The Norwegian Directorate for Health (Ed.), *En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11*. <https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/301/Norkost-3-en-landsovmfattende-kostholdsundersokelse-blant-menn-og-kvinner-i-norge-i-alderen-18-70-ar-2010-11-IS-2000.pdf>. In Norwegian.
- The Norwegian Directorate of Health. (2014). Strategy for the reduction of salt intake in the population. Recommendation from the National Nutrition Council. The Norwegian Directorate of Health (Ed.). <https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/321/Tiltaksplan-salt-2014-18-reduksjon-av-saltinntaket-i-befolkningen-IS-2193.pdf>. In Norwegian.
- The Norwegian Directorate of Health, & University of Oslo. (2015). Norwegian Food Composition Database., from <http://www.matvaretabellen.no/haddock-raw-04.110>, Retrieved 12.02.2015
- Thermo Scientific. (2008). User Guide ROSS Sodium Ion Selective Electrode.
- Toldrá, F., & Barat, J. M. (2012). Strategies for salt reduction in foods. *Recent Patents on Food, Nutrition and Agriculture*, *4*(1), 19-25.
- Tornberg, E., Andersson, A., Arasson, G., & von Seth, G. (1993). *Water and fat distribution in pork in relation to sensory properties*. Wallingford: CAB International.
- Tortora, G. J., & Derrickson, B. (2006). *Principles of Anatomy and Physiology*: John Wiley and Sons.
- USDA Agricultural Marketing Service. (2012). Grading, Certification and Verification: USDA Quality Standards. Washington DC, : USDA.
- Valous, N. A., Mendoza, F., Sun, D.-W., & Allen, P. (2009). Texture appearance characterization of pre-sliced pork ham images using fractal metrics: Fourier analysis dimension and lacunarity. *Food Research International*, *42*(3), 353-362. doi: 10.1016/j.foodres.2008.12.012
- Veliyulin, E., & Aursand, I. G. (2007). (1)H and (23)Na MRI studies of Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*) fillet pieces salted in different brine concentrations. *Journal of the Science of Food and Agriculture*, *87*(14), 2676-2683. doi: 10.1002/jsfa.3030
- Veliyulin, E., Egelanddal, B., Marica, F., & Balcom, B. J. (2009). Quantitative ²³Na magnetic resonance imaging of model foods. *Journal of Agricultural and Food Chemistry*, *57*(10), 4091-4095.
- Vella, D., Marccone, M., & Duizer, L. M. (2012). Physical and sensory properties of regional sea salts. *Food Research International*, *45*(1), 415-421. doi: 10.1016/j.foodres.2011.11.013
- Venugopal, V., & Shahidi, F. (1996). Structure and composition of fish muscle. *Food Reviews International*, *12*(2), 175-197. doi: 10.1080/87559129609541074

REFERENCES

- Vestergaard, C., Erbou, S. G., Thauland, T., Adler-Nissen, J., & Berg, P. (2005). Salt distribution in dry-cured ham measured by computed tomography and image analysis. *Meat Science*, *69*(1), 9-15. doi: <http://dx.doi.org/10.1016/j.meatsci.2004.06.002>
- Watt, B. K., & Merrill, A. L. (1963). *Composition of foods. Agricultural handbook No. 8*. Washington DC: US Department of Agriculture.
- WHO. (2012). Guideline: Sodium intake for adults and children. . In WHO (Ed.). Geneva: World Health Organization (WHO).
- WHO. (2013). Global action plan for the prevention and control of noncommunicable diseases 2013-2020. In W. H. O. (WHO) (Ed.).
- Wiskus, K. J., Addis, P. B., & Ma, R. T.-I. (1976). Distribution of β R, α R and α W Fibers in Turkey Muscles. *Poultry Science*, *55*(2), 562-572. doi: 10.3382/ps.0550562
- Wold, J. P., Johansen, I.-R., Haugholt, K., Tschudi, J., Thielemann, J., Segtnan, V., . . . Wold, E. (2006). Non-contact transreflectance near infrared imaging for representative on-line sampling of dried salted coalfish (bacalao). *Journal of Near Infrared Spectroscopy*, *14*(1). doi: 10.1255/jmirs.587
- Wold, S., Esbensen, K., & Geladi, P. (1987). Principal Component Analysis. *Chemom. Intell. Lab. Systems*, *2*, 37-52.
- Wu, H., Zhang, Y., Long, M., Tang, J., Yu, X., Wang, J., & Zhang, J. (2014). Proteolysis and sensory properties of dry-cured bacon as affected by the partial substitution of sodium chloride with potassium chloride. *Meat Science*, *96*(3), 1325-1331. doi: <http://dx.doi.org/10.1016/j.meatsci.2013.10.037>
- Xiong, Y. L., & Brekke, C. J. (1991). Gelation properties of chicken myofibrils treated with calcium and magnesium chlorides. *Journal of Muscle Foods*, *2*(1), 21-36. doi: 10.1111/j.1745-4573.1991.tb00438.x
- Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Raghavan, S., & Marshall, M. R. (2010). Correlation between astaxanthin amount and a* value in fresh Atlantic salmon (*Salmo salar*) muscle during different irradiation doses. *Food Chemistry*, *120*(1), 121-127. doi: <http://dx.doi.org/10.1016/j.foodchem.2009.09.086>
- Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Ralat, M., & Marshall, M. R. (2009). Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon. *Food Chemistry*, *116*(4), 828-835. doi: <http://dx.doi.org/10.1016/j.foodchem.2009.03.029>
- Åsli, M., & Mørkøre, T. (2012). Brines added sodium bicarbonate improve liquid retention and sensory attributes of lightly salted Atlantic cod. *LWT - Food Science and Technology*, *46*(1), 196-202. doi: <http://dx.doi.org/10.1016/j.lwt.2011.10.007>

PART II

Paper I

Innovative Nondestructive Measurements of Water Activity and the Content of Salts in Low-Salt Hake Minces

Kirsti Greiff,^{*,†,§} Ana Fuentes,[#] Ida G. Aursand,[†] Ulf Erikson,[†] Rafael Masot,[⊥] Miguel Alcañiz,[⊥] and Jose M. Barat[#]

[†]SINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway

[§]Department of Biotechnology, Norwegian University of Science and Technology, Sem Sælandsvei 6/8, N-7491 Trondheim, Norway

[#]Departamento de Tecnología de Alimentos (DTA), Universitat Politècnica de València, Cno de Vera s/n, 46022 València, Spain

[⊥]Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Centro Mixto Universitat Politècnica de València, Cno de Vera s/n, 46022 València, Spain

S Supporting Information

ABSTRACT: Impedance spectroscopy (IS), low-field proton nuclear magnetic resonance (LF ¹H NMR), chloride titration, ion chromatography, and an ion selective electrode were used to investigate the physicochemical parameters and measure sodium and potassium contents in low-salt brines and fish. Salt solutions (0–3 w/w, %) and model products of minced hake with added NaCl (0.5–3.0 w/w, %), or a mixture of NaCl and KCl (50/50 w/w, %), were analyzed. Good correlation was observed between the sodium content determined by using the ion selective electrode method and ion chromatography ($R^2 = 0.97$). In both salt solutions and fish minces, the impedance spectroscopy measurements could detect the difference in salt contents in mince with salt contents down to 0.5%. The NMR transversal relaxation time T_2 measurements clearly distinguished samples with 0, 0.5, and 1.0–3.0% salt, based on principal component analysis (PCA). Therefore, LF ¹H NMR seems to be a suitable technique for studies of low-salt products.

KEYWORDS: impedance spectroscopy, low sodium, low salt, low-field NMR, T_2 relaxation, hake

■ INTRODUCTION

A high consumption of sodium has been directly associated with a greater likelihood of increased blood pressure, which in turn has been directly related to the development of cardiovascular and renal diseases.¹ For these reasons, national and international bodies have set targets for a reduction in sodium consumption.² Salt is commonly employed in fish processing, because it helps increase shelf life, reduces water activity (a_w), and has important effects on water-holding capacity, fat binding, color, flavor, and texture.^{3–5} Fish is used as a main ingredient in numerous seafood products, such as fish sausages, surimi, and surimi-based products such as fish puddings.

The development of low-sodium fish products without affecting product quality and safety is of interest, especially considering the otherwise good nutritional characteristics of fish. The partial substitution of NaCl by KCl has been shown to be one of the best alternatives for reducing sodium content.^{6–8} Indeed, both salts have similar properties, and the health effects of increased potassium intake are continuously evaluated by international health authorities.^{9–11} Replacement of NaCl by high concentrations of KCl may have a negative influence on the flavor intensity and produce bitter tastes.¹²

Parameters such as a_w and salt content have important implications for product shelf life and consumer safety. In this regard, the development of rapid, accurate, and nondestructive methods for monitoring these parameters, independently of the sodium replacement, is of industrial interest as is the accurate determination of the sodium content in food. The increasing

use of salt replacers such as potassium chloride⁸ makes it necessary to find new rapid techniques for determining the sodium content directly, because measuring the chloride content no longer represents the sodium content in the food.

Analytical methods for the determination of salt include flame atomic absorption spectrophotometry (FAAS), inductively coupled plasma/MS (ICP/MS), ionic chromatography, and sodium selective electrodes.¹³ Other methods, such as the Volhart method (AOAC method 971.27)¹⁴ and potentiometric titration,¹⁵ measure the chloride contents, and the sodium content is then calculated stoichiometrically.

To meet the objective of developing fast and nondestructive methods to monitor product quality as affected by sodium reduction, electronic sensors based on impedance spectroscopy (IS) may be an option. The relationship between sodium chloride content and impedance measurements has already been demonstrated.^{16–19} In the IS technique, an electrical sinusoidal stimulus is applied to the electrodes to measure the impedance of the sample at different frequencies. The module and phase of the impedance can vary significantly according to the charges present (free ions) and types of microstructure and electrolytes, as well as texture, geometry, and the electrodes used.¹⁸ However, this technique has not yet been applied to

Received: December 10, 2013

Revised: February 24, 2014

Accepted: March 1, 2014

Published: March 11, 2014

food products in which sodium has been replaced by other cations.

The effect of salting can also be determined indirectly, for example, by using low-field (LF) ^1H NMR to monitor changes in proton relaxation behavior as a result of salt addition. In foods, the NMR proton signals basically originate from small molecules such as water and fat. Changes in tissue microstructure due to salting will affect proton exchange with the surrounding environment. For example, tissue swelling after the addition of salt leads to a more open microstructure, causing higher water mobility. Several studies have been carried out in which LF NMR has been used to monitor changes during fish salting processes.^{20–26} However, because none of these studies have dealt with low-salt tissues, it would be of interest to explore the method further as a potential tool for low-salt applications.

The objectives of the present research are to (1) evaluate the application of impedance spectroscopy to monitor physicochemical parameters in salted fish products with and without sodium replacement, (2) establish a fast and consistent method to measure sodium and potassium contents in fish products, and (3) assess the feasibility of employing LF NMR in low-salt tissues.

MATERIALS AND METHODS

Chemicals. Ammonium chloride (NH_4Cl), ammonium hydroxide (NH_4OH), ammonium hydrogen fluoride (NH_4FHF <1%, LD₅₀ mg/kg not found), chloroform (CHCl_3), ethanol ($\text{C}_2\text{H}_5\text{OH}$), sulfuric acid (H_2SO_4), potassium sulfate (K_2SO_4), copper sulfate (CuSO_4), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH) (Scharlau, S.A., or Thermo Fisher Scientific, USA). All chemicals were of analytical-reagent grade.

Experimental Protocol. Experiments using the impedance system were carried out in two phases. In the first phase, the system's capability to distinguish between different types and quantities of salts was evaluated. The second phase evaluated the impedance system for discriminating between fish samples salted with different salt mixtures and quantities of salt.

Phase I: Salt Solutions. Different brines were prepared by using NaCl, KCl, and a mixture of NaCl/KCl (50:50, w/w, %) at different contents. The total salt contents assessed were 0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% (g salt/100 g distilled water). NaCl and KCl reagents (analytical-reagent grade) were obtained from Panreac Química S.A.U. (Barcelona, Spain). The brines were prepared the day before analysis to ensure that all of the salt was completely dissolved. The parameters measured in brines were a_w , pH, conductivity, and sodium and chloride contents. Sodium content in brines was determined by a Na-selective electrode. All measurements were done in triplicate. Impedance spectroscopy measurements were also carried out on the same brine solutions.

Phase II: Fish Minces. Fresh hake (*Merluccius paradoxus/capensis*) was used as raw material. The fish were caught June 13, 2012, by trawling off the coast of South Africa (FAO fishing area 47, Atlantic Southeast) and were obtained June 19, 2012, from a local supermarket in Valencia (Spain). The fish specimens were placed in styrofoam boxes with ice and transported immediately to the laboratory. Upon arrival at the laboratory, two fish were headed and gutted. Then, the fish were filleted and skinned, and the flesh was chopped with a standard food processor at low speed (Minirobot D81, Moulinex, Group SEB Iberica, Barcelona, Spain). Samples were prepared by mixing the fish mince (fish mince and salt, 200 g total) with an exact amount of salt before homogenization for 1 min in the food processor. The amount of salt added to the fish mince had been preweighed to achieve an exact salt content (NaCl or NaCl/KCl) in the final sample of 0.0, 0.5, 1.0, 2.0, or 3.0% salt (g NaCl or NaCl/KCl/100 g salted fish mince). The homogenized fish minces were divided into five plastic containers (40 g in each). Three of the plastic containers were

used for the physicochemical analyses and impedance spectroscopy, whereas the remaining two containers were used for LF NMR measurements. According to the results obtained by Sánchez-Alonso,²⁷ the mince composition does not suffer significant alterations during the frozen storage period. The samples were stored at $-18\text{ }^\circ\text{C}$ and thawed to $4\text{ }^\circ\text{C}$ during 18 h before analysis. Moisture, lipid, protein, ash, a_w , pH, chloride, sodium, and potassium contents were determined in the same subsamples as were subjected to impedance spectroscopy. The minces assigned for LF NMR analysis were kept frozen for 86 days before the measurements were carried out.

ANALYTICAL METHODS

Physicochemical Analyses. Moisture, lipid, protein, and ash contents were assayed according to AOAC Methods 950.46, 991.36, 928.08, and 920.153, respectively,²⁸ whereas pH and conductivity of brines were determined by using a multimeter MM 40 (Crison Instruments, S.A., Barcelona, Spain). The pH measurements of fish were carried out using a digital pH-meter micropH 2001 (Crison Instruments) with a puncture electrode (Crison S231). Water activity was assessed in brines and fish minces with a fast water activity meter (GBX FAsT/lab, Romans sur Isère Cedex, France).

The chloride and sodium contents in brines were measured directly in the solutions, using a chloride analyzer (Sherwood model 926, Cambridge, UK) and a Dual Star pH/ISE meter (Thermo Fisher Scientific, Waltham, MA, USA) with a Na-selective electrode (Ross Sodium Ion Selective Electrode, Thermo Fisher Scientific), respectively. Chloride, sodium (by two different analytical methods), and potassium contents of fish minces were measured in an extract of the sample. For preparing the extract, 1.5 g of the mince was homogenized in ultrapure water using an Ultraturrax T-25 (IKA, Labortechnik, Staufen, Germany) at 9000 rpm for 1 min. Then, samples were warmed to $90\text{ }^\circ\text{C}$ for 30 min, cooled to room temperature, transferred to a volumetric flask, and diluted to 200 mL with ultrapure water. Finally, samples were filtered through a cellulose filter paper (Whatman no. 1, Whatman International Ltd., Maidstone, UK). For chloride and sodium determinations, an aliquot of the extract was measured at room temperature by using the chloride analyzer and the Na-selective electrode as described above. The Na-selective electrode method was a modification of the Kivikari method.²⁹ In this study the direct calibration method was used, contrary to the method of Kivikari, where the known addition method was used. A calibration curve was made by using three standards of analytical grade NaCl from Panreac Química S.A.U. Sodium ion strength adjustor (sodium ionic strength adjustor, Thermo Fisher Scientific) was added to all solutions to ensure that samples and standards had similar ionic strengths. Sodium and potassium contents of the samples were determined by ion chromatography (Compact IC 761, Metrohm Ltd., Herisau, Switzerland) by using an ion exchange column (Metrosep C2, 250/4.0, Metrohm Ltd.). The separation was monitored by using a regulated ($20\text{ }^\circ\text{C}$) conductivity detector, and IC Net 2.3 (Metrohm Ltd.) software was used for data collection and processing. Prior to analysis, samples were filtered through $0.45\text{ }\mu\text{m}$ nylon syringe filters. The isocratic elution was carried out using a solution of tartaric acid (4.0 mM)/dipicolinic acid (0.75 mM) at a flow rate of 1 mL/min. Samples were injected using a $20\text{ }\mu\text{L}$ loop injector. The content of each cation was determined by interpolation in the corresponding calibration curve. The calibration was established using a triplicate set of standard solutions of Na^+ (Fluka, Buchs, Switzerland) and K^+ (Sigma-Aldrich, St. Louis, MO, USA).

Impedance Spectroscopy. The impedance spectroscopy measurement system was developed by the Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) at the Universidad Politécnica de Valencia (UPV).¹⁸ It consists of a software application that runs on a PC, electronic equipment, and an electrode (for more information see the Supporting Information).

Using the software application, the user chooses the frequencies and the amplitudes of the sinusoidal voltage signals. For each of the frequencies the electronic equipment generates the corresponding sinusoidal voltage waveform to the electrode. The current (I) and

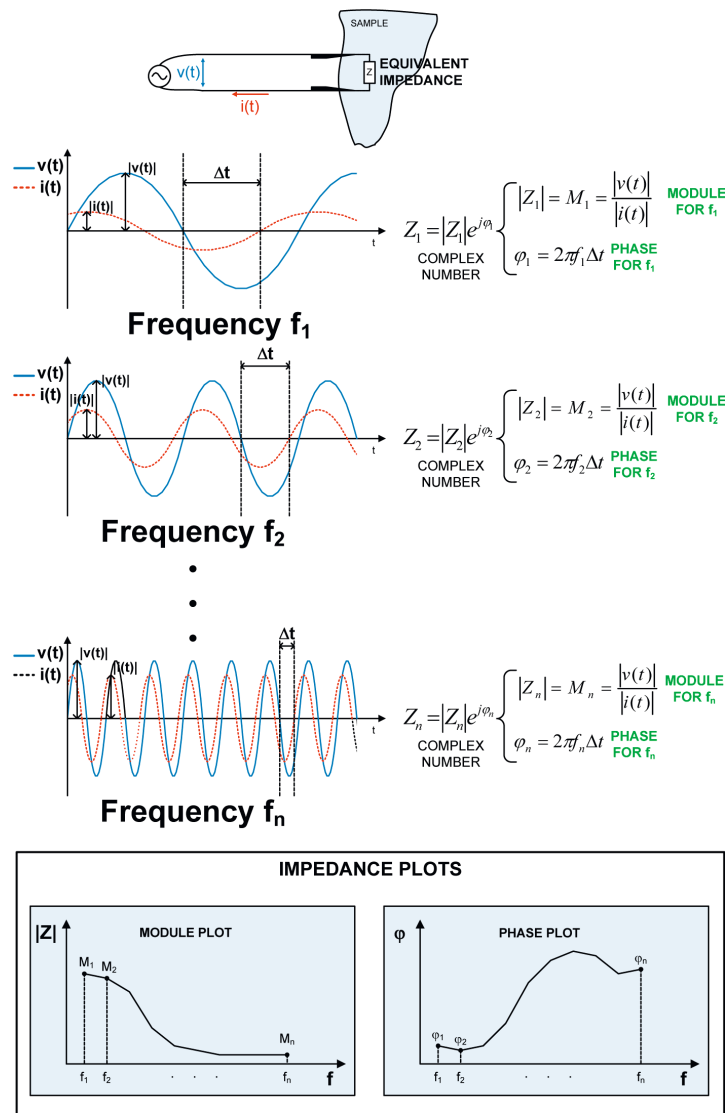


Figure 1. Scheme of impedance measurement and registered signals: module $|Z|$; phase (ϕ); $v(t)$, voltage signal; $i(t)$, current signal; f , frequency of the signals; Δt , time interval between the zero crossing of the voltage and current signals.

voltage (V) signals at the electrode are then sampled, and the collected data are sent to the PC, where a discrete Fourier transform analysis (DFT) is performed to determine their amplitude and phase. The module $|Z|$ and the phase (ϕ) of the impedance are then calculated using eq 1, where $v(t)$ is the voltage signal, $i(t)$ the current signal, f the frequency of the signals, and Δt the time interval between the zero crossing of the voltage and current signals (Figure 1).

$$Z = |Z|e^{j\phi} \begin{cases} |Z| = \frac{|v(t)|}{|i(t)|} & \text{module} \\ \phi = 2\pi f \Delta t & \text{phase} \end{cases} \quad (1)$$

The electronic equipment includes a digital processing block based on two CPLDs and three random-access memories (RAM), one digital-to-analog converter, two analog-to-digital converters, and some analog signal adaptation circuits.¹⁸

The sensor employed in this study is a double electrode designed at IDM-UPV. The sensor consists of two steel needles 1.5 cm long and 1 mm in diameter, separated by a distance of 1 cm in a nonconductive frame. This design keeps the separation between both needles constant during measurements.

The impedance measurements were taken by inserting the sensors into the middle of the plastic containers ($n = 3$) containing the solutions or the fish minces. Ten parallel measurements were

Table 1. Physicochemical Parameters of Brine Solutions Prepared with Different Salts (S: KCl (K), NaCl:KCl (Na:K), and NaCl (Na)) and Contents (C)

S	C (g salt/100 g brine)	a_w^a	conductivity ^a (mS/cm)	Na ^a (g/L)	Cl ^a (g/L)
K	0.1	0.999 ± 0.000aA	2.10 ± 0.11A	(0.34 ± 0.02) × 10 ⁻³ aA	1.03 ± 0.06aA
	0.5	0.993 ± 0.001aB	10.27 ± 1.15B	(0.76 ± 0.00) × 10 ⁻³ aB	3.50 ± 0.14aB
	1.0	0.996 ± 0.003aB	19.31 ± 1.24C	(0.89 ± 0.01) × 10 ⁻³ aC	5.78 ± 0.13aC
	1.5	0.990 ± 0.002aC	31.20 ± 0.28D	(1.26 ± 0.02) × 10 ⁻³ aD	8.30 ± 0.26aD
	2.0	0.985 ± 0.001aC	41.67 ± 1.21E	(1.37 ± 0.02) × 10 ⁻³ aE	10.54 ± 0.09aE
	2.5	0.980 ± 0.002aCD	51.23 ± 3.61F	(1.69 ± 0.04) × 10 ⁻³ aF	12.64 ± 0.13aF
	3.0	0.984 ± 0.002aD	63.30 ± 5.30G	(2.18 ± 0.16) × 10 ⁻³ aG	14.68 ± 0.33aG
Na:K	0.1	0.995 ± 0.002bA	2.07 ± 0.04A	0.26 ± 0.00bA	1.08 ± 0.08bA
	0.5	0.989 ± 0.002bB	9.90 ± 0.42B	0.77 ± 0.00bB	3.74 ± 0.21bB
	1.0	0.991 ± 0.001bB	19.33 ± 1.26C	1.67 ± 0.02bC	6.44 ± 0.17 bC
	1.5	0.985 ± 0.004bC	30.17 ± 3.06D	2.60 ± 0.01bD	9.12 ± 0.19bD
	2.0	0.985 ± 0.001bC	38.27 ± 0.81E	3.45 ± 0.01bE	11.86 ± 0.2 bE
	2.5	0.980 ± 0.002bCD	50.63 ± 3.10F	4.23 ± 0.07bF	14.72 ± 0.3 bF
	3.0	0.981 ± 0.001bD	63.10 ± 4.27G	5.17 ± 0.02bG	16.52 ± 0.15bG
Na	0.1	0.998 ± 0.002bA	2.57 ± 0.68A	0.39 ± 0.00cA	1.05 ± 0.08cA
	0.5	0.989 ± 0.001bB	10.44 ± 1.50B	1.60 ± 0.01cB	3.63 ± 0.21cB
	1.0	0.984 ± 0.004bB	19.37 ± 0.97C	3.56 ± 0.02cC	6.72 ± 0.28cC
	1.5	0.980 ± 0.002bC	28.37 ± 1.51D	5.53 ± 0.03cD	10.01 ± 0.19cD
	2.0	0.981 ± 0.002bC	39.87 ± 2.00E	7.65 ± 0.00cE	12.64 ± 0.05cE
	2.5	0.983 ± 0.001bCD	50.80 ± 3.10F	9.45 ± 0.19cF	16.20 ± 0.24cF
	3.0	0.984 ± 0.002bCD	59.10 ± 3.05G	11.07 ± 0.06cG	18.78 ± 0.26cG
			F Ratio^b		
S		22.45***	0.61ns	69815.08***	600.85***
C		80.43***	589.51***	14371.42***	10525.71***
S × C		7.90***	0.29ns	5104.16***	69.82***

^aMean values ± SD ($n = 3$). ANOVA F ratio for each of the two factors (S and C) and its interaction in the physicochemical parameters. Different lower case letters indicate significant differences ($p < 0.05$) for factor S (salt composition). Different capital letters indicate significant differences ($p < 0.05$) for factor C (salt content). ^b p values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, nonsignificant.

performed in each plastic container. The penetration depth of the electrodes was constant in all analyses (1.5 cm). All measurements were carried out at room temperature.

Preliminary impedance spectroscopy measurements showed that information given by low frequencies was not relevant for this study. Therefore, all of the measurements were carried out in the range of 10 kHz–1 MHz. Seventeen frequencies were chosen in this range; thus, a set of 34 values (17 module values and 17 phase values) was obtained for each sample.

Low-Field ¹H NMR. LF ¹H NMR measurements were made on all fish minces. After thawing, approximately 2 g samples were taken from each subsample of fish mince ($n = 2$) and placed in NMR tubes (diameter = 10 mm). There were analyzed in three parallels from each subsample with fish mince in the LF ¹H NMR measurements. The tubes were immediately placed in ice and kept there until the NMR measurements were carried out. The measurements were performed using a Bruker minispec mq 20 (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz. The instrument was equipped with a 10 mm temperature-variable probe. A built-in heating element was connected to the temperature control unit (BVT3000, Bruker Optik GmbH). The temperature in the probe was regulated to 4 °C by blowing compressed air through the sample holder. Transversal (T_2) relaxation was measured using the Carr–Purcell–Meiboom–Gill pulse sequence (CPMG).^{30,31} The T_2 measurements were performed with a time delay between the 90° and 180° pulses (τ) of 150 μ s. Data from 4000 echoes were acquired from 16 scan repetitions. The repetition time between two succeeding scans was set to 3 s. All even echoes were sampled. The NMR transverse relaxation data were analyzed using two different calculation methods: (1) Biexponential analysis of T_2 relaxation data was performed by fitting of the following equation

to the experimental CPMG curves, similar to that reported by Erikson et al.²¹ and Lambelet et al.³²

$$\text{signal} = A_{21} e^{-t/T_{21}} + A_{22} e^{-t/T_{22}} \quad (2)$$

T_{21} and T_{22} are the relaxation time components, and A_{21} and A_{22} are the corresponding amplitudes; 4000 data points were used, and the calculations were made using MatLab (The Mathworks Inc., Natick, MA, USA). Because the absolute relaxation amplitudes are proportional to the amount of water and fat in the sample, the relative amplitudes within samples were used. The T_{21} populations are calculated as $A_{21}/(A_{21} + A_{22})$.

For the biexponential fitting, the populations sum to 100%. Three parallel samples from each fish mince ($n = 2$) were averaged. (2) Multivariate data analysis was performed for all raw relaxation (CPMG) curves. These curves were normalized by setting the first sampled echo to a value of 100 and thereafter scaling the rest of the echo train. The first 600 data points were used for the principal component analysis (PCA).

Statistical Analyses. Statistical treatment of the data was performed using the Statgraphics Centurion (Statpoint Technologies, Inc., Warranton, VA, USA). A multifactor analysis of variance (ANOVA) was conducted for each evaluated parameter to test whether there were significant differences between the samples. These analyses were performed for the salt solutions and fish mince samples (phases I and II); in both cases, the physicochemical parameters were considered as dependent variables in these analyses. The type of cations and salt content, as well as its interaction, were the factors. The Tukey test (least significant difference) was used to test for differences between averages at the 5% significance level.

To evaluate the measurement techniques used in this paper, different multivariate analyses³³ were carried out using the software

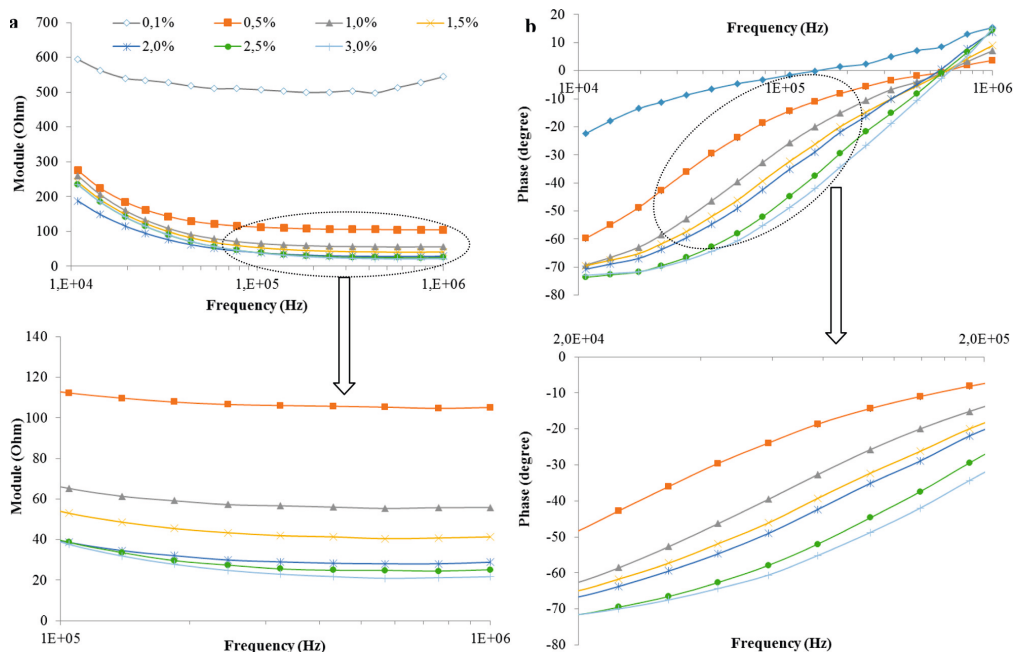


Figure 2. Mean values of modulus (a) and phase (b) of impedance spectra for the KCl solutions with different salt contents (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g salt/100 g distilled water, respectively).

SOLO PLS_Toolbox (eigenvector Research, Inc., Wenatchee, WA, USA).

PCA was used to discriminate the salt content level for NaCl, KCl, and mixtures. Typically, PCA projects a multidimensional data set onto a new coordinate base formed by the orthogonal directions with data maximum variance. The eigenvectors of the data matrix are called principal components (PCs), and they are uncorrelated between them. The PCs are ordered so that PC1 displays the greatest amount of variance, followed by the next greatest, PC2, and so forth. The main features of PCA are the coordinates of the data in the new base (scores plot) and the contribution to each component of the sensors (loads plot).

To create predictive models of physicochemical parameters, partial least square (PLS) regressions were applied to both impedance spectroscopy and NMR measurements. The main objective of PLS is to predict one or more parameters (dependent variables Y) from a set of measured data (independent variables X). First, the set of independent variables is projected onto a new coordinate space by maximizing the covariance between Y and X . The axes of this new space are called latent variables (LVs). The important information that correlates Y and X is contained in the first LVs. Then a prediction model is built by applying a multiple regression to a reduced number of the LVs. PLS prediction models for a_w , and Na, K, NaCl, and solute contents (g/100 g) as well as solutes content in the water phase (g solutes/100 g liquid phase) were created using a set of experimental data (calibration set). First, cross-validation was used to select the number of LVs. The model was then validated with a new set of experimental data (validation set).

In the case of impedance measurements PCA and PLS regressions were performed using impedance module and phase values obtained for the 17 frequencies in the range from 10 kHz to 1 MHz. In the case of NMR measurements, the relaxation times for each defined frequency were used.

RESULTS AND DISCUSSION

Phase I: Salt Solutions. Physicochemical Parameters.

The results of the physicochemical analyses carried out for the salt solutions are shown in Table 1. As expected, the a_w of brines decreased with increasing brine content regardless of type of salt, and the conductivity increased as salt content increased. Conductivity correlates with the total dissolved solids independent of the solute composition. In water, ions pass the electricity from one to another; therefore, the more Na^+ , K^+ , and Cl^- the solution contains, the more electricity is carried and the higher the conductivity. This explains the fact that the conductivity was affected by the amount of salt but not by the sodium replacement. The initial conductivity of distilled water employed for preparing the salt solutions was 0.025 ± 0.003 mS/cm. The value increased with increasing salt content, from 2 to 60 mS/cm for the lowest and highest salt contents, respectively. Table 1 also shows the resulting contents of Na^+ and Cl^- after different salt additions to distilled water. In the solution prepared from KCl only, sodium was present in the range of 0.3–2.2 mg/L. The observed differences in chloride content depending on the type of salt are due to the different atomic masses of sodium and potassium (23 and 39 atomic mass units, respectively), owing to the fact that the salts were added equally by weight.

Impedance Spectroscopy. Module and phase impedance spectra of KCl solutions are shown in Figure 2, panels a and b, respectively. Differences in both module and phase of impedance were observed to depend on salt content.

The module of the impedance decreased as the salt content increased, and the values were much higher for the lowest content (0.1% KCl) than for the other contents. Similar

differences between salt contents were observed for NaCl and the mixture of NaCl:KCl (data not shown). These results are in agreement with those observed for the conductivity parameters for the brine (Table 1). The results are in accordance with previous studies on impedance spectroscopy.^{16–18} The correlation can be explained by the conductance of an aqueous solution as a function of the ion content of the samples, and in fact impedance measurements are related to the ions' capability of movement under the influence of an electrical field in this aqueous solution. In the present study, the behavior observed for NaCl solutions was similar to what was observed for KCl and NaCl:KCl solutions, which would indicate that impedance values were highly correlated with solute content. These results were confirmed by ANOVAs carried out for each impedance value (module and phase of impedance for each frequency), which established significant differences for solute content ($p < 0.001$) but not for the type of salt ($p > 0.05$) (ANOVA data not shown).

A PCA was performed with the data obtained in the impedance measurements (Figure 3). The statistical analysis

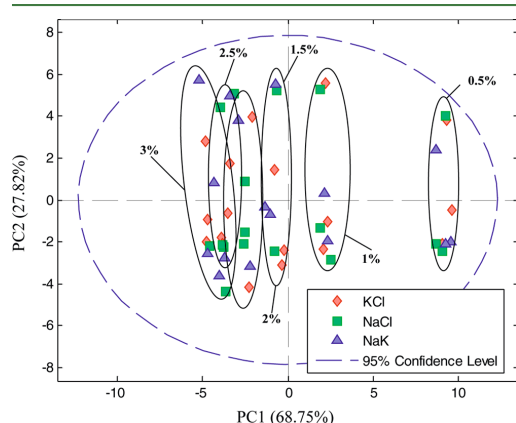


Figure 3. PCA score plot of data obtained from the impedance spectroscopy measurements in solutions with different types of salt (NaCl, KCl, and NaCl:KCl (NaK), 50:50 w/w %) and contents (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g salt/100 g distilled water, respectively).

was able to reduce the initial variables (34 variables, 17 values of module and 17 values of phase of impedance) into a set of values of linearly uncorrelated variables called PCs, the number of principal components being less than or equal to the number of original variables. Most of the variation in the sample was explained by PC1 (68.75%) and PC2 (27.82%). According to the results obtained, the impedance spectroscopy method could distinguish between salt contents; however, it was difficult to establish a correct classification of solutions according to the type of salt (Figure 3).

Phase II: Fish Mince. *Physicochemical Analyses.* The composition of the frozen/thawed raw material was determined. Moisture, protein, lipid, and ash contents for unsalted hake were 80.2 ± 0.1 (Table 2), 15.6 ± 1.3 , 0.5 ± 0.2 , and 1.20 ± 0.02 g/100 g, respectively. These results are similar to those reported in other studies carried out with the same fish species.^{27,34}

The results of the physicochemical analyses for salted hake mince are summarized in Table 2. As expected, adding salt to

Table 2. Physicochemical Parameters of Fish Mince Prepared with Different Salts (S: NaCl (Na) and NaCl:KCl (Na:K) and Contents (C)

S	C (g salt/100 g fish mince)	moisture ^a (%)	a_w ^a	pH ^a	chloride ^a (g/100 g)	sodium ^a (ISE) (g/100 g)	sodium ^a (IC) (g/100 g)	potassium ^a (g/100 g)	ionic strength ^a (mol/kg solvent)
fish mince	0	80.15 ± 0.08	0.992 ± 0.004	6.97 ± 0.13	0.21 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.35 ± 0.00	0.11 ± 0.00
Na:K	0.5	$80.37 \pm 0.24aA$	$0.987 \pm 0.004aA$	$6.81 \pm 0.01aA$	$0.43 \pm 0.01aA$	$0.12 \pm 0.01aA$	$0.12 \pm 0.01aA$	$0.48 \pm 0.09aA$	$0.18 \pm 0.03aA$
Na:K	1.0	$79.13 \pm 0.41aA$	$0.984 \pm 0.003aAB$	$6.71 \pm 0.04aA$	$0.69 \pm 0.00aB$	$0.19 \pm 0.00aB$	$0.19 \pm 0.05aA$	$0.56 \pm 0.12aA$	$0.26 \pm 0.04aB$
Na:K	2.0	$79.57 \pm 0.09aA$	$0.980 \pm 0.005aB$	$6.83 \pm 0.02aA$	$1.29 \pm 0.06aC$	$0.43 \pm 0.04aC$	$0.42 \pm 0.03aB$	$0.79 \pm 0.11aB$	$0.47 \pm 0.03aC$
Na:K	3.0	$78.32 \pm 0.41aB$	$0.974 \pm 0.001aC$	$6.69 \pm 0.02aB$	$1.70 \pm 0.03aD$	$0.57 \pm 0.02aD$	$0.68 \pm 0.08aC$	$1.17 \pm 0.14aC$	$0.69 \pm 0.05aD$
Na	0.5	$79.09 \pm 0.94aA$	$0.985 \pm 0.002bA$	$6.76 \pm 0.05bA$	$0.50 \pm 0.01bA$	$0.19 \pm 0.00bA$	$0.26 \pm 0.04bA$	$0.40 \pm 0.004bA$	$0.22 \pm 0.01bA$
Na	1.0	$80.47 \pm 0.88aA$	$0.982 \pm 0.001bAB$	$6.78 \pm 0.03bA$	$0.82 \pm 0.06bB$	$0.35 \pm 0.01bB$	$0.38 \pm 0.07bA$	$0.30 \pm 0.03bB$	$0.30 \pm 0.03bB$
Na	2.0	$78.67 \pm 0.14aA$	$0.978 \pm 0.002bB$	$6.67 \pm 0.04bA$	$1.34 \pm 0.11bC$	$0.76 \pm 0.06bC$	$0.78 \pm 0.02bB$	$0.40 \pm 0.04bB$	$0.52 \pm 0.03bC$
Na	3.0	$77.77 \pm 0.06aB$	$0.969 \pm 0.001bC$	$6.67 \pm 0.04bB$	$1.90 \pm 0.03bD$	$1.01 \pm 0.00bD$	$1.09 \pm 0.11bC$	$0.39 \pm 0.04bC$	$0.71 \pm 0.03bD$
S		2.79ns	6.82****	6.42****	28.40****	52.67****	116.86****	112.43****	8.26****
C		15.07****	31.63****	10.26****	819.47****	720.83****	153.16****	21.40****	300.80****
S × C		7.80****	0.67ns	12.58****	2.71ns	60.63****	6.65****	17.82****	0.17ns

^aMean values \pm SD ($n = 3$). Different lower case letters indicate significant differences ($p < 0.05$) for factor S (salt composition). Different capital letters indicate significant differences ($p < 0.05$) for factor C (salt content).

the mince led to a reduction in moisture, from about 80.2% (mince without additions) to 78.3 and 77.8%, for minces containing sodium and potassium chloride (Na:K) and minces containing sodium chloride (Na), respectively. Due to the increase in mineral contents (up to 3.0 g/100 g mince) the a_w decreased from 0.992 (mince without additions) to 0.974 (Na:K) and 0.969 (Na). The moisture and a_w were significantly lower in minces containing 3.0% salt compared to minces containing less. Both the type of salt and their contents had a significant effect on the a_w , compared to the brines, where the a_w correlates only with the contents of salt. As expected, slightly higher water activities were found in the NaCl:KCl minces than in minces containing NaCl. Water activity decreases with increasing number of colligative units dissolved per volume. As K^+ is a larger ion than Na^+ , replacing NaCl with an equal amount by weight of KCl will lead to a lower number of dissolved ions (colligative units) per volume and thus an increase in a_w of the product.

The pH of the unsalted mince (pH 6.97) was reduced after preparation of the mince with different salts (Na) and (Na:K) and contents (Table 2). The pH values of the raw material employed in this study are in accordance with the results obtained in other studies^{34,35} for fresh hake. A decrease in pH was observed when salt was added to our minces, a little more pronounced in the case of Na than with most Na:K mixtures. Similar results have been observed in a study by Leroy and Joffraud,³⁶ indicating that the pH decreases in fish flesh by the addition of salt due to the increase of the ionic strength of the solution inside the cells. Another explanation might be that an increased amount of chloride ions would open the myosin filament and the more dissociable acidic groups would be water-accessible.³⁷ Samples containing Na exhibited lower pH than the corresponding Na:K samples: pH 6.76 vs 6.81, respectively. Similar results with fish products subjected to partial sodium replacement have also been observed.³⁸

The measured contents of sodium, potassium, and chloride are shown in Table 2. The sodium (0.05–0.06 g/100 g) and potassium contents (0.35 g/100 g) of fresh fish mince (Table 2) agree with those reported in another study³⁹ for deboned hake. The chloride content in mince without additions was 0.21 g/100 g. When only NaCl was added to the minces, the potassium levels remained almost constant at 0.30–0.40 g/100 g, resembling the level in mince without additions.

Table 2 shows a comparison between sodium contents in the different minces as determined by the ion selective electrode and by ion chromatography. Good correlation was observed between the sodium content determined by the ion selective electrode method and ion chromatography, which was confirmed by a simple regression carried out on the data obtained by both methodologies ($y = 1.066x + 7.961$, $R^2 = 0.967$).

Impedance Spectroscopy Measurements. Impedance spectroscopy was used to detect changes in the fish mince by adding different salt contents and types of salt. A PCA was performed on the impedance spectroscopy measurements in fish mince samples with different type of salts.

The discrimination between the different salt contents observed in the PCA plot for fish minces was better than the one obtained for salt solutions (Figure 4). The percentage of variance explained by the first principal component in Figure 4 is 90.17%, whereas in Figure 3 PC1 explains only 68.75% of the total variance. This means that the correlation between impedance spectroscopy data and salt content is stronger in

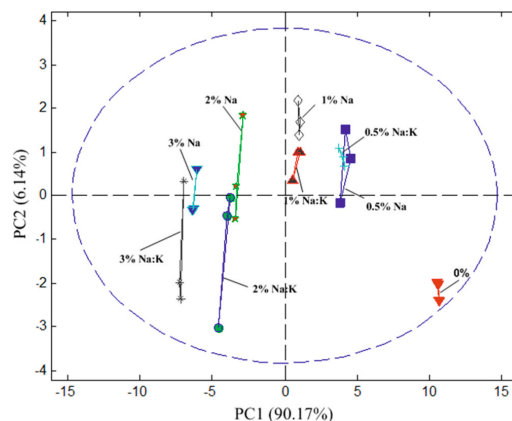


Figure 4. PCA score plot of data obtained from the impedance spectroscopy measurements in hake minces with different salt contents (NaCl (Na) or NaCl/KCl (Na:K) 0.0, 0.5, 1.0, 1.5, 2.0, and 3.0 g/100 g salted mince, respectively).

fish samples than in solutions. A possible explanation for this behavior is the salting-in effects on muscle proteins.³⁷ At salt contents lower than 0.5 M, the swelling of myofibrils starts and reaches a maximum at 0.8–1 M.³⁷ This usually causes a decrease in myofibril volume, because the myofibril tends to dissolve. However, in our study, the highest contents in the minces corresponded to 0.65 and 0.55 M for the minces with 3.0% NaCl and NaCl:KCl, respectively. The conformational changes, together with the increase in the conductivity, could be responsible for the different behavior in the IS observed among our hake minces and in the solutions. At some contents, the method also distinguished between types of cations (Na^+ or Na^+/K^+) in the fish mince, a behavior that can be explained by the different effects of sodium (kosmotrope, water-structure maker) and potassium (chaotrope, water-structure breaker) in actin and myosin.⁴⁰ Further work is needed to reveal significant differences between cations in the minces.

LF NMR. A LF NMR T_2 relaxation method was used to study the relaxation behaviors in the mince when different types of salt were added to the mince in different amounts. The two transversal relaxation times with corresponding populations obtained from fitting of NMR data are shown in Table 3. In fish muscle, typically two or three relaxation components are reported (ref 41 and references cited therein). The two major ones have relaxation times in the range of 40–60 ms (T_{21}) and 150–400 ms (T_{22}), similar to those of the present research. The mean T_{21} and T_{22} relaxation times for the unsalted hake mince were 54 and 219 ms, respectively. The interpretation of such data has been controversial, but it is now becoming more accepted that the observed changes in relaxation behavior are due primarily to chemical and diffusive proton exchange between water molecules and biopolymers (e.g., proteins).^{42,43} A number of studies have nevertheless shown that these processes are linked to the morphology of the sample that in turn can be affected by, for example, processing, such as salting and mincing.^{23,25,41}

After addition of 0.5% NaCl or NaCl/KCl to the mince, the proton relaxation times, found by bioexponential fitting, increased to 59–61 ms in the case of T_{21} , whereas the T_{22}

Table 3. Biexponential Fitting of LF ^1H NMR T_2 Relaxation Data Obtained in Fish Mince and Fish Mince Prepared with Different Salts (S: NaCl and NaCl:KCl) and Concentrations (C)

S	C (g salt/100 g/ fish mince)	T_{21}^a (ms)	T_{22}^a (ms)	T_{21} pop a (%)	T_{22} pop a (%)
fish mince	0	54 ± 1	219 ± 7	86 ± 3	14 ± 3
NaCl/KCl	0.5	61 ± 1aA	226 ± 10aA	87 ± 2aA	13 ± 2aD
NaCl/KCl	1.0	67 ± 1aB	286 ± 46aB	96 ± 1aBD	4 ± 1aAC
NaCl/KCl	2.0	71 ± 1aD	496 ± 42aD	99 ± 0aBC	1 ± 0aBC
NaCl/KCl	3.0	68 ± 2aBC	342 ± 15aC	98 ± 0aCD	2 ± 0aAB
NaCl	0.5	59 ± 1bA	215 ± 10aA	85 ± 1bA	15 ± 1bD
NaCl	1.0	69 ± 1bC	366 ± 48bB	98 ± 1bB	2 ± 1bC
NaCl	2.0	67 ± 0bB	427 ± 34bD	99 ± 0aCD	1 ± 0aAB
NaCl	3.0	68 ± 0aBCD	423 ± 34bC	99 ± 0aD	1 ± 0aA
F Ratiob					
S		9.20**	4.99*	0.30ns	0.30ns
C		214.59***	132.32***	609.87***	609.87***
S × C		22.49***	17.43***	12.14***	12.14***

^aMean values ± SD ($n = 2$). Different lower case letters indicate significant differences ($p < 0.05$) for factor S (salt composition). Different capital letters indicate significant differences ($p < 0.05$) for factor C (salt concentration). ^b F values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns: non significant.

value remained largely unchanged. Addition of more salt led to increases in both T_{21} and T_{22} relaxation times, with mean values of 67–71 and 286–496 ms, respectively.

By comparison, when frozen/thawed Atlantic salmon fillets were salted to 2.7% NaCl in the head part of the fillet, T_{21} increased from 47 ms (unsalted) to 48 ms (salted), whereas the tail part of the fillet had 2.9% NaCl and T_{21} increased from 47 ms (unsalted) to 50 ms (salted), respectively. No significant changes were observed in T_{22} (140–150 ms (head part) and 140–169 ms (tail part)).²¹ Similar values were obtained when fillets of the same species were salted in a 15% NaCl brine.²⁰ Thus, it seems that the magnitude of change in T_{21} can be similar in highly concentrated brines (whole, lean fillets) as in our lean hake mince. Notably, the mincing of cod fillets does not alter the magnitude of the T_{21} values.⁴⁴ A stronger effect of salting of cod was, however, reported,²⁴ where T_{21} values increased from 51 ms (raw material) to 86–94 ms after presalting by different methods (12% salt).

A PCA score plot of the relaxation time curves is presented in Figure 5. Most of the variation in the sample was explained by PC1 (76.60%) and PC2 (20.54%), and it separates minces with 0, 0.5, and 1.0–3.0% salt. Otherwise, the relaxation data did not reveal any clear trends between the magnitudes of the relaxation times at increasing salt contents above 0.5%. The increase in relaxation times when 0.5% salt was added, reflecting higher water proton mobility, suggests that a more open mince microstructure was formed. This was possibly caused by the binding of chloride ions to myosin filaments, which would induce electrostatic repulsive forces, causing an increase of filament spacing.³⁷

In contrast to the increase in T_{21} as a result of the addition of 0.5% salt, the corresponding population (T_{21} pop) did not change accordingly. The T_{21} pop values remained similar to those in the mince without additions (85–87%). With further addition of salt, the values increased to 96–99%, regardless of type and amount of salt (1.0, 2.0, or 3.0% salt) with a corresponding decrease in T_{22} pop. The latter population with high mobility decreased to 1–4%. The changes in T_2 populations reflect a shift of the proton populations, increasing the amount of protons with higher mobility and decreasing the

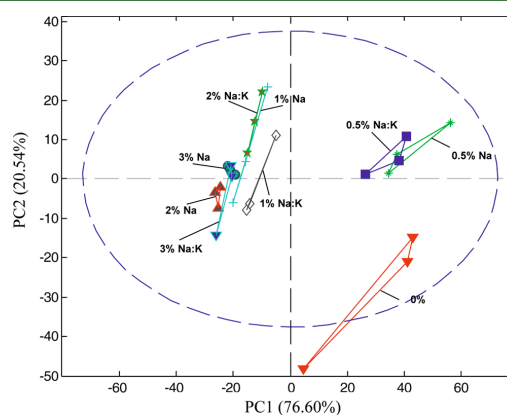


Figure 5. PCA score plot of LF ^1H NMR T_2 relaxation data obtained from fish mince with salt content (NaCl (Na) or NaCl/KCl (Na:K)) 0.0, 0.5, 1.0, 1.5, 2.0, and 3.0 g/100 g salted mince, respectively).

amount of protons with lower mobility. This may be explained by the changes in muscle structure due to the salting-in effect previously discussed.

On the basis of principal component analyses of the NMR T_2 relaxation data, a clear separation between samples with 0, 0.5, and 1.0–3.0% of salt was obtained. However, the LF NMR method was unable to distinguish between minces with different types of cations. In summary, the fact that the most pronounced changes in relaxation behavior occurred at low contents of salt (0–0.5%) indicates that LF ^1H NMR can be a suitable tool for indirect studies of structural changes in low-salt systems.⁴¹

PLS Results. To create predictive models of physicochemical parameters, PLS regression was applied to both impedance spectroscopy and LF NMR measurements. Table 4 shows the values of the determination coefficient (R^2), the root-mean-square error of prediction (RMSEP), and the number of LVs corresponding to the prediction models built for a_w , Na^+ (mg/

Table 4. Parameters of the PLS Models of Physicochemical Parameters from the Impedance Measurements^a

	LV	R ²	RMSEP
a_w	5	0.886	0.003
	1	0.812	0.003
Na (mg/100 g)	4	0.480	276.154
K (mg/100 g)	4	0.094	269.090
NaCl (g/100 g)	4	0.425	0.730
KCl (g/100 g)	3	0.393	0.628
solute content (g/100 g)	5	0.950	0.210
	1	0.953	0.203
solute content in liquid phase (g/100 g)	4	0.946	0.286

^aLV, number of latent variables; R², coefficient of determination; RMSEP, root mean square error of prediction.

100 g), K⁺ (mg/100 g), NaCl (g/100 g), gram salts (g/100 g), and gram solutes/100 g liquid phase using impedance spectroscopy data. Models for a_w , gram salts (g/100 g), and gram solutes/100 g liquid phase show very good behavior with R² values close to or higher than 0.9. However, the results obtained for Na⁺ (mg/100 g), K⁺ (mg/100 g), and NaCl (g/100 g) demonstrate that the proposed technique is not able to discriminate between the different types of salt. As shown in Figure 3, in the PCA plots most of the total variance corresponds to PC1. In the module and phase plots a similar discrimination between the different salt content levels could be obtained considering the module and phase values for all of the frequencies or considering just the module value for one frequency (for example, 1 MHz). On this basis, new PLS models were built for a_w and gram salts (g/100 g) using only one LV. The R² values for these new models are similar to those obtained using the number of LVs established by cross-validation. This opens the possibility to limit impedance measurements to the module at a single frequency so that the measurement process would be greatly shortened and the prediction could be made using a simple regression. There were no significant correlations between the LF NMR measurements and the physicochemical results. In conclusion, the PLS models of impedance spectroscopy measurements showed good correlations with R² values close to or higher than 0.9 for a_w , solute content, and solute content in the liquid phase. However, the results obtained for Na⁺ (mg/100 g), K⁺ (mg/100 g), and NaCl (g/100 g) demonstrate that the proposed technique is not able to discriminate between the different types of salt.

In conclusion, good correlations were observed between the sodium content determined by using the ion selective electrode method and ionic chromatography, which was confirmed by a simple regression, carried out using the data obtained with both methodologies. In both salt solutions and fish minces, the impedance spectroscopy measurements could separate between different salt contents down to 0.5%. However, the results obtained for cation determinations demonstrate that the proposed technique is not able to discriminate between the different types of salt. Furthermore, impedance spectroscopy measurements showed good correlations for a_w , solute content, and solute content in the liquid phase. The NMR transversal relaxation time T₂ clearly distinguishes samples with 0, 0.5, and 1.0–3.0% salt, based on PCA. We conclude that LF ¹H NMR can be a suitable technique for studies of low-salt products. However, the LF NMR method was unable to distinguish between minces with different types of cations.

■ ASSOCIATED CONTENT

5 Supporting Information

System block diagram. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*(K.G.) Mail: SINTEF Fisheries and Aquaculture, Brattørkaia 17C, 7465 Trondheim, Norway. Phone: +47 47 90 38 91. Fax: +47 93 27 07 01. E-mail: kirsti.greiff@sintef.no.

Funding

This research was conducted when K.G. visited Universidad Politécnica de Valencia (UPV) as a part of the project Low Salt Products, Project 185063/O10, supported by the Research Council of Norway.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank our co-workers at UPV, Isabel Fernández-Segovia, Arantxa Rizo, and Lupis Hernandez, and Marte Schei at SINTEF Fisheries and Aquaculture for their support and valuable participation in discussions regarding planning of the experiments, production of fish mince, and guidance related to the use of the different measuring techniques.

■ REFERENCES

- He, F. J.; MacGregor, G. A. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J. Hum. Hypertens.* **2002**, *16*, 761–70.
- WHO. *Reducing Salt Intake in Populations*; report of a WHO Forum and Technical Meeting; World Health Organization: Paris, France, 2006.
- Martínez-Alvarez, O.; Gómez-Guillén, M. C. Effect of brine salting at different pHs on the functional properties of cod muscle proteins after subsequent dry salting. *Food Chem.* **2006**, *94*, 123–129.
- Thorarinsdottir, K. A.; Arason, S.; Bogason, S. G.; Kristbergsson, K. The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). *Int. J. Food Sci. Technol.* **2004**, *39*, 79–89.
- Fuentes, A.; Fernández-Segovia, I.; Barat, J. M.; Serra, J. A. Physicochemical characterization of some smoked and marinated fish products. *J. Food Process. Preserv.* **2010**, *34*, 83–103.
- Aliño, M.; Fuentes, A.; Fernández-Segovia, I.; Barat, J. M. Development of a low-sodium ready-to-eat desalted cod. *J. Food Eng.* **2011**, *107*, 304–310.
- Fuentes, A.; Fernández-Segovia, I.; Barat, J. M.; Serra, J. A. Influence of sodium replacement and packaging on quality and shelf life of smoked sea bass (*Dicentrarchus labrax* L.). *LWT—Food Sci. Technol.* **2011**, *44*, 917–923.
- Toldrá, F.; Barat, J. M. Strategies for salt reduction in foods. *Recent Pat. Food Nutr. Agric.* **2012**, *4*, 19–25.
- Geleijnse, J. M.; Witterman, J. C. M.; Stijnen, T.; Kloos, M. W.; Hofman, A.; Grobbee, D. E. Sodium and potassium intake and risk of cardiovascular events and all-causes mortality: the Rotterdam study. *Eur. J. Epidemiol.* **2007**, *22*, 763–777.
- FSAI. *Salt and Health: Review of the Scientific Evidence and Recommendations for Public Policy in Ireland*; Dublin, Ireland, 2005.
- EFSA. Opinion of the Scientific Panel of Dietetic Products, Nutrition and Allergies on request from the Commission related to the tolerable upper intake level of potassium. *EFSA J.* **2005**, *193*, 1–19.
- Desmond, E. Reducing salt: a challenge for the meat industry. *Meat Sci.* **2006**, *74*, 188–196.
- Ehling, S.; Tefera, S.; Earl, R.; Cole, S. Comparison of analytical methods to determine sodium content of low-sodium foods. *J. AOAC Int.* **2010**, *93*, 628–637.

- (14) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; AOAC: Arlington, VA, USA, 1990.
- (15) Pérez-Olmos, R.; Herrero, R.; Lima, J. L. F. C.; Montenegro, M. C. B. S. M. Sequential potentiometric determination of chloride and nitrate in meat products. *Food Chem.* **1997**, *59*, 305–311.
- (16) Chanet, M.; Rivière, C.; Eynard, P. Electric impedance spectrometry for the control of manufacturing process of comminuted meat products. *J. Food Eng.* **1999**, *42*, 153–159.
- (17) Guerrero, L.; Gobantes, I.; Oliver, M. À.; Arnau, J.; Dolors Guàrdia, M.; Elvira, J.; Riu, P.; Grèbol, N. S.; Monfort, J. M. Green hams electrical impedance spectroscopy (EIS) measures and pastiness prediction of dry cured hams. *Meat Sci.* **2004**, *66*, 289–294.
- (18) Masot, R.; Alcañiz, M.; Fuentes, A.; Schmidt, F. C.; Barat, J. M.; Gil, L.; Baigts, D.; Martínez-Mañez, R.; Soto, J. Design of a low-cost non-destructive system for punctual measurements of salt levels in food products using impedance spectroscopy. *Sensor Actuat. A-Phys.* **2010**, *158*, 217–223.
- (19) Rizo, A.; Fuentes, A.; Fernández-Segovia, I.; Masot, R.; Alcañiz, M.; Barat, J. M. Development of a new salmon salting–smoking method and process monitoring by impedance spectroscopy. *LWT—Food Sci. Technol.* **2013**, *51*, 218–224.
- (20) Aursand, I. G.; Gallart-Jornet, L.; Erikson, U.; Axelson, D. E.; Rustad, T. Water distribution in brine salted cod (*Gadus morhua*) and salmon (*Salmo salar*): a low-field ^1H NMR study. *J. Agric. Food Chem.* **2008**, *56*, 6252–6260.
- (21) Aursand, I. G.; Veliyulin, E.; Böcker, U.; Ofstad, R.; Rustad, T.; Erikson, U. Water and salt distribution in Atlantic salmon (*Salmo salar*) studied by low-field ^1H NMR, ^1H and ^{23}Na MRI and light microscopy: effects of raw material quality and brine salting. *J. Agric. Food Chem.* **2008**, *57*, 46–54.
- (22) Erikson, U.; Veliyulin, E.; Singstad, T. E.; Aursand, M. Salting and desalting of fresh and frozen-thawed cod (*Gadus morhua*) fillets: a comparative study using ^{23}Na NMR, ^{23}Na MRI, low-field ^1H NMR, and physicochemical analytical methods. *J. Food Sci.* **2004**, *69*, FEP107–FEP114.
- (23) Aursand, I. G.; Erikson, U.; Veliyulin, E. Water properties and salt uptake in Atlantic salmon fillets as affected by ante-mortem stress, rigor mortis, and brine salting: a low-field ^1H NMR and $^1\text{H}/^{23}\text{Na}$ MRI study. *Food Chem.* **2010**, *120*, 482–489.
- (24) Guðjónsdóttir, M.; Arason, S.; Rustad, T. The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydrated cod – a low-field NMR study. *J. Food Eng.* **2011**, *104*, 23–29.
- (25) Guðjónsdóttir, M.; Gunnlaugsson, V. N.; Finnbogadóttir, G. A.; Sveinsdóttir, K.; Magnússon, H.; Arason, S.; Rustad, T. Process control of lightly salted wild and farmed Atlantic cod (*Gadus morhua*) by brine injection, brining, and freezing – a low field NMR study. *J. Food Sci.* **2010**, *75*, E527–E536.
- (26) Guðjónsdóttir, M.; Lauzon, H. L.; Magnússon, H.; Sveinsdóttir, K.; Arason, S.; Martinsdóttir, E.; Rustad, T. Low field nuclear magnetic resonance on the effect of salt and modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage. *Food Res. Int.* **2011**, *44*, 241–249.
- (27) Sánchez-Alonso, I.; Martínez, I.; Sánchez-Valencia, J.; Careche, M. Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) by low field NMR. *Food Chem.* **2012**, *135*, 1626–1634.
- (28) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th ed.; AOAC: Arlington, VA, USA, 1997.
- (29) Kivikari, R. Analysis of sodium in meat products using an Na-selective electrode (in Finnish). In *Proceedings of Meat Day Seminar* 536; 1996; pp 64–66
- (30) Carr, H. Y.; Purcell, E. M. Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Am. J. Physiol.* **1954**, *94*, 630–638.
- (31) Meiboom, S.; Gill, D. Modified spin-echo method for measuring nuclear times. *Rev. Sci. Instrum.* **1958**, *29*, 688–691.
- (32) Lambelet, P.; Renevey, F.; Kaabi, C.; Raemy, A. Low-field nuclear magnetic resonance relaxation study of stored or processed cod. *J. Agric. Food Chem.* **1995**, *43*, 1462–1466.
- (33) Martens, H.; Martens, M. *Multivariate Analysis of Quality. An Introduction*; Wiley: New York, 2001.
- (34) Orban, E.; Nevigato, T.; Di Lena, G.; Masci, M.; Casini, I.; Caproni, R.; Rampacci, M. Total volatile basic nitrogen and trimethylamine nitrogen levels during ice storage of European hake (*Merluccius merluccius*): a seasonal and size differentiation. *Food Chem.* **2011**, *128*, 679–682.
- (35) Pastoriza, L.; Sampedro, G.; Herrera, J. J.; Cabo, M. L. Influence of sodium chloride and modified atmosphere packaging on microbiological, chemical and sensorial properties in ice storage of slices of hake (*Merluccius merluccius*). *Food Chem.* **1998**, *61*, 23–28.
- (36) Leroi, F.; Joffraud, J. J. Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *J. Food Prot.* **2000**, *63*, 1222–1227.
- (37) Offer, G.; Trinick, J. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Sci.* **1983**, *8*, 245–281.
- (38) Fuentes, A.; Fernández-Segovia, I.; Serra, J. A.; Barat, J. M. Effect of partial sodium replacement on physicochemical parameters of smoked sea bass during storage. *Food Sci. Technol. Int.* **2012**, *18*, 207–217.
- (39) Martínez-Valverde, I.; Jesús Periago, M.; Santaella, M.; Ros, G. The content and nutritional significance of minerals on fish flesh in the presence and absence of bone. *Food Chem.* **2000**, *71*, 503–509.
- (40) Puolanne, E.; Halonen, M. Theoretical aspects of water-holding in meat. *Meat Sci.* **2010**, *86*, 151–165.
- (41) Erikson, U.; Standal, I. B.; Aursand, I. G.; Veliyulin, E.; Aursand, M. Use of NMR in fish processing optimization: a review of recent progress. *Magn. Reson. Chem.* **2012**, *50*, 471–480.
- (42) Halle, B. Molecular theory of field-dependent proton spin-lattice relaxation in tissue. *Magn. Reson. Med.* **2006**, *56*, 60–72.
- (43) Belton, P. Spectroscopic approaches to the understanding of water in foods. *Food Rev. Int.* **2011**, *27*, 170–191.
- (44) Andersen, C. M.; Jørgensen, B. M. On the relation between water pools and water holding capacity in cod muscle. *J. Aquat. Food Prod. Technol.* **2004**, *13*, 13–23.

Paper II

1 **Gradual reduction in sodium content in cooked ham, with corresponding change in**
2 **sensorial properties measured by sensory evaluation and a multimodal *machine vision***
3 ***system.***

4

5 Kirsti Greiff^{ab}, John Reidar Mathiassen^a, Ekrem Misimi^a, Margrethe Hersleth^c og Ida G.
6 Aursand^a.

7

8 ^a *SINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway*

9 ^b *Department of Biotechnology, Norwegian University of Science and Technology, Sem*

10 *Sælandsv. 6/8, N-7491, Trondheim, Norway*

11 ^c *Nofima AS, Oslovegen 1, 1430 Ås, Norway*

12

13 Kirsti Greiff, SINTEF Fisheries and Aquaculture, Brattørkaia 17C, 7465 Trondheim, Norway

14 Tel: +47 47 90 38 91, Fax: +47 93 27 07 01, E-mail: kirsti.greiff@sintef.no

15

16

17

18

19

20

21 END PAGE 1

22 **ABSTRACT**

23 The European diet today generally contains too much sodium (Na^+). A partial substitution of
24 NaCl by KCl has shown to be a promising method for reducing sodium content. The aim of
25 this work was to investigate the sensorial changes of cooked ham with reduced sodium
26 content. Traditional sensorial evaluation and objective multimodal *machine vision* were used.

27 The salt content in the hams was decreased from 3.4% to 1.38%, and 25% of the Na^+
28 was replaced by K^+ . The salt reduction had highest influence on the sensory attributes salty
29 taste, after taste, tenderness, hardness and color hue. The multimodal machine vision system
30 showed changes in lightness, as a function of reduced salt content. Compared to the reference
31 ham (3.4% salt), a replacement of Na^+ -ions by K^+ -ions of 25% gave no significant changes in
32 WHC, moisture, pH, expressed moisture, the sensory profile attributes or the surface lightness
33 and shininess. A further reduction of salt down to 1.7-1.38% salt, led to a decrease in WHC
34 and an increase in expressible moisture.

35

36 **Keywords:** Ham, sodium reduction, quality, sensory evaluation, machine vision

37 **END PAGE 2**

38 **Introduction**

39 A high consumption of sodium has been directly associated with a greater likelihood of
40 increased blood pressure, which in turn has been directly related to the development of
41 cardiovascular and renal diseases [1]. For these reasons, national and international bodies
42 have set targets for a reduction in sodium consumption down to 2 g/day [2-4].

43 Due to the low fat content, cooked ham is a good source of animal protein in the diet.
44 Usually when manufacturing cooked ham, the pork meat pieces are tumbled with brine
45 (containing water, salt, nitrite and other ingredients such as phosphate and polysaccharides).
46 During the tumbling with brine, the salt soluble myofibrillar proteins are extracted and they
47 form a network. The network gels during the heating and chilling process [5]. Reducing salt
48 contents in cooked ham increases cooking loss [6] and thereby the production costs.

49 Sodium chloride is added to a wide variety of foods to increase shelf life and
50 palatability (e.g. soups, meat products, bread, sauces and snacks). When it comes to sensory
51 quality, sodium chloride contributes to saltiness and the overall flavor and suppresses
52 bitterness [7]. Accordingly, the development of palatable sodium-reduced products is
53 important in order to guide consumers towards more healthy food choices. Partial substitution
54 of NaCl by KCl has shown to be one of the best alternatives for reducing sodium content [5,
55 8]. Studies have shown that KCl induces a bitter taste at high concentrations, in dry-cured
56 loins [9], fermented sausage[10] and cooked ham [11]. Those results show that the maximum
57 level of KCl replacement may vary among different type of product.

58 It is essential to characterize and understand the sensory effects of sodium reduction in
59 foods. Sensory analysis is the most appropriate approach to fully describe the sensory
60 perception of foods and descriptive profiling has been a popular sensory technique for
61 cognitive descriptions of products in many years. These techniques give a complete sensory
62 description of products and make it possible to identify underlying ingredient and process

63 variables [12]. For measurement of flavor no instruments which exist today can replace the
64 human senses and describe the sensory perception. For appearance and texture attributes, the
65 situation is different, as there are several examples of studies where instrumental data
66 representing appearance and texture attributes, show a strong correlation with data from
67 sensory trained panels [13-16].

68 In particular, classification of ham qualities (pork and turkey) is possible based on
69 color and textural features extracted from digital color images. Logistic regression, used on
70 these features, could to a large degree explain consumers' responses for visually-based
71 sensorial attributes [13]. The qualities of pork ham could be distinguished based on visual
72 texture characteristics extracted from fractal analysis of digital color images [16]. This
73 showed that the fractal-based features are able to quantify quality-specific visual texture
74 characteristics. A combination of geometric and texture features, extracted from digital color
75 images, were input to multivariate classifiers, including artificial neural networks (ANNs),
76 and were capable of predicting meat tenderness – as measured by shear force [17]. The eating
77 quality of beef could be predicted from wavelet surface texture and other texture features
78 extracted from digital color images. For the optical setup, a polarizer filter was placed on the
79 lens and the meat pieces were surface-dried to mitigate some specular reflection [14]. Other
80 machine vision approaches have been designed specifically to improve the objective analysis
81 of color and texture in translucent or semi-transparent muscle foods [18] These works and
82 others [15, 19] show that computer vision, with optimal illumination in combination with
83 appropriate feature extraction and classifiers, can be used to predict sensorial properties from
84 digital color images.

85 The effect of reduced salt/sodium content on cooked ham has previously been
86 investigated by different authors [5, 20, 21], but nobody, as far as we know, have used the
87 combination of sensorial Descriptive Analysis (DA) and multimodal *machine vision system* to

88 investigate the changes in the sensorial properties in cooked ham with effect of reduced
89 salt/sodium content. In this paper, we apply a novel dual-polarization multimodal machine
90 vision system that, to our knowledge, is new to meat imaging and imaging of other muscle
91 foods such as fish [22].

92 The aim of this study was to investigate sensory quality of cooked pork ham
93 containing gradually reduced salt/sodium content and a partial replacement of sodium (Na^+)
94 by (K^+). The product quality parameters addressed were physicochemical and sensory
95 properties obtained by sensory descriptive analyses and a multimodal *machine vision system*.

96

97 **Materials and Methods**

98 **Samples and Chemicals**

99 **Cooked ham preparation.** The preparation of ham Eight different formulas of cooked hams
100 were manufactured by Espeland AS, Ålgård, Norway 26-28 February 2013. Each of the
101 formulas contained 20 kg of fresh coarsely grounded (perforated disc with hole size 30 mm),
102 lean pork meat (*M. semimembranosus*, *M. adductor*, *M. semitendinosus*, *M. biceps femoris*
103 *and M. psoas major*), obtained from a local meat processing company (PrimaGruppen,
104 Norway) four days after slaughtering. The brine solution for each formulation was prepared
105 by dissolving 200 g commercial phosphate mixture (57% P_2O_5)(A. B. Corneliussen AS,
106 Norway), 150 g gelatin (Gelita Sweden AB, Sweden), 60 g carrageenan (CEAMSA,
107 Pontverda, Spain), 450 g Na-lactate (Purac Biochem, Gorichem, The Netherlands) and 150 g
108 maltodextrin (Avebe U.A., The Netherlands) in 2.5 L tap water ($t = 2-4$ °C). All sample
109 except from the reference ham (Na 100) were added 3.3 g sodium nitrite (Merck, KGaA
110 Darmstadt, Germany), were the commercial food salt containing nitrite salt (Hoff Norske
111 Potetindustrier, Gjøvik, Norway) was added to the reference ham (Na100). Sodium chloride
112 (Hoff Norske Potetindustrier, Gjøvik, Norway) and potassium chloride (Culinar Lyckeby,

113 Sweden) were added to the brine to achieve the expected Na^+ and K^+ content as shown in
114 **Table 1**. The mole ratio of sodium (Na^+) and potassium (K^+) was kept constant ($\text{Na}^+ : \text{K}^+ = 3:1$)
115 in all formulas containing potassium (NaK), where the contribution of all sodium and
116 potassium in all ingredients were taken into account. The salt (NaCl) content (g/100g) was
117 calculated as: added Na^+ (including natural sodium content in the meat) $\times 2.5$ (recalculation
118 factor for NaCl from Na^+), according to the provision of food information to consumers [23].
119 The reference cooked ham did not contain potassium, only sodium (Na 100), and the amount
120 of sodium in the formulation was at the same level as in a commercially cooked ham, 1.24 g
121 $\text{Na}^+/\text{100g}$, corresponding to 3.1% salt. The amount of total salt content in the formulation was
122 gradually decreased to 20% of the amount of the salt in the reference cooked ham,
123 corresponding to 1.3% salt (NaK20). The brine was added to the pork meat in a tumbler
124 (Fatosá, Barcelona, Spain) without vacuum and massaged for 2 \times 25 min, stored 20 h at 2°C,
125 and then tumbled for 15 more min. The meat matrix was stuffed into a plastic casing (160
126 mm in diameter, Viscofan S. A., Navarra, Spain). Two parallel production batches of the
127 hams with formulation NaK 100 and NaK 60 were prepared to control the reproducibility of
128 the production. Seven hams (3.0 kg) from each formulation were produced. The hams were
129 pressed in molds and kept at 0-2°C for 20 h before cooking. The hams were cooked in a
130 cooking chamber (Fessmann, Germany) until they reached a core temperature of 78 °C,
131 chilled down in tap water and kept in a cold-storage chamber at 0-2°C. Two of the hams per
132 formula were sliced in 20 slices (16 ± 1 g) 12 days after production, and alternate of the slices
133 were subjected to sensorial evaluation and analysis of machine vision, respectively. Sensorial
134 evaluation and machine vision analyses were carried out directly after slicing. Two of the
135 hams per formula were sliced and packed in modified atmosphere (30% CO_2 and 70% N_2)
136 and stored at 4°C for bacterial analyzes. The remaining cooked hams were stored at 4°C for

137 15 days after production before chemical analysis. In addition, one slice (approximately 100
138 g) was used for further analysis.

139

140 Replicated formulas of cooked ham were manufactured for Na100, NaK80 and NaK60,
141 respectively, for the supplementary image acquisition.

142

143 **Chemicals.** Ammonium chloride (NH_4Cl), ammonium hydroxide (NH_4OH) and ammonium
144 hydrogen fluoride ($\text{NH}_4\text{FHF} < 1\%$, LD_{50} mg/kg not found) of analytical-reagent grade
145 (Thermo Fisher Scientific, USA). Chemicals of food grade: sodium tripoly phosphate
146 ($\text{Na}_5\text{P}_3\text{O}_{10}$) (A. B. Corneliussen AS, Norway), Na-lactate ($\text{C}_3\text{H}_5\text{NaO}_3$) (Purac Biochem,
147 Gorichem, The Netherlands) and sodium nitrite (NNaO_2)(Merck, KGaA Darmstadt,
148 Germany).

149

150 **Imaging Setup and Image Acquisition.**

151 The imaging system consisted of a ColorRanger multimodal line-scan camera (SICK IVP
152 AB, Linköping, Sweden), two high-intensity white LED linear array lights (Banner
153 Engineering, Minneapolis, MN, USA), with polarizers on the LED arrays and in front of the
154 camera lens. Additionally, a nematic liquid crystal industrial-grade polarization rotator
155 (ARCOptix SA, Neuchâtel, Switzerland) was placed in front of the polarizer on the camera
156 lens. The polarization rotator is controlled by the image acquisition PC and enables
157 effectively to rotate the polarizer on the lens by means of an electric signal. The purpose of
158 rotating the polarizer direction on the lens polarizer relative to the polarizer on the LEDs, was
159 to be able to capture light reflected from the object for two polarization states.

160 The experiment sought to separately image the subsurface color and the surface color
161 of the ham slices, in order to separately image the light that was scattered in the subsurface

162 from the light that was directly reflected off the surface. Subsurface imaging revealed the bulk
163 color of the ham near the surface, whereas surface imaging revealed the surface roughness,
164 shininess and other effects such as "mother-of-pearl" appearance commonly seen in some
165 hams. To separate the subsurface from the surface image, two images – I_{\parallel} LED camera
166 polarizers oriented parallel to each other were acquired, and I_{\perp} with the polarizers oriented
167 perpendicular to each other. Image I_{\parallel} will image both the subsurface and the surface
168 components, whereas I_{\perp} will image the subsurface components only, and hence the difference
169 $I_{\parallel} - I_{\perp}$ between the two images will image only the surface components of the light. This
170 principle of light interactions as a function of polarization state is illustrated in images in **Fig.**
171 **1.**

172 The extracted imaging features were simply the mean of the red, green and blue (r, g, b)
173 values, as acquired with the ColorRanger, over the entire ham slice and for both polarizer
174 orientations. Thus, for each ham slice the $(r_{\parallel}, g_{\parallel}, b_{\parallel})$ and $(r_{\perp}, g_{\perp}, b_{\perp})$ values were obtained. The
175 sum of $(r_{\perp}, g_{\perp}, b_{\perp})$ indicated the lightness of the sample and an increase either in r_{\perp}, g_{\perp} or b_{\perp} ,
176 indicated increased lightness. An average of nine slices per formulation of cooked hams, were
177 used in the image acquisition experiment.

178 **Supplementary Image Acquisition.** Image acquisition of the hams used for the
179 sensory evaluation was done with a polycarbonate window in the imaging setup, which
180 unfortunately affected the polarization of the imaged light. This resulted in suboptimal
181 images. Therefore, only the blue channel images (b_{\parallel} and b_{\perp}) were used for analysis and a
182 supplementary image acquisition was done. Here, the polycarbonate window was replaced
183 with an optical grade AR-coated BK7 glass window. Replicated formulas of cooked ham
184 were manufactured for Na100, NaK80 and NaK60, respectively, which were used *for*
185 *validation of the multimodal imaging acquisition system.* The formulation, production
186 process, and storage were similar to the cooked ham preparation as described above. An

187 example set of images is seen in **Fig. 2**. The images in **Fig. 2** are included in order to provide
188 the reader with a visual understanding of the possibilities of using multi-modal imaging of
189 cooked ham with dual polarization states.

190

191 **Chemical analysis**

192 **Water activity** (a_w) was determined in cooked hams with a fast water activity-meter (GBX
193 FAsT/lab, Romans sur Isère Cedex, France). The **pH** measurements on light and dark muscle,
194 in brine and cooked ham were carried out using a digital pH-meter WTW pH3110 (Weilheim,
195 Germany) with a puncture electrode (WTW A 120513078, Weilheim, Germany). The
196 moisture content was determined by drying three parallel samples of 5 g of minced cooked
197 ham at 105 °C for 24 hours [24]. **Water holding capacity (WHC)** was determined on minced
198 cooked ham by low-speed centrifugation as described by Eide, Børresen [25] with a
199 centrifugation force of 210 g. The WHC is expressed as the percentage of water retained in
200 the mince after centrifugation for 5 min. The analyses were run in quadruplicate. Analyses
201 **sodium** contents in cooked hams were measured in an extract of the sample, using a Dual
202 StarTM pH/ISE Meter (Thermo Fisher Scientific, Waltham, MA, USA) with a Na-selective
203 electrode (Ross® Sodium Ion Selective Electrode, Thermo Fisher Scientific, Waltham, MA
204 USA). For preparing the extract, 7.5g of ham was homogenized in ultra-pure water using an
205 Ultra-turrax T-25 D (IKA, Labortechnik, Staufen, Germany) at 9000 rpm for 1 min. Then,
206 samples were warmed up to 90°C for 30 min, cooled down to room temperature, transferred
207 to a volumetric flask and diluted to 200 mL with ultra-pure water. Finally, samples were
208 filtered through a cellulose filter paper (Whatman n° 1, Whatman International Ltd.,
209 Maidstone, UK). The Na-selective electrode method was a modification of the Kivikari [26]
210 method. In this study the direct calibration method was used, whereas Kivikari, used the
211 known addition method. A calibration curve was made by using three standards of analytical-

212 grade NaCl from (Merck KGaA, Darmstadt, Germany) and Sodium ionic strength adjustor
213 (Thermo Fisher Scientific, Waltham, MA, USA) was added to all solutions to ensure that
214 samples and standards had similar ionic strength.

215 The modified method of Grau and Hamm [27] was used to measure **expressible**
216 **moisture (EM)** for cooked hams. Samples were punched out with a hollow drill (25 mm in
217 diameter) from cooked slices of hams (15 mm thick). Each sample was placed in the middle
218 of ten filter papers (Whatman No. 1) and pressed down with a flat-ended cylindrical plunger
219 (80 mm diameter), by single compression test mode and a test speed of 0.8 mm/s until 50%
220 compression of total height, using a Texture Analyzer T.A.XT2 (Stable Micro System,
221 Surrey, U.K.). Expressible moisture was determined as the amount of water released per gram
222 of meat and was expressed in percentage.

223

224 **Microbial analysis**

225 Two hams per formula were sliced and packed in modified atmosphere (30% CO₂ and
226 70% N₂) and stored at 4°C. The total viable bacterial count (TVC) was analyzed 6, 35, 42, 49
227 and 56 days after production. Ten grams of cooked ham were sampled from a new package
228 for each salt level, diluted (100-10 000 times) with 0.1% pepton water (Merk 1.07214.100).
229 The samples were pour plated in Petrifim Aerobic Count Plates TF6400 (3M™ Petrifilm™)
230 and incubated for 30°C for 48 h. The average numbers of TVC were expressed as colony
231 forming units per gram (cfu/g).

232

233 **Sensory evaluation**

234 Descriptive Analysis (DA) was conducted by a trained sensory panel according to
235 Generic Descriptive Analysis as described by [12]. All assessors (n=9) were selected and
236 trained in accordance with ISO 8586-1 (ISO, 1993) and the test was done in a sensory

237 laboratory designed in accordance with ISO 8589 (ISO, 2007). Each assessor evaluated all
238 samples using EyeQuestion for direct recording of data (v3.8.7, Logic8, Elst(Gld), The
239 Netherlands). A list of 21 attributes was generated from a brainstorming session with the
240 assessors. This list included attributes representing appearance (color-hue, color intensity,
241 whiteness, color evenness, shiny, marbling, cohesiveness, visible moist on surface), odor
242 (sour, pork meat, metal), taste/ flavor (sourness, sweetness, saltiness, bitterness, pork meat,
243 metal, after flavor) and texture (hardness, juiciness, tenderness). Attributes were evaluated
244 using a continuous, non-structured scale ranging from no intensity (1) on the left to high
245 intensity (9) on the right. The assessors had previous experience with analyses of meat
246 products including ham and were calibrated on selected attributes in a pre-test. DA was
247 performed during 6 sessions on one day, in which 8 different hams were served in two
248 replicates (totally 16 samples). The hams were sliced by a machine to a weight of 16 ± 1 g,
249 and each assessor got one slice served per sample. Appearance attributes were evaluated on
250 the surface of the ham slice, while the texture was evaluated biting over a rolled slice. The
251 serving order was randomized across all sessions. All samples were expectorated and unsalted
252 crackers and lukewarm water was available for rinsing.

253

254 **Data analysis**

255 The influence of the different levels of sodium on physicochemical properties was
256 studied through one-way analysis of variance (ANOVA), (Minitab 16, Minitab Inc.) In cases
257 there the effect was defined as significant ($p < 0.05$), the means were compared using Tukeys
258 test to find the significant difference between the samples containing different amounts of
259 salt.

260 PanelCheck 1.3.2 (www.panelcheck.com) was used to evaluate the panel performance
261 in the pre-test. For determination of sensory attributes discriminating between samples, a two-

262 way ANOVA with product as a fixed factor, panelist as a random factor and product x
263 panelist as an interaction factor was performed. Tukey's Multiple Comparisons Test was
264 applied to determine which products that was significantly different. The significance level
265 was defined to $p < 0.05$. (The ANOVA was run by SAS 9.2, SAS Institute, Inc., Cary, NS,
266 USA).

267

268 **Results and Discussion**

269 **Chemical analysis**

270 The results of the chemical analyses of cooked ham at different salt/sodium levels are
271 summarized in **Table 2**. The salt contents (%) in the cooked ham were calculated as measured
272 Na^+ -content multiplied with 2.5. The measured sodium content in the reference ham (Na 100
273 added as Na^+ - ions only) was 1.35 g Na^+ /100 g, this result was slightly higher than estimated
274 in the formulation of the sample, 1.24 g Na^+ /100g. The rest of the hams, were prepared with
275 different levels of total salt including a constant $\text{Na}^+:\text{K}^+$ mol ratio of 3:1, and the measured
276 sodium content were in good agreement with the estimated in the formulation.

277 As expected, a decrease in WHC was observed in hams were the salt content was
278 reduced by 60% (NaK40) and 80% (NaK20) (on molar basis), corresponding to a measured
279 salt content of 1.73 and 1.38% salt, respectively. These results are in accordance with Hamm
280 (1972) and Offer & Knight (1988) who found that sodium chloride increases the water-
281 binding of meat [28, 29]. Albarracín, Sánchez [30] explained the increasing in WHC in meat
282 by increasing salt content where the anions (Cl^-) preferentially bind to protein molecules. The
283 moisture content in hams added a mixture of sodium and potassium salt without reduction of
284 the total salt, 2.70% salt (NaK100) was significant lower than in cooked ham containing
285 1.38% salt (NaK20).

286 The pH in the raw material was within the range of 5.5- 5.8. These pH values are
287 similar to typical ultimate pH values in Norwegian pork [31]. The pH of the hams increased
288 from pH 6.15 ± 0.02 to 6.25 ± 0.01 with decreasing sodium content. The pH in hams with the
289 lowest levels of sodium, 1.73% salt (NaK40) and 1.38% salt (NaK20), were significant higher
290 than the reference containing 3.38% salt (Na100) and the cooked ham with the mixture of
291 sodium and potassium salt (NaK100). These findings are in accordance with Puolanne,
292 Ruusunen [32], who found an average decrease, about 0.1 pH-units/%-units of salt, in cooked
293 sausages.

294 The expressible moisture increased with decreasing salt/sodium content, and those
295 hams containing 1.38% salt (NaK20) had significantly higher expressible moisture than those
296 containing 3.38% salt (Na 100).

297 The a_w decreased slightly from 0.97 to 0.96 with increasing salt content, although the
298 differences were not significant (data not shown). According to Mossel, Corry [33], such
299 levels of a_w in cooked hams were too high to represent a hurdle for unwanted spoilage- and
300 pathogenic microorganisms.

301 Compared to the reference ham (Na100), a replacement of Na^+ -ions by 25% of K^+ -
302 ions gave no significant changes in WHC, moisture, pH or expressible moisture. This
303 replacement results in a total sodium content of approximately 1.1 g /100g cooked ham. This
304 finding is in accordance with Zanardi, Ghidini [34] who found no effects on pH and water
305 activity in reduced sodium when replacing (Na^+) by a mixture of KCl, CaCl_2 and MgCl_2 in
306 Cacciatore salami compared to the traditional recipe.

307 **Microbial stability**

308 The total aerobic microbial count is a common indicator of shelf-life. The total aerobic
309 counts were relatively low during the entire storage period and varied between 1-3 log cfu/g.

310 There were no significant changes in total aerobic microbial counts presumably due to the
311 addition of salt or storage time in our cooked hams (data not shown).

312 **Descriptive sensory profile**

313 The sensory profile of the eight cooked hams for all the twenty one different attributes
314 is presented in **Fig. 3. Table 3** list the mean value of the most important attributes together
315 with corresponding *p* values.

316 Compared to the reference ham (Na100), a replacement of Na⁺ ions by K⁺ ions of 25%
317 and reduction to 60% (NaK40) of total salt had no effect on the color hue. However, a
318 reduction of total salt content by 80% and replacement of Na⁺ ions by K⁺ ions of 25%
319 (NaK20) showed a significant lower color hue. There were significant differences between
320 samples for cohesiveness, but there were no clear correlation between the salt content and the
321 cohesiveness score. A possible explanation might be the test production. In small scale test
322 production without vacuum, it can be difficult to avoid air and small gaps between the muscle
323 pieces.

324 To summarize, a reduction to 40% (NaK60) of total salt, corresponding to 2.04% salt,
325 was possible without significantly influencing the salty taste. The changes in saltiness in this
326 experiment correspond to another low-salt ham study, Ruusunen, Särkkä-Tirkkonen [5] found
327 that hams with 1.4% salt were less salty than those containing 1.7 - 2.6% salt. The reduced
328 saltiness can be explained by the reduced content of Cl⁻ anions in the cooked ham since Cl⁻
329 anions have an effect on the receptor cells and consequently on the perception of salt taste
330 [35]. There were no significant differences in metal flavor and metal odor except for a higher
331 value for metal flavor for one of the parallels of cooked ham containing the highest amount of
332 potassium chloride (NaK100_1) compared to the cooked ham containing the lowest salt
333 content (NaK20). These results indicate that a three to one replacement of Na⁺ ions with K⁺
334 ions might be an acceptable level, regarding the issue of bitter (no significant differences

335 between samples) and metallic flavor (NaK20 significant different from the other samples).
336 Previous studies have shown that KCl may induce a bitter taste at high concentrations. In dry-
337 cured loins [9] and fermented sausages [10], the maximum level was 40% replacement of
338 sodium with potassium. In a study of cooked ham they showed that 2.0% NaCl had a higher
339 score than a 50% replacement with KCl [11]. Another study found that it was possible to
340 replace one-third of NaCl with KCl without altering the sensory properties of smoked salmon
341 [36]. Those results show that the maximum level of KCl replacement may be dependent on
342 product type.

343 **Table 3** shows that a reduction to 60% (NaK40) of total salt had no effect on the after
344 taste. However, a reduction of total salt content by 80% and replacement of Na⁺ ions by K⁺
345 ions by 25% (NaK20) gave a less pronounced aftertaste compared to all other hams. This can
346 be explained by the fact that salt has a flavor enhancing effect in meat products [37], and the
347 salt content can also effect on biochemical and enzymatic processes that in turn may impact
348 flavor and/or structure of a product [30]. These findings are in accordance with Ruusunen,
349 Vainionpää [38], who found that ground meat patties with salt content down to 0.6% NaCl,
350 had weak flavor intensity.

351 Compared to the reference ham (Na100), a reduction down to 60% (NaK40) of total
352 salt, corresponding to 1.73% salt, had no effect on tenderness or hardness in the cooked hams.
353 However, a reduction of total salt content by 80% and replacement of Na⁺ ions by K⁺ ions of
354 ¼ (NaK20), corresponding to a salt content of 1.38%, gave a more tender and less hardness
355 on cooked ham. These results were as expected and in accordance with Ruusunen, Vainionpää
356 [39], who found that added salt increased the firmness in Bologna type sausages added 1.10,
357 1.35 to 1.60% salt, respectively and Sofos [40] found that a reduction in salt content of more
358 than 20% (<2.0% NaCl) in Frankfurters resulted in softer and less firm texture. It is important
359 to keep in mind that the effects of proteins may differ in cooked ham where small pieces of

360 lean meat are connected together whereas sausages have it an emulsified fat protein network.
361 In both cases the solubility and swelling of the myosin are nevertheless important. In cooked
362 ham, molecular bonds are able to form inside the exudate matrix (gel cohesion) and between
363 the exudate and the muscle [20], and when the salt concentration is reduced, the protein
364 extractability is limited due to the solubility of the myosin [28, 29].

365 Compared to the reference ham (Na100), a replacement of Na⁺-ions by K⁺-ions of
366 25% gave no significant changes in sensory profile attributes. This replacement corresponds
367 to a total sodium content of approximately 1.1 g /100 g cooked ham. These findings are in
368 accordance with other studies [9-11, 36].

369 **Multimodal machine vision system**

370 The main imaging experiment, using the multimodal machine vision system, was done using
371 the same hams as with the chemical and sensorial measurements

372 Machine vision was used to objectively study changes in color and texture in the
373 surface. The change in lightness, as a function of reducing the salt content, is clearly seen in
374 **Fig. 4a.**

375 The intensity of the subsurface backscattered reflected blue light b_{\perp} shows a clear increase as
376 the salt content is reduced. This is confirmed by sensorial evaluation, indicating an increase in
377 whiteness and decrease in color-hue with decreased salt content. A possible explanation for
378 this behavior might be the decreased swelling of the salt soluble proteins and a weaker heat
379 induced gel in cooked ham with 1.38% salt (NaK 20) as discussed in above in section 3.3.

380 Due to this theory, the surface of the cooked ham with less salt might be rougher, and the
381 interaction of light will change, as has been shown previously [13]. Another explanation for
382 the increased whiteness due to decreased salt content may be that salt affects the formation of
383 heat stable nitric oxide myoglobin, the "cured meat color" [41].

384 A reduction in surface shininess, as measured by $b_{\parallel}-b_{\perp}$, using imaging, was only
385 weakly linked to reduction in salt content, **Fig. 4b**. Despite the challenges in the imaging
386 setup in the main experiment, the improvements from the supplementary experiment in **Fig. 2**
387 show that imaging with two polarization orientations can provide images that potentially can
388 quantify subtle changes in visual appearance.

389 Since we used only simple features, namely the $(r_{\parallel},g_{\parallel},b_{\parallel})$ and $(r_{\perp},g_{\perp},b_{\perp})$ values
390 computed as the mean over each slice, we see greater potential in dual-polarization imaging if
391 more advanced features such as those described in the literature [13-15, 17, 19] would be
392 applied to dual-polarization images using the multimodal machine vision system described in
393 this paper. We would also suggest that automatic segmentation between the dark and light
394 portions of the mean would help improve the quantification of the visual appearance
395 characteristics in a way that human sensorial evaluators do with their combined vision and
396 decision process.

397

398 **Conclusions**

399 Compared to the reference ham (3.4% salt), a replacement of 25% Na⁺-ions by K⁺-ions gave
400 no significant changes in WHC, moisture, pH, expressed moisture, the sensory profile
401 attributes or the surface lightness and shininess. However, a reduction of salt content by 60%
402 and 80% on molar basis or more (down to 1.7-1.4% salt) led to a decrease in WHC and an
403 increase in expressible moisture. The salt reduction had highest influence on the sensory
404 attributes salty taste, after taste, tenderness, hardness and color hue. To summarize, a
405 reduction to 40% of total salt, corresponding to 2.04% salt, was possible without significantly
406 influencing the salty taste. Further reduction down to 1.4% salt led to increased softness and
407 reduced harness in the cooked hams. When reduction of salt by more than 60% (down to

408 1.7% salt) together with a replacement of 25% Na⁺-ions by K⁺-ions, the low-salt ham
409 manufacture procedure has to be modified. The multimodal machine vision system showed
410 changes in lightness, as a function of reduced salt content.

411

412 **Acknowledgments**

413 The work is financed by the SALTO project no. 210431/O10 and Low salt project no.
414 185063/O10, supported by the Norwegian industry and the Research Council of Norway. The
415 authors thank Marte Schei and Anne Blikra for their contribution in production and analyzing
416 of the hams, MSc Aleksander Eilertsen and research scientist Morten Bondø for their
417 assistance in setting up the electronics and programming of the machine vision system.

418

419 **References**

- 420 1. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of
421 randomized trials. Implications for public health. *J Hum Hypertens*. 2002;16(11):761-70.
- 422 2. WHO. Reducing salt intake in populations. Report of a WHO Forum and Technical Meeting. World
423 Health Organization, Paris, 2006.
- 424 3. The Norwegian Directorate of Health. Strategy for the reduction of salt intake in the population.
425 Recommendation from the National Nutrition Council. <http://helsedirektoratet.no/folkehelse/ertering/strategier-og-satsninger/Documents/saltstrategi.pdf>; The Norwegian Directorate of Health; 2011.
- 427 4. FSAI. Food Safety Authority of Ireland, Salt and health: review of the scientific evidence and
428 recommendations for public policy in Ireland. 2005.
- 429 5. Ruusunen M, Särkkä-Tirkkonen M, Puolanne E. Saltiness of coarsely ground cooked ham with reduced
430 salt content. *Agricultural and food science in Finland*. 2001;10:27-31.
- 431 6. Pietrasik Z, Gaudette NJ. The impact of salt replacers and flavor enhancer on the processing
432 characteristics and consumer acceptance of restructured cooked hams. *Meat Sci*. 2014;96(3):1165-70.
- 433 7. Breslin PAS, Beauchamp GK. Suppression of Bitterness by Sodium: Variation Among Bitter Taste
434 Stimuli. *Chem Senses*. 1995;20(6):609-23.
- 435 8. Olson D. Salt for processing probably can be cut by only one quarter. *The National Provisioner*. 1982;7-
436 10.
- 437 9. Gou P, Guerrero L, Fau - Gelabert J, Gelabert J, Fau - Arnau J, Arnau J. Potassium chloride, potassium
438 lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. 1996(0309-
439 1740 (Print)).
- 440 10. Gelabert J, Gou P, Guerrero L, Arnau J. Effect of sodium chloride replacement on some characteristics
441 of fermented sausages. *Meat Sci*. 2003;65(2):833-9.
- 442 11. Frye CB, Hand LW, Calkins CR, Mandigo RW. Reduction or Replacement of Sodium Chloride in a
443 Tumbled Ham Product. *J Food Sci*. 1986;51(3):836-7.
- 444 12. Lawless HT, Heymann H. Sensory evaluation of food - principles and practices (2nd ed.). New York:
445 Springer; 2010.
- 446 13. Iqbal A, Valous NA, Mendoza F, Sun D-W, Allen P. Classification of pre-sliced pork and Turkey ham
447 qualities based on image colour and textural features and their relationships with consumer responses. *Meat Sci*.
448 2010;84(3):455-65.
- 449 14. Jackman P, Sun DW, Du CJ, Allen P. Prediction of beef eating qualities from colour, marbling and
450 wavelet surface texture features using homogenous carcass treatment. *Pattern Recognition*. 2009;42(5):751-63.
- 451 15. Jackman P, Sun DW, Allen P. Prediction of beef palatability from colour, marbling and surface texture
452 features of longissimus dorsi. *J Food Eng*. 2010;96(1):151-65.
- 453 16. Valous NA, Mendoza F, Sun D-W, Allen P. Texture appearance characterization of pre-sliced pork ham
454 images using fractal metrics: Fourier analysis dimension and lacunarity. *Food Res Int*. 2009;42(3):353-62.
- 455 17. Chandraratne MR, Samarasinghe S, Kulasiri D, Bickerstaffe R. Prediction of lamb tenderness using
456 image surface texture features. *J Food Eng*. 2006;77(3):492-9.
- 457 18. Alçiçek Z, Balaban MÖ. Development and application of “The Two Image” method for accurate object
458 recognition and color analysis. *J Food Eng*. 2012;111(1):46-51.

- 459 19. Mendoza F, Valous NA, Allen P, Kenny TA, Ward P, Sun D-W. Analysis and classification of
460 commercial ham slice images using directional fractal dimension features. *Meat Sci.* 2009;81(2):313-20.
- 461 20. Bombrun L, Gatellier P, Carlier M, Kondjoyan A. The effects of low salt concentrations on the
462 mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking.
463 *Meat Sci.* 2014;96(1):5-13.
- 464 21. Aaslyng MD, Vestergaard C, Koch AG. The effect of salt reduction on sensory quality and microbial
465 growth in hotdog sausages, bacon, ham and salami. *Meat Sci.* 2014;96(1):47-55.
- 466 22. Mathiassen JR, Misimi E, Bondø M, Veliyulin E, Østvik SO. Trends in application of imaging
467 technologies to inspection of fish and fish products. *Trends in Food Science & Technology.* 2011;22(6):257-75.
- 468 23. Regulation EU no 1169/2011, Regulation EU no 1169/2011 (25.Oct 2011).
- 469 24. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed.
470 Arlington, Virginia: AOAC; 1990.
- 471 25. Eide O, Børresen T, Strøm T. Minced Fish Production From Capelin (*Mallotus villosus*). A New
472 Method for Gutting, Skinning and Removal of Fat from Small Fatty Fish Species. *J Food Sci.* 1982;47(2):347-9.
- 473 26. Kivikari R. Analysis of sodium in meat products using an Na-selective electrode. (In Finnish). Proc. of
474 Meat Day Seminar 1996, Nr, 536. Proc. of Meat Day Seminar 1996. 1996.
- 475 27. Grau R, Hamm R. Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. *Die*
476 *Naturwissenschaften.* 1953;40(1):29-30.
- 477 28. Hamm R. *Kolloidchemie des Fleisches*: Parey, Berlin; 1972.
- 478 29. Offer G, Knight P. The structural basis of water-holding in meat. R.A. Lawrie DimS-, editor. *Capters 3-*
479 *4*: London: Elsevier Applied Science; 1988.
- 480 30. Albarracín W, Sánchez IC, Grau R, Barat JM. Salt in food processing; usage and reduction: a review.
481 *Int J Food Sci Technol.* 2011;46(7):1329-36.
- 482 31. Gjerlaug-Enger E, Aas L, Ødegård J, Vangen O. Genetic parameters of meat quality traits in two pig
483 breeds measured by rapid methods. *Animal.* 2010;4(11):1832-43.
- 484 32. Puolanne EJ, Ruusunen MH, Vainionpää JI. Combined effects of NaCl and raw meat pH on water-
485 holding in cooked sausage with and without added phosphate. *Meat Sci.* 2001;58(1):1-7.
- 486 33. Mossel DAA, Corry JET, Struijk CB, Bair RM. *Essentials of the Microbiology of Foods. A Textbook*
487 *for Advanced Studies*: John Wiley & Sons, Chichester, England; 1995.
- 488 34. Zanardi E, Ghidini S, Conter M, Ianieri A. Mineral composition of Italian salami and effect of NaCl
489 partial replacement on compositional, physico-chemical and sensory parameters. *Meat Sci.* 2010;86(3):742-7.
- 490 35. Murphy C, Cardello AV, Brand JG. Tastes of fifteen halide salts following water and NaCl: Anion and
491 cation effects. *Physiology & Behavior.* 1981;26(6):1083-95.
- 492 36. Almli V, Hersleth M. Salt replacement and injection salting in smoked salmon evaluated from
493 descriptive and hedonic sensory perspectives. *Aquaculture International.* 2013;21(5):1091-108.
- 494 37. Desmond E. Reducing salt: A challenge for the meat industry. *Meat Sci.* 2006;74(1):188-96.
- 495 38. Ruusunen M, Vainionpää J, Lyly M, Lähteenmäki L, Niemistö M, Ahvenainen R, et al. Reducing the
496 sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. *Meat Sci.*
497 2005;69(1):53-60.

- 498 39. Ruusunen M, Vainionpää J, Puolanne E, Lyly M, Lähteenmäki L, Niemistö M, et al. Effect of sodium
499 citrate, carboxymethyl cellulose and carrageenan levels on quality characteristics of low-salt and low-fat bologna
500 type sausages. *Meat Sci.* 2003;64(4):371-81.
- 501 40. Sofos JN. Effects of Reduced Salt (NaCl) Levels on Sensory and Instrumental Evaluation of
502 Frankfurters. *J Food Sci.* 1983;48:1692-9.
- 503 41. Honikel K-O. The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.*
504 2008;78(1-2):68-76.
- 505

506 **Figure caption**

507

508 **Figure 1** Illustration of the components of light interacting with the imaged raw material,
509 imaged using parallel polarizers (left), crossed polarizers (middle) and the difference between
510 the two (right).

511 **Figure 2** Images of a slice of ham, from supplementary Image Acquisition using parallel
512 polarizers (left), crossed polarizers (middle) and the difference between the two (right).

513 **Figure 3** Descriptive sensory profile of the eight cooked hams prepared with different levels
514 of total salt.

515 **Figure 4a** Mean subsurface reflectance b_{\perp} for the hams with varying salt content.

516 **Figure 4b** Mean surface reflectance $b_{\parallel}-b_{\perp}$ for the hams with varying salt content.

517

518 Table 1. Sample ID, description of the sample, estimated sodium, potassium and NaCl content in the preparation of the eight cooked hams.
 519 Each formulation varied in its amount of total salt. The reference cooked ham contained nitrite salt (Na 100 Reference). In the rest of the
 520 formulas (NaK100, NaK80, NaK60, NaK40 and NaK20) the amount of total salt content was gradually decreased to 20% of the amount of
 521 the salt in the reference cooked ham. To keep the mole ratio of sodium (Na⁺) and potassium (K⁺) constant (Na⁺: K⁺ = 3:1), all contribution of
 522 sodium and potassium from the raw material and the ingredients was added when calculation of the mole ratio.

Sample ID	Description	Estimated sodium content (g/100 g ham)	Estimated potassium content (g/100 g ham)	NaCl** (%)	% reduction of sodium compared to the reference
Na 100	Reference ham	1.24	0.28	3.1	0
NaK 100_1*	100% of the amount of salt in the reference	1.06	0.61	2.6	14
NaK 100_2*	100% of the amount of salt in the reference	1.06	0.61	2.6	14
NaK 80	80% of the amount of salt in the reference	0.93	0.53	2.3	25
NaK 60_1*	60% of the amount of salt in the reference	0.80	0.46	2.0	35
NaK 60_2*	60% of the amount of salt in the reference	0.80	0.46	2.0	35
NaK 40	40 % of the amount of salt in the reference	0.67	0.38	1.7	46
NaK 20	20% of the amount of salt in the reference	0.53	0.31	1.3	57

523 * Produced twice to control the reproducibility of the production

524 ** The salt content (%) is calculated by using the formula: added sodium (Na⁺), including natural Na⁺ content in the meat x 2.5 (recalculation factor for NaCl from Na⁺)

525

526 Table 2. Water holding capacity (WHC), moisture, pH, sodium, salt and expressible moisture of cooked ham prepared with different levels
 527 of salt at constant Na⁺:K⁺ mole ratio 3:1. The reference cooked ham contained nitrite salt (Na 100 Reference). In the rest of the formulas
 528 (NaK100, NaK80, NaK60, NaK40 and NaK20) the amount of salt was gradually decreased to 20% of the amount of salt in the reference
 529 ham. Mean values \pm SD (n=3)

Group	WHC (%)	Moisture (%)	pH	Sodium (g/100g)	Salt (g/100g)	Expressible moisture (%)
Na 100	78.45 \pm 1.03 ^{abc}	74.58 \pm 0.23 ^{bc}	6.16 \pm 0.02 ^{ab}	1.35 \pm 0.17 ^a	3.38 \pm 0.43 ^a	0.96 \pm 0.30 ^{abc}
NaK 100_1	82.57 \pm 1.68 ^a	73.63 \pm 0.26 ^c	6.15 \pm 0.04 ^{ab}	1.08 \pm 0.01 ^b	2.70 \pm 0.03 ^b	0.86 \pm 0.16 ^{ab}
NaK 100_2	80.59 \pm 0.50 ^{ab}	73.67 \pm 0.15 ^c	6.14 \pm 0.04 ^a	1.03 \pm 0.04 ^{bc}	2.58 \pm 0.10 ^{bc}	0.84 \pm 0.16 ^a
NaK 80	81.55 \pm 1.13 ^{ab}	74.40 \pm 0.14 ^{bc}	6.19 \pm 0.02 ^{abcd}	0.92 \pm 0.01 ^{cd}	2.25 \pm 0.03 ^{cd}	1.07 \pm 0.23 ^{de}
NaK 60_1	80.83 \pm 0.16 ^{ab}	73.83 \pm 0.19 ^a	6.21 \pm 0.02 ^{bcde}	0.82 \pm 0.01 ^{de}	2.05 \pm 0.03 ^{de}	1.55 \pm 0.39 ^{de}
NaK 60_2	79.54 \pm 0.88 ^{abc}	75.14 \pm 0.18 ^{ab}	6.21 \pm 0.02 ^{bed}	0.81 \pm 0.01 ^{de}	2.03 \pm 0.03 ^{de}	1.12 \pm 0.17 ^{abcd}
NaK 40	76.31 \pm 0.25 ^{cde}	75.43 \pm 0.28 ^{ab}	6.24 \pm 0.02 ^{cdef}	0.69 \pm 0.01 ^{ef}	1.73 \pm 0.03 ^{ef}	1.49 \pm 0.23 ^{cde}
NaK 20	72.71 \pm 1.83 ^{de}	75.40 \pm 0.46 ^{ab}	6.25 \pm 0.01 ^{def}	0.55 \pm 0.01 ^f	1.38 \pm 0.03 ^f	1.96 \pm 0.35 ^e
p-value	0.000	0.000	0.000	0.000	0.000	0.000

530 ^{a-f} Different upper-case letters within a column indicate significant differences (p<0.005) between different groups. Means that do not share a common letter are
 531 significantly different.

532

533 Table 3. Sensory properties in the cooked hams

Group	Appearance				Taste and odour				Texture	
	Colour hue	Cohesiveness	Whiteness	Shiny	Salty taste	Metal flavour	Metal odour	After taste	Tenderness	Hardness
Na100	6.67 ^{abc}	6.97 ^{ab}	5.61 ^{ab}	3.71	6.18 ^{abcd}	4.74 ^{abc}	4.94 ^{abc}	5.92 ^{abc}	5.36 ^a	4.78 ^{ab}
NaK 100_1	6.84 ^{abc}	7.12 ^{ab}	5.59 ^{ab}	4.21	6.53 ^{ab}	5.12 ^a	5.19 ^a	6.21 ^{ab}	5.02 ^a	5.03 ^a
NaK 100_2	6.97 ^{ab}	7.42 ^a	5.49 ^{ab}	3.76	6.58 ^a	4.83 ^{abc}	4.91 ^{abc}	6.36 ^a	5.11 ^a	4.91 ^{ab}
NaK 80	6.97 ^{ab}	7.18 ^a	5.61 ^{ab}	4.09	6.18 ^{abcd}	4.92 ^{ab}	4.84 ^{abc}	6.14 ^{abc}	5.09 ^a	5.02 ^a
NaK 60_1	6.77 ^{abc}	6.94 ^{ab}	5.79 ^{ab}	3.32	5.55 ^{bcd}	4.76 ^{abc}	4.94 ^{abc}	5.52 ^{bc}	5.40 ^a	4.73 ^{ab}
NaK 60_2	6.49 ^{abcd}	7.27 ^a	5.72 ^{ab}	3.98	5.26 ^{de}	4.64 ^{abc}	5.08 ^{ab}	5.54 ^{bc}	5.88 ^{abc}	4.50 ^{abc}
NaK 40	6.77 ^{abc}	6.66 ^{abc}	5.91 ^{ab}	3.76	4.90 ^{ef}	4.34 ^{abc}	4.63 ^{abc}	5.73 ^{abc}	6.13 ^{abc}	4.06 ^{cd}
NaK 20	5.99 ^d	6.01 ^{bc}	6.20 ^a	3.36	4.06 ^f	4.03 ^{bc}	4.28 ^{bc}	4.73 ^d	6.83 ^{bc}	3.58 ^{de}
p-value	0.0010	0.0013	n.s.	n.s.	<0.0001	0.0065	0.0098	<0.0001	0.0001	<0.0001

534 ^{a-f} Different upper-case letters within a column indicate significant differences (p<0.005) between different groups. Means that do not share a common letter are

535 significantly different. Non-significant: n.s.

536

537

Fig.1

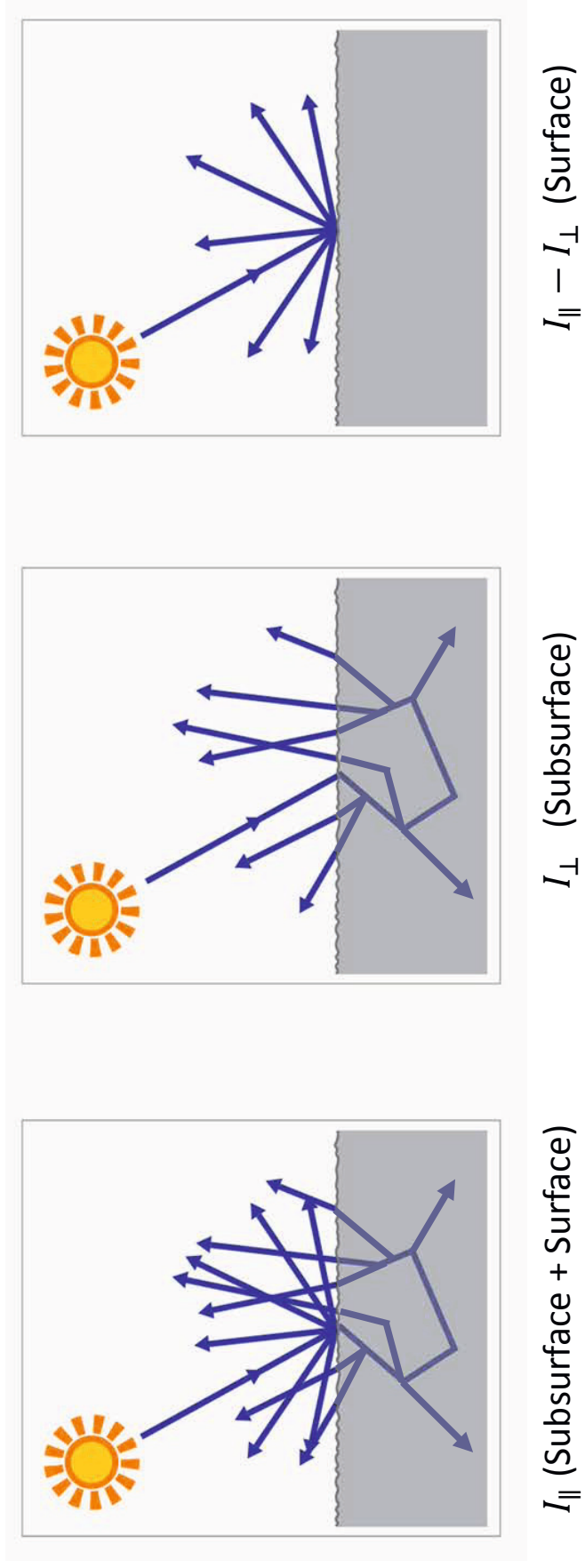


Fig. 2



$I_{||}$ (Subsurface + Surface)

I_{\perp} (Subsurface)

$I_{||} - I_{\perp}$ (Surface)

Fig. 4a

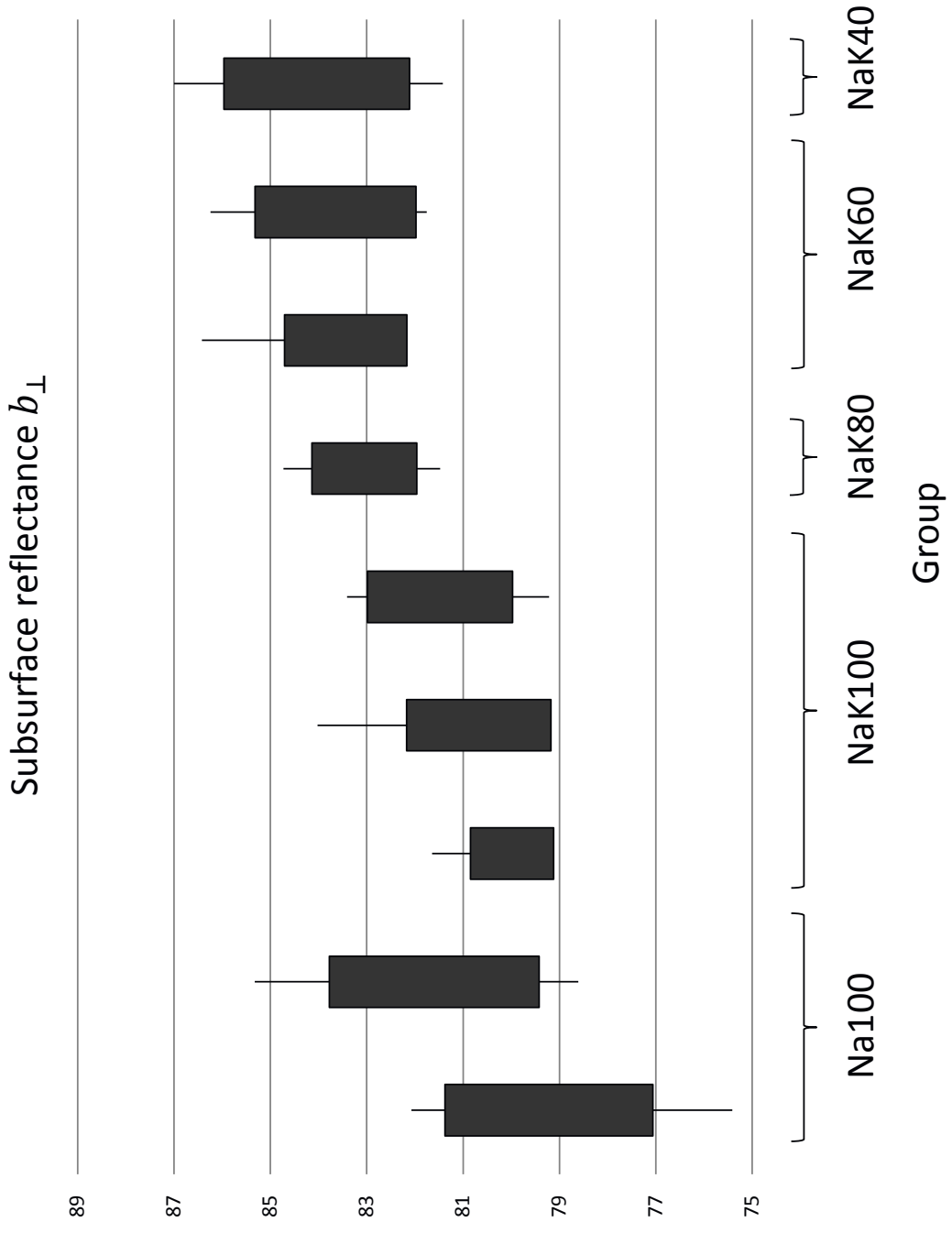
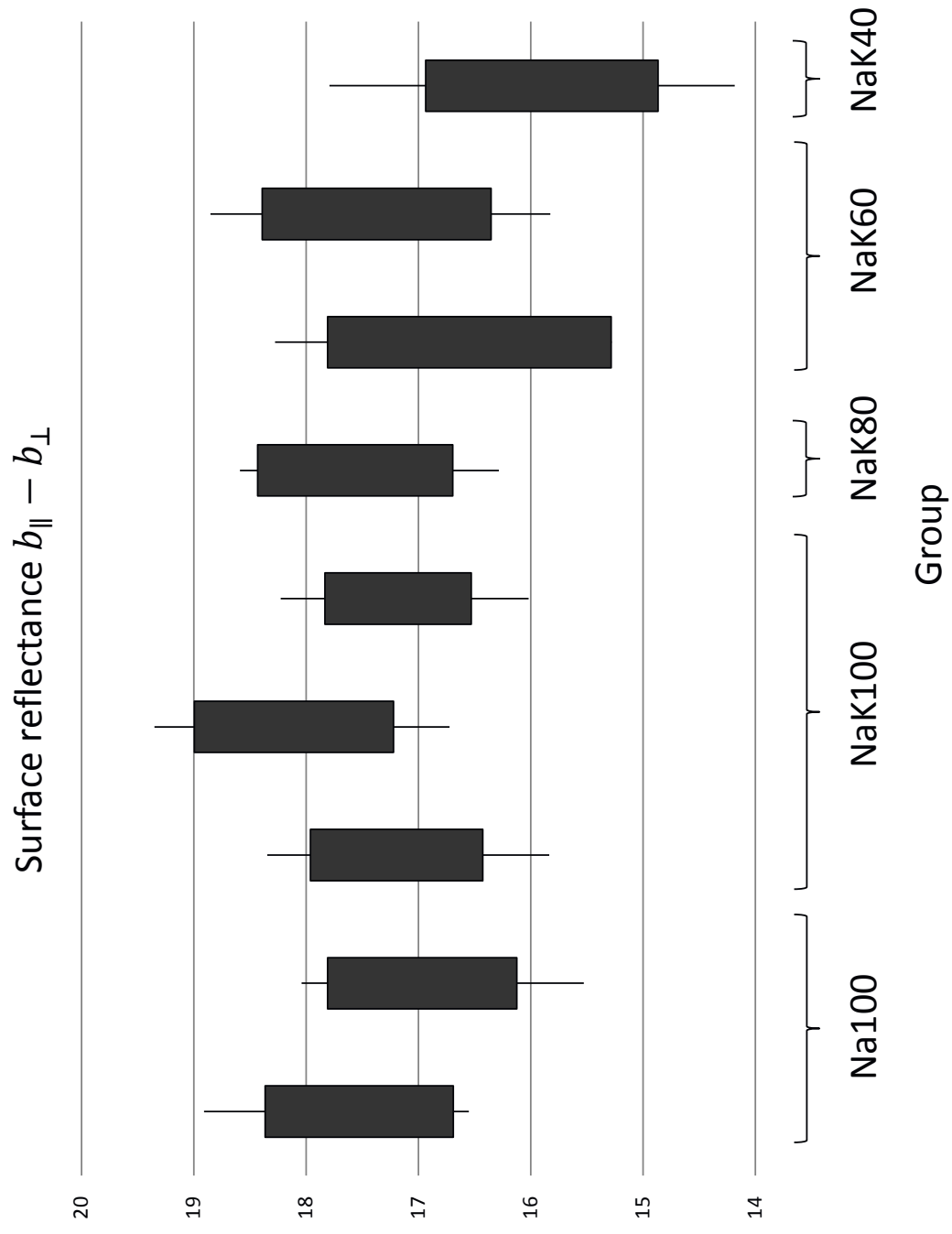


Fig. 4b



Paper III

1 **Sodium reduction in minced fish products by adding Magnesium and Potassium**
2 **Chloride; effect on physicochemical properties.**

3

4 Kirsti Greiff^{a, c}, Ida G. Aursand^a, Ulf Erikson^a, Kjell D. Josefsen^b and Turid Rustad^c

5

6

7 *^aSINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway*

8 *^bSINTEF Materials and Chemistry, R. Birkelandsvei 2B, N-7465 Trondheim, Norway*

9 *^cDepartment of Biotechnology, Norwegian University of Science and Technology, Sem*

10 *Sælandsv. 6/8, N-7491, Trondheim, Norway*

11

12 Running title header: Sodium reduction in minced fish.

13

14 *corresponding author:

15 Kirsti Greiff, SINTEF Fisheries and Aquaculture, Brattørkaia 17C, 7465 Trondheim, Norway

16 Tel: +4747903891, Fax: +47 93 27 07 01, E-mail: kirsti.greiff@sintef.no

17

18 **Abstract**

19 Cooked minces made from both fresh and frozen haddock with NaCl, KCl, or MgCl₂ at
20 concentrations from 0.07 – 0.34 mol/kg fish mince were prepared and analysed for
21 physicochemical properties. The properties of the minces varied both with the type and
22 amount of added salt. Mince added the same molar amount of NaCl or KCl had fairly similar
23 properties, but pH in minces made with KCl was slightly higher. Mince made with MgCl₂
24 had lower pH than the corresponding minces made with NaCl. Mince made from fresh raw
25 material with 0.07 mol/kg MgCl₂ had significantly lower WHC than the rest of the minces
26 made from fresh raw material, while minces made with 0.34 mol MgCl₂/kg had lower
27 moisture and breaking force than the corresponding minces with KCl or NaCl. When fresh
28 fillets were replaced by frozen, cooking loss increased. WHC for minces made with NaCl
29 decreased significantly, but WHC did not change much for minces made with KCl or MgCl₂.
30 Cooking loss decreased with increasing salt content both when fresh and frozen raw material
31 was employed, and the results indicate that this could be due to the increased ionic strength.

32 Key words: haddock; low sodium; potassium chloride; magnesium chloride; low field NMR

33

34 **1. Introduction**

35 A high intake of sodium (Na) is associated with an increased risk of high blood pressure,
36 which is a significant risk factor in the development of cardiovascular disease (CVD) and
37 stroke (Cook et al., 2007; He & MacGregor, 2002). The daily Na-intake in Europe and USA
38 is estimated to 4-5 g/day (The Norwegian Directorate of Health, 2011), and salt (NaCl) added
39 to food products during production, preparation and at the table is the main source of this Na.
40 Guidelines published by public health and regulatory authorities (FSA, 2004; The Norwegian
41 Directorate of Health, 2011; WHO, 2006) recommend a reduction of Na intake to 2 g/day or
42 less. Salt is commonly employed in fish processing, due to its preservative effects, taste,
43 positive technological effects and low cost (Fuentes, Fernández-Segovia, Serra, & Barat, 2010;
44 Martínez-Alvarez & Gómez-Guillén, 2006).

45 The development of safe high quality low-sodium fish products is of interest,
46 especially considering the good nutritional characteristics of fish. It is often possible to reduce
47 the addition of salt in "traditional" recipes to some extent, but further reduction then requires
48 the addition of salt substitutes to maintain palatability, texture, processing yield and shelf-life
49 (Kilcast & Angus, 2007). Salts contribute to formation of a viscous protein paste, that creates
50 a dense protein network, a gel, by solubilising the myofibrillar proteins (Kilcast & Angus,
51 2007). Several authors have reported that when NaCl is partially replaced with other salts like
52 KCl, MgCl₂ or CaCl₂ this will affect the enzyme activity, protein matrix and texture
53 (Andreetta-Gorelkina, Greiff, Rustad, & Aursand, 2014; Barat, Pérez-Esteve, Aritoy, &
54 Toldrá, 2012; Martínez-Alvarez & Gómez-Guillén, 2013). Partial substitution of NaCl by KCl
55 is one of the best alternatives for reducing sodium content (Aliño, Fuentes, Fernández-
56 Segovia, & Barat, 2011; Fuentes et al., 2010; Toldrá & Barat, 2012). MgCl₂ is used in low
57 concentrations in commercial "low-sodium" salts on the market (Barat et al., 2012). Most of

58 the studies on the effect of salt and "low-sodium" salts on physicochemical properties have
59 employed meat and meat batters (Barat et al., 2012; Nayak, Kenney, Slider, Head, & Killefer,
60 1998; Offer & Trinick, 1983; Ruusunen & Puolanne, 2005; Toldrá & Barat, 2012) or heavily
61 salted cod (Aliño et al., 2011; Martínez-Alvarez, Borderías, & Gómez-Guillén, 2005;
62 Martínez-Alvarez & Gómez-Guillén, 2005). In a study of salt substitution in surimi gels,
63 Tahergorabi, Beamer, Matak, and Jaczynski (2012) found that hardness increased when NaCl
64 or KCl was added to the gel, but there are limited results available on cooked fish mince.
65 Low-field ^1H NMR (LF-NMR) has been used to indirectly determine the effect of salting by
66 monitoring the changes in proton relaxation behavior as a result of the salting process
67 (reviewed by Erikson, Standal, Aursand, Veliyulin, & Aursand, 2012), However, none of
68 these studies dealt with salt replacers such as MgCl_2 and, it was of interest to explore the LF-
69 NMR method further as a tool for measuring proton relaxation behavior in low-salt
70 applications.

71 The aim of this study was to investigate the effect of different concentrations of cations
72 (Na^+ , K^+ , Mg^{2+}) and fresh and frozen raw material on cook loss, WHC, pH and water mobility
73 measured by LF-NMR of raw and cooked sodium reduced haddock mince.

74 **2. Materials and methods**

75 *2.1 Raw material*

76 Haddock (*Melanogrammus aeglefinus*) was caught by Danish seine on Vesterålsbanken
77 North-Norway in March 2011. The catch was pumped on board by a vacuum pump, stored
78 alive on-board before electrostunning, bleeding and gutting. The fish were iced in styrofoam
79 boxes and shipped to Trondheim (cold storage). The core temperature in the fish was $<4^\circ\text{C}$
80 during the experiment. Six days after catch the fish were evaluated, filleted and skinned. The

81 fish were considered of very good quality. Gutted mean weight was 1.8 ± 0.6 kg. Half of the
82 fishes were packed in plastic bags and frozen at -28°C , the other half were filleted and
83 minced the day after filleting. After 67 days, the frozen fish were thawed with drainage in a
84 cold room ($+4^{\circ}\text{C}$) for 67 h. The thawed fish were filleted and the minces prepared in the same
85 manner as with the fresh fish.

86 2.2 *Experimental design*

87 The compositional model variables were molar concentration of salt and type of salt (MgCl_2 ,
88 NaCl , KCl), giving in total nine different minces, see Table 1. The salts were analytical-
89 reagent grade; $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ (VWR International BVBA, Leuven, Belgium), NaCl (Merck
90 KGaA, Darmstadt, Germany) and KCl (Merck KGaA, Darmstadt, Germany) and added in
91 equimolar concentrations at three levels: 0.07, 0.17 and 0.34 mol/kg mince (corresponding to
92 0.4, 1.0 and 2.0 % NaCl). The content of coarsely ground fillets and added water was $61.7 \pm$
93 0.6 and 36.8 ± 0.4 g/100 g mince, respectively. The crystal water in $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was taken
94 into account when calculating the amount of added water.

95 2.3 *Process and heat treatment*

96 The fillets were coarsely ground (perforated disc, hole size 5 mm), (Hobart A 200 N, Hobart
97 manufacturing, Braunton, UK) and mixed (45 sec) with salt before ice water was added. The
98 mixture was homogenized (1.5 min) in a food processor (BRAUN Multiquick 7, Braun
99 GmbH, Kronberg, Germany), interrupted at one interval to scrape the sides of the bowl. Two
100 parallel minces from each sample were produced and blended into one batch. The fish mince
101 was divided in three plastic trays (~ 460 g in each tray) and cooked in a convection oven
102 (Rational SCC 61, Rational AG, Landsberg a. Lech, Germany) (100°C , 100 % RH) to a core
103 temperature of 80°C . After cooling the minces were packed in plastic bags and stored cool

104 (4°C) for 24 h before further analysis. The minces (n=3) were subjected to physicochemical
105 analyses and LF-NMR.

106 The experiment was part of a larger study of minces added different salts with the aim to
107 investigate the effect of cations Na⁺, K⁺ and Mg²⁺ on physicochemical properties and protein
108 solubility of raw and cooked haddock mince. The protein solubility results are reported by
109 Andreetta-Gorelkina et al. (2014).

110 2.4 Analytical methods

111 The **pH** of the raw and cooked minces was measured directly with a pH-meter (WTW
112 PH3110, Weilheim, WTW, Germany) equipped with a glass electrode (SenTix Sp, A,
113 Weilheim, WTW, Germany). Moisture content was determined by drying 5 g cooked fish
114 mince at 105 °C for 24 h to constant weight according to AOAC method (AOAC, 1997).
115 **WHC** of ground fillets and cooked mince was determined by low-speed centrifugation (210 x
116 g) as described by Eide, Børresen, and Strøm (1982). The analyses were run in quadruplicate
117 and WHC expressed as the percentage of water retained in the mince after centrifugation for 5
118 min. **Cooking loss** was determined by weighing the mince before cooking and 24 h after
119 cooking and chilling. The cooked mince was stored on a grid for 1 min before weighing.
120 Cooking loss was calculated as percent weight difference between the heated and unheated
121 minces (n=3).

122 The **sodium (Na⁺) and chloride (Cl⁻)** content of cooked minces were determined in mince
123 extracts. Sodium was determined at ambient temperature with a Na-selective electrode
124 (Ross® Sodium Ion Selective Electrode, Thermo Fisher Scientific, USA) and a Dual Star
125 pH/ISE meter (Thermo Fisher scientific, Waltman, MA, USA), under constant stirring
126 (Kivikari, 1996) as modified by Greiff et al. (2014). The analytical uncertainty of the method
127 was determined by analysing three extracts from each of three replicates of the same cooked

128 mince. This showed very low variation (1-2%). For the rest of the minces, only one extract
129 from each of three replicates of the same formulation and were analysed. The Cl⁻ content was
130 determined by end point titration of halides according to the titration application issued by
131 Radiometer Analytical SAS (TTEP01-04AFD/2001-05A). The titration end-point was
132 assessed potentiometrically using an automatic titrator (TitraLab980) coupled with a silver
133 electrode (M295Ag) and a reference electrode (REF 921) (all equipment from Radiometer
134 Analytical ASA, Copenhagen, Denmark).

135 The **breaking force** of the cooked minces was measured by a single compression using a
136 T.A.XT2 Texture Analyzer XT.plus using Exponent Software (Exponent Stable Micro
137 System Ltd., Godalming, UK.) equipped with a flat-ended cylindrical plunger (12 mm
138 diameter), by a modification of the method described by Einen and Thomassen (1998) on
139 salted fillets. The probe was pressed into the mince at constant speed (1 mm/s) until it reached
140 60 % of the initial sample height. The texture profile curve was recorded continuously. The
141 breaking force is given as the average of six measurements of each mince.

142 *2.5 Proton relaxation behavior*

143 **LF ¹H NMR samples preparation and analysis.** LF-NMR measurements were made on all
144 cooked minces. Three subsamples (about 1 × 1 × 3 cm, approx. 2-3 g) from each mince were
145 placed in NMR tubes (diameter 10 mm). The tubes were immediately placed on ice for about
146 30 min before equilibration to 1°C in a water bath (Julabo Labortechnik GmbH, Seelbach,
147 Germany) and analysis on a minispec mq 20 (Bruker Optik GmbH, Rheinstetten, Germany)
148 with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20
149 MHz. The instrument was equipped with a 10 mm temperature-variable probe. A built-in
150 heating element was connected to the temperature control unit (BVT3000, Bruker Optik
151 GmbH). The probe temperature was controlled at 4°C by blowing compressed air through the

152 sample holder. Proton transversal (T_2) relaxation was measured using the Carr-Purcell-
153 Meiboom-Gill pulse sequence (CPMG) (Carr & Purcell, 1954; Meiboom & Gill, 1958). The
154 T_2 measurements were performed with a time delay between the 90° and 180° pulses (τ) of
155 $150 \mu\text{s}$. Data from 6000 echoes were acquired from 16 scan repetitions. The repetition time
156 between two succeeding scans was set to 4 s. All even echoes were sampled.

157 **Low-field NMR data processing.** The NMR transverse relaxation data were analysed using
158 two different calculation methods. (1) Multivariate data analysis was performed for all raw
159 relaxation (CPMG) curves. These curves were normalized by setting the first sampled echo to
160 a value of 100, and thereafter scaling the rest of the echo-train. The first 1000 data points were
161 used for the principal component analysis (PCA) (Jolliffe, 1997) using an in-house program
162 written in Visual Basic. Each row (n) represented a single mince sample and each column (m)
163 represented a signal amplitude from an echo in the CPMG echo train. Four principal
164 components (k) were used. The input matrix was not mean-centred. (2) Biexponential analysis
165 of T_2 relaxation data was performed by using MatLab (The Mathworks Inc., Natick, MA) to
166 fit the following equation to the experimental CPMG curves, as reported by Erikson,
167 Veliyulin, Singstad, and Aursand (2004) and Lambelet, Renevey, Kaabi, and Raemy (1995):

$$168 \quad \text{Signal} = A_{21}e^{-t/T_{21}} + A_{22}e^{-t/T_{22}} \quad (\text{Eq. 1})$$

169 where T_{21} and T_{22} were the relaxation components, and A_{21} and A_{22} were the corresponding
170 amplitudes. Since the absolute relaxation amplitudes are proportional to the amount of water
171 and fat in the sample, the relative amplitudes within samples were used. T_{21} populations were
172 calculated as: $A_{21}/(A_{21} + A_{22})$. For the biexponential fitting, the T_{21} and T_{22} populations sum
173 up to 100 %, therefore, only T_{21} population values are given here. Three parallel samples from
174 each mince were averaged at both sampling time points.

175 2.6 *Statistical analysis*

176 Statistical analyses were performed using the software Minitab© (v.17.1.0, Coventry, UK).
177 One-way ANOVA was used to test for significant differences based on salt concentrations or
178 type of added cations (Na^+ , K^+ or Mg^{2+}). Where significant differences were found, the Tukey
179 test (least significant difference) was used as a post hoc test. The 2-sample t-test was used to
180 find the difference between WHC in minces made with fresh and frozen raw material.

181 **3. Results and discussion**

182 3.1 *Physicochemical Parameters*

183 The moisture and WHC of the fresh ground haddock (before addition of water and salt), was
184 $82.6 \pm 2.2\%$ and $83.3 \pm 2.4\%$, respectively, similar to results reported by Aubourg & Medina
185 (1999) and Olsson, Seppola, and Olsen (2007) on the same fish species. pH in the fresh
186 ground haddock was 6.52 ± 0.04 , which is within the range reported for cod (Gudjonsdottir et
187 al., 2010). The moisture content, pH and hardness in the raw mince (after addition of water
188 and salt) and the cooked minces are summarized in Table 2. The sodium content in cooked
189 minces added 0.07, 0.17 and 0.34 mol NaCl/kg, were 209 ± 5 , 434 ± 12 and 795 ± 12 mg/100
190 g, respectively. This is in good agreement with the expected values taking into account that
191 raw haddock meat contains approx. 96 mg Na/100 g (NFSA, 2014). The chloride content in
192 the cooked minces varied from 317 ± 7 in 0.07 K to 2356 ± 4 mg/100 g in 0.34 Mg, in good
193 agreement with the expected values taking into account the natural content of chloride in fish,
194 approx. 138 mg/100g (Abbas Bakhiet, Khogali, & Ahmed, 2013).

195 3.2 *Effect of different types and concentrations of salts on pH in raw and cooked mince.*

196 The pH of the raw mince after addition of salt depended on the type and amount of salt added
197 (Table 2). Compared to fresh ground haddock, pH increased slightly in mince with 0.07 mol

198 KCl /kg, was fairly similar in raw mince with 0.07 mol NaCl /kg and decreased significantly
199 in mince with 0.07 mol MgCl₂/kg. Upon further addition of salt, pH in the minces decreased
200 for all types of salts, most for minces added MgCl₂. In raw mince added 0.34 mol/kg, pH was
201 one unit lower than in freshly ground haddock.

202 A decrease of pH in fish flesh and animal meat with increasing addition of salt (NaCl) has
203 frequently been observed (Abbas Bakhiet & Khougalie, 2011; Leroi & Joffraud, 2000;
204 Puolanne, Ruusunen, & Vainionpää, 2001). The effect has been ascribed to an increase in the
205 ionic strength (Leroi & Joffraud, 2000) and interactions between the salt ions and proteins
206 leading to exposure of previously buried charged and/or hydrophilic groups and thus a change
207 in the overall pKa of the proteins (Puolanne et al., 2001). However, Schwabe (1967) also
208 found that when neutral salts were added to acidic solutions, pH decreased linearly with
209 increasing salt concentration. The rate of decrease depended on the type of salt ions due,
210 among others, to the charge of the ions, their size and hydration number. For the salts relevant
211 here, the rate of pH decrease with increasing salt concentration was lowest for KCl and
212 highest for MgCl₂. This is accordance with the rate sequence observed here. The pH in all
213 minces increased slightly (0.05-0.23 pH units) after cooking (Table 2) as also observed for
214 cooked sausages with NaCl (Puolanne et al., 2001).

215 *3.3 Effect of salt on water holding capacity, cooking loss, moisture and water mobility*

216 The **WHC** in the cooked fish minces added different amounts of salts are shown in Fig.1.
217 WHC tended to increase slightly with increasing addition of the different salts, but only the
218 mince added 0.07 mol MgCl₂/kg had WHC significantly lower than the rest of the minces.
219 Neither ionic strength nor pH appears to explain this result. It may be related to the ability of
220 divalent Mg²⁺-ion to form cross-bridges between peptide chains (Aliño et al., 2009), but that
221 this effect of Mg²⁺ is masked by the increased ionic strength when the addition of MgCl₂ is
10

222 increased. Addition of KCl and NaCl gave similar WHC, in agreement with Larsen &
223 Elvevoll's (2008) claim that KCl can replace NaCl without affecting the technological quality
224 parameters. Minces added NaCl had slightly higher WHC than the corresponding minces
225 added KCl, possibly because NaCl can lead to more muscle swelling than KCl due to cation
226 effects in accordance with the Hofmeister series (Damodaran, 2008). The **moisture** content of
227 cooked mince added 0.34 mol MgCl₂/kg was significantly lower than in all the other minces.
228 Weinberg, Regenstein, & Baker, (1984), observed poor moisture retention in cod containing
229 MgCl₂.

230 Moisture loss during cooking (**cooking loss**) decreased with increasing addition of salt. The
231 decrease correlated both with increase in the concentration of chloride ions and increase in
232 ionic strength (Fig 2 a-d). Larsen & Elvevoll, (2008), found that increasing the chloride
233 concentration in cod fillets` from 600 to 800 mg/100g increased yield by 8 %.

234 The LF-NMR T₂ relaxation method was used to study the proton **relaxation**
235 **behaviors** in the cooked minces. The two transversal relaxation times with corresponding
236 populations obtained from fitting of NMR data, are shown in Table 3. In fish muscle,
237 typically two or three relaxation components are reported. The two major ones have
238 relaxation times of 40-60 ms (T₂₁) and 150-400 ms (T₂₂) (see Erikson et al., 2012). In the
239 present study, the relaxation times were slightly higher; 64-93 ms (T₂₁) and 402-598 ms (T₂₂).
240 A possible explanation for the higher relaxation times is changes in the protein matrix due to
241 the added salt and water and the cooking. The interpretation of such data have been
242 controversial, but it is becoming more accepted that the observed changes in relaxation
243 behavior is primarily due to chemical and diffusive proton exchange between water molecules
244 and biopolymers (e.g. proteins) (Belton, 2011; Halle, 2006). Several studies have shown that
245 these processes are linked to the morphology of the sample, which in turn can be affected by

246 processing such as salting (Aursand, Erikson, & Veliyulin, 2010; Erikson et al., 2012;
247 Gudjonsdottir et al., 2010).

248 Increasing the sodium content from 0.07 (0.4 % NaCl) to 0.17 mol/kg (1.0 % NaCl)
249 increased the proton relaxation times from 65 to 92 ms in case of T_{21} , but a further increase of
250 Na-concentration to 0.34 mol/kg did not affect the T_{21} values. The T_{21} values in 0.34 Na
251 mince (93 ms) were higher than in the 0.34 K (68 ms) and 0.34 Mg mince (59 ms). Both the
252 T_{21} and T_{22} values were higher in 0.17 Mg mince (83 ± 4 ms and 523 ± 35 ms) than in 0.07
253 Mg mince (68 ± 8 ms and 466 ± 64 ms) and 0.34 Mg mince (59 ± 2 ms and 405 ± 27 ms).
254 Part of the explanation might be the effect of the divalent cation (Mg^{2+}) on muscle structure
255 (Aliño et al., 2009), as discussed above, and thus on water mobility. By comparison, addition
256 of 0.5% NaCl or NaCl/KCl to raw mince of hake increased the T_{21} proton relaxation time to
257 59-61 ms whereas the T_{22} value remained largely unchanged. Further addition of salt
258 increased both T_{21} and T_{22} relaxation times, with mean values of 67-71 ms and 286-496 ms,
259 respectively (Greiff et al., 2014).

260 3.4 *Breaking force*

261 The **breaking force** increased with increasing Na^+ content as also observed for cooked pork
262 sausages within the same range of NaCl contents (Puolanne et al., 2001). However, the
263 hardness of cod frankfurter decreased with increasing NaCl content (Cardoso, Mendes, &
264 Nunes, 2009). The disagreement may be related to differences in protein extractability at
265 different ionic strengths between fish species, as discussed by Jafarpour & Gorczyca (2012).
266 The 0.34 Mg mince was softer than 0.34 K and 0.34 Na minces. Nayak et al. (1998) found
267 that low levels of $MgCl_2$ decreased the hardness on beef batter, possibly due to the amount of
268 proteins solubilised by the salt. A significant decrease in salt soluble proteins in raw haddock

269 mince added 0.34 mol/kg $MgCl_2$ compared to 0.34 mol/kg KCl or NaCl was observed by
270 Andreetta-Gorelkina et al. (2014).

271 3.5 *The effect of frozen raw material on WHC and cooking loss.*

272 As for minces made from fresh raw material, increased addition of NaCl or KCl had no effect
273 on WHC in cooked minces made from frozen raw material (Fig.1), but WHC in minces
274 prepared from frozen raw material and added NaCl decreased significantly compared to the
275 corresponding minces prepared from fresh raw material and added NaCl (p-value < 0.05 for
276 0.07 and 0.34 Na, p-value < 0.10 for 0.17 Na). This effect was much less pronounced in
277 minces added KCl and not present in minces added $MgCl_2$. The use of frozen raw material
278 increased the cooking loss compared to fresh raw material (Fig.2 a-d). As for fresh raw
279 material (Section 3.3), the cooking loss decreased with increasing concentration of chloride
280 ions and increased ionic strength (Fig.2 b,d). However, the results fit best when the cooking
281 loss seen as an effect of ionic strength (Fig. 2).

282 4. **Conclusion**

283 Replacing NaCl with the same molar concentration of KCl had no or only small effects on
284 pH, moisture, breaking force WHC and cooking loss in cooked minces made from fresh raw
285 material. However, replacing NaCl with an equal molar concentration of $MgCl_2$ affected
286 several of the physicochemical properties and in particular WHC, cooking loss and pH.
287 Cooking loss decreased with increased salt content, and the results indicate that the
288 concentration of ionic strength was more important than the type of cations. Mince prepared
289 with frozen raw material had a much higher cooking loss than the corresponding minces
290 prepared with fresh material, particularly at low salt concentrations.

291 **5. Acknowledgements**

292 The work is financed by the Low Salt project number 185063/O10, supported by the Research
293 Council of Norway and Norwegian industry. The authors would like to thank Marte Schei for
294 her contributions in production and analysing the fish minces.

295 **Literature cited**

- 296 Abass Bakhiet, H. H., & Khogali, F. A. E. (2011). Effect of different salt concentrations on
 297 chemical composition of the fish hydrocynus spp. *Online Journal of Animal and*
 298 *Feed Research*, 1(6), 461-464.
- 299 Abass Bakhiet, H. H., Khogali, F. A. H., & Ahmed, F. A. M. (2013). Effect of Different
 300 Salt Concentrations on Mineral Content of the Fish (Hydrocynus Spp.). *Journal of*
 301 *Life Science and Biomedicine*, 3(1), 01-03.
- 302 Aliño, M., Fuentes, A., Fernández-Segovia, I., & Barat, J. M. (2011). Development of a
 303 low-sodium ready-to-eat desalted cod. *Journal of Food Engineering*, 107(3-4), 304-
 304 310. doi: 10.1016/j.jfoodeng.2011.07.012
- 305 Aliño, M., Grau, R., Toldrá, F., Blesa, E., Pagán, M. J., & Barat, J. M. (2009). Influence of
 306 sodium replacement on physicochemical properties of dry-cured loin. *Meat Science*,
 307 83(3), 423-430. doi: 10.1016/j.meatsci.2009.06.022
- 308 Andreetta-Gorelkina, I. V., Greiff, K., Rustad, T., & Aursand, I. G. (2014). Reduction of
 309 salt in haddock mince: Effect of different salts on the solubility of proteins. *J*
 310 *Aquatic Food Product Technology*, 26.
- 311 AOAC. (1997). Official Methods of Analysis of the Association of Official Analytical
 312 Chemists (16th ed.). Arlington, Virginia: AOAC.
- 313 Aubourg, S. P., & Medina, I. (1999). Influence of storage time and temperature on lipid
 314 deterioration during cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)
 315 frozen storage. *Journal of the Science of Food and Agriculture*, 79(13), 1943-1948.
 316 doi: 10.1002/(SICI)1097-0010(199910)79:13<1943::AID-JSFA461>3.0.CO;2-J
- 317 Aursand, I. G., Erikson, U., & Veliyulin, E. (2010). Water properties and salt uptake in
 318 Atlantic salmon fillets as affected by ante-mortem stress, rigor mortis, and brine
 319 salting: A low-field 1H NMR and 1H/23Na MRI study. *Food Chemistry*, 120(2),
 320 482-489. doi: http://dx.doi.org/10.1016/j.foodchem.2009.10.041
- 321 Barat, J. M., Pérez-Esteve, E., Aritoy, M.-C., & Toldra, F. (2012). Partial replacement of
 322 sodium in meat and fish products by using magnesium salts. A review. *Plant Soil*.
 323 doi: 10.1007/s11104-012-1461-7
- 324 Belton, P. (2011). Spectroscopic Approaches to the Understanding of Water in Foods. *Food*
 325 *Reviews International*, 27(2), 170-191. doi: 10.1080/87559129.2010.535234
- 326 Cardoso, C. M., Mendes, R., & Nunes, M. L. (2009). Instrumental texture and sensory
 327 characterisitic of cod frankfurter sausages. *International Journal of Food Properties*,
 328 12, 625-643.
- 329 Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear
 330 magnetic resonance experiments. *Am. J. Physiology*, 94, 630-638.
- 331 Cook, N. R., Cutler, J. A., Obarzanek, E., Buring, J. E., Rexrode, K. M., Kumanyika, S. K.,
 332 Whelton, P. K. (2007). Long term effects of dietary sodium reduction on
 333 cardiovascular disease outcomes: observational follow-up of the trials of
 334 hypertension prevention (TOHP). *BMJ*, 334(7599), 885-888.
- 335 Damodaran, S. (2008). Amino Acids, Peptides, and Proteins. In O. R. Fennema, S.
 336 Damodaran & K. L. Parkin (Eds.), *Fennema's Food Chemistry* (Vol. Fourth ed, pp.
 337 217-329). Florida: CRC Press.

- 338 Eide, O., Børresen, T., & Strøm, T. (1982). Minced Fish Production From Capelin
339 (Mallotus villosus). A New Method for Gutting, Skinning and Removal of Fat from
340 Small Fatty Fish Species. *Journal of Food Science*, 47(2), 347-349. doi:
341 10.1111/j.1365-2621.1982.tb10078.x
- 342 Einen, O., & Thomassen, M. (1998). Starvation piror to slaughter in Atlantic salmon
343 (*Salmo salar*) II. White muscle compositioin and evalutaion og freshness, texture and
344 colour characteristics in raw and cooked fillets. *Aquaculture*, 169, 37-53.
- 345 Erikson, U., Standal, I. B., Aursand, I. G., Veliyulin, E., & Aursand, M. (2012). Use of
346 NMR in fish processing optimization: a review of recent progress. *Magnetic
347 Resonance in Chemistry*, 50(7), 471-480. doi: 10.1002/mrc.3825
- 348 Erikson, U., Veliyulin, E., Singstad, T. E., & Aursand, M. (2004). Salting and desalting of
349 fresh and frozen-thawed cod (*Gadus morhua*) fillets: A comparative study using
350 ²³Na NMR, ²³Na MRI, low-field ¹H NMR, and physicochemical analytical methods.
351 *Journal of Food Science*, 69(3).
- 352 Fuentes, A., Fernández-Segovia, I., Serra, J. A., & Barat, J. M. (2010). Development of a
353 smoked sea bass product with partial sodium replacement. *LWT - Food Science and
354 Technology*, 43(9), 1426-1433. doi: 10.1016/j.lwt.2010.04.013
- 355 FSA, (2004). Nutrition statement. Common statement of representatives of national food
356 safety agencies and institutions involved in nutrition in the European countries and
357 Norway: Standards Agency, UK.
- 358 Greiff, K., Fuentes, A., Aursand, I. G., Erikson, U., Masot, R., Alcañiz, M., & Barat, J. M.
359 (2014). Innovative Nondestructive Measurements of Water Activity and the Content
360 of Salts in Low-Salt Hake Minces. *Journal of Agricultural and Food Chemistry*,
361 62(12), 2496-2505. doi: 10.1021/jf405527t
- 362 Gudjonsdottir, M., Gunnlaugsson, V. N., Finnbogadottir, G. A., Sveinsdottir, K.,
363 Magnusson, H., Arason, S., & Rustad, T. (2010). Process Control of Lightly Salted
364 Wild and Farmed Atlantic Cod (*Gadus morhua*) by Brine Injection, Brining, and
365 Freezing-A Low Field NMR Study. *Journal of Food Science*, 75(8), E527-E536.
366 doi: 10.1111/j.1750-3841.2010.01808.x
- 367 Halle, B. (2006). Molecular theory of field-dependent proton spin-lattice relaxation in
368 tissue. *Magnetic Resonance in Medicine*, 56(1), 60-72. doi: 10.1002/mrm.20919
- 369 He, F. J., & MacGregor, G. A. (2002). Effect of modest salt reduction on blood pressure: a
370 meta-analysis of randomized trials. Implications for public health. *J Hum
371 Hypertens*, 16(11), 761-770. doi: 10.1038/sj.jhh.1001459
- 372 Jafarpour, A., & Gorczyca, E. M. (2012). Contribution of Sarcoplasmic Proteins to
373 Myofibrillar Proteins Gelation. *Journal of Food Science*, 77(2), R73-R81. doi:
374 10.1111/j.1750-3841.2011.02521.x
- 375 Jolliffe, I. T. *Principal Component Analysis*; Springer-verlag: Berlin, Germany, 1997.
- 376 Kilcast, D. a. A., F. (Ed.). (2007). *Reducing salt in foods, practical strategies*. UK: CRC
377 Press, Cambridge.
- 378 Kivikari, R. (1996). Analysis of sodium in meat products using an Na-selective eletrode.
379 (In Finnish). Proc. of Meat Day Seminar 1996, Nr, 536. In D. o. M. T. University of
380 Helsinki, Reports from Meat Technology (Ed.), (pp. 64-66).

- 381 Lambelet, P., Renevey, F., Kaabi, C., & Raemy, A. (1995). Low-Field Nuclear Magnetic
382 Resonance Relaxation Study of Stored or Processed Cod. *Journal of Agricultural*
383 *and Food Chemistry*, 43(6), 1462-1466. doi: 10.1021/jf00054a009
- 384 Larsen, R., & Elvevoll, E. O. (2008). Water uptake, drip losses and retention of free amino
385 acids and minerals in cod (*Gadus morhua*) fillet immersed in NaCl or KCl. *Food*
386 *Chemistry*, 107(1), 369-376. doi: <http://dx.doi.org/10.1016/j.foodchem.2007.08.031>
- 387 Leroi, F., & Joffraud, J. J. (2000). Salt and smoke simultaneously affect chemical and
388 sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial
389 design. *Journal of Food Protection*, 63(9), 1222-1227.
- 390 Martínez-Alvarez, O., Borderías, A. J., & Gómez-Guillén, M. C. (2005). Sodium
391 replacement in the cod (*Gadus morhua*) muscle salting process. *Food Chemistry*,
392 93(1), 125-133. doi: 10.1016/j.foodchem.2004.10.014
- 393 Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2013). Influence of mono- and divalent
394 salts on water loss and properties of dry salted cod fillets. *LWT - Food Science and*
395 *Technology*, 53(2), 387-394. doi: <http://dx.doi.org/10.1016/j.lwt.2013.04.013>
- 396 Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2005). The effect of brine composition
397 and pH on the yield and nature of water-soluble proteins extractable from brined
398 muscle of cod (*Gadus morhua*). *Food Chemistry*, 92(1), 71-77. doi:
399 10.1016/j.foodchem.2004.04.049
- 400 Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2006). Effect of brine salting at different
401 pHs on the functional properties of cod muscle proteins after subsequent dry salting.
402 *Food Chemistry*, 94(1), 123-129.
- 403 Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear times.
404 *Rev. Sci. Instrum*, 29, 688-691.
- 405 Nayak, R., Kenney, P. B., Slider, S., Head, M. K., & Killefer, J. (1998). Cook Yield,
406 Texture and Gel Ultrastructure of Model Beef Batters as Affected by Low Levels of
407 Calcium, Magnesium and Zinc Chloride. *Journal of Food Science*, 63(6), 945-950.
408 doi: 10.1111/j.1365-2621.1998.tb15829.x
- 409 NFSA, (2014). Norwegian Food Composition Database (2014). The Norwegian Directorate
410 of Health and University of Oslo. Norwegian Food Safety
411 Authority. www.matvaretabellen.no.
- 412 Offer, G., & Trinick, J. (1983). On the Mechanism of Water Holding in Meat: The Swelling
413 and Shrinking of Myofibrils. *Meat Science*, 8, 245-281. doi: 0309-1740/83/0008-
414 0245/\$03.00
- 415 Olsson, G. B., Seppola, M. A., & Olsen, R. L. (2007). Water-holding capacity of wild and
416 farmed cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) muscle
417 during ice storage. *LWT - Food Science and Technology*, 40(5), 793-799. doi:
418 <http://dx.doi.org/10.1016/j.lwt.2006.04.004>
- 419 Puolanne, E. J., Ruusunen, M. H., & Vainionpää, J. I. (2001). Combined effects of NaCl
420 and raw meat pH on water-holding in cooked sausage with and without added
421 phosphate. *Meat Science*, 58(1), 1-7.
- 422 Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat*
423 *Science*, 70(3), 531-541.
- 424 Schwabe, K. (1967). Azidität konzentrierter elektrolytlösungen. *Electrochimica Acta*,
425 12(1), 67-93. doi: [http://dx.doi.org/10.1016/0013-4686\(67\)85007-2](http://dx.doi.org/10.1016/0013-4686(67)85007-2)

- 426 Tahergorabi, R., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2012). Salt substitution in
427 surimi seafood and its effects on instrumental quality attributes. *LWT - Food*
428 *Science and Technology*, 48(2), 175-181. doi:
429 <http://dx.doi.org/10.1016/j.lwt.2012.03.004>
- 430 The Norwegian Directorate of Health. (2011). Strategy for the reduction of salt intake in the
431 population. Recommendation from the National Nutrition Council. In Department
432 for national public health (Ed.).
433 [http://helsedirektoratet.no/folkehelse/ertering/strategier-og-](http://helsedirektoratet.no/folkehelse/ertering/strategier-og-satsninger/Documents/saltstrategi.pdf)
434 [satsninger/Documents/saltstrategi.pdf](http://helsedirektoratet.no/folkehelse/ertering/strategier-og-satsninger/Documents/saltstrategi.pdf).
- 435 Toldrá, F., & Barat, J. M. (2012). Strategies for salt reduction in foods. *Recent Patents on*
436 *Food, Nutrition and Agriculture*, 4(1), 19-25.
- 437 TTEP01-04AFD/2001-05A. Chloride in food products (2001). *Titration Application -*
438 *Precipitation Titrations*. Radiometer Analytical SAS: Villeurbanne Cedex, France.
- 439 Weinberg, Z. G., Regenstein, J. M., & Baker, R. C. (1984). Effects of different salts on
440 water retention and heat initiated binding properties of comminuted cod muscle.
441 *Journal of Food Biochemistry*, 8(3), 215-227. doi: 10.1111/j.1745-
442 4514.1984.tb00325.x
- 443 WHO. (2006). Reducing salt intake in populations. Report of a WHO Forum and Technical
444 Meeting: World Health Organization, Paris.

455 **Table 1.** Type and concentration of salt added to the mince formulations. The salts (KCl, MgCl₂ and NaCl) were added in equimolar
 456 amounts at three levels, 0.07, 0.17 and 0.34 mol pr kg mince.

Sample ID	Salt	Molar Concentration (mol/kg mince)	Salt concentration (w/w %)	Estimated Ionic strength due to the added salts (mol/kg solute)
0.07 K	KCl	0.07	0.5	0.07
0.07 Mg	MgCl ₂	0.07	0.7	0.21
0.07 Na	NaCl	0.07	0.4	0.07
0.17 K	KCl	0.17	1.3	0.17
0.17 Mg	MgCl ₂	0.17	1.6	0.51
0.17 Na	NaCl	0.17	3.2	0.17
0.34 K	KCl	0.34	2.5	0.34
0.34 Mg	MgCl ₂	0.34	3.2	1.02
0.34 Na	NaCl	0.34	2.0	0.34

457

458 **Table 2** Moisture, pH and breaking force in haddock mince (from fresh raw material) with different salts at different concentrations.

Sample	Moisture cooked mince (%)	pH raw mince	pH cooked mince	Breaking force cooked mince (g)
0.07 K	86.3 ± 0.1 ^d	6.63 ± 0.02 ^a	6.80 ± 0.07 ^a	192 ± 7 ^c
0.07 Mg	86.6 ± 0.2 ^{cd}	6.09 ± 0.01 ^e	6.14 ± 0.09 ^d	235 ± 7 ^{bc}
0.07 Na	86.2 ± 0.2 ^d	6.51 ± 0.03 ^{bc}	6.65 ± 0.02 ^b	250 ± 10 ^{bc}
0.17 K	86.6 ± 0.1 ^{bcd}	6.54 ± 0.00 ^b	6.67 ± 0.02 ^{ab}	217 ± 16 ^c
0.17 Mg	87.3 ± 0.1 ^a	5.71 ± 0.02 ^f	5.94 ± 0.03 ^e	230 ± 8 ^c
0.17 Na	86.9 ± 0.0 ^{abc}	6.46 ± 0.01 ^c	6.56 ± 0.05 ^{bc}	214 ± 10 ^c
0.34 K	86.9 ± 0.3 ^{abc}	6.49 ± 0.03 ^c	6.61 ± 0.01 ^{bc}	290 ± 54 ^{ab}
0.34 Mg	85.4 ± 0.2 ^e	5.52 ± 0.01 ^g	5.73 ± 0.08 ^f	202 ± 3 ^c
0.34 Na	87.1 ± 0.3 ^{ab}	6.33 ± 0.02 ^d	6.50 ± 0.01 ^c	313 ± 15 ^a
<i>p</i> -value	***	***	***	***

459 *Mean value ± SD (n=3). Different letters (a-g) indicate significant differences (p < 0.05).

460

461 **Table 3**

462 Biexponential fitting of LF ¹H NMR T₂ relaxation data obtained in cooked haddock mince (from fresh raw material) with different
463 salts and concentrations.

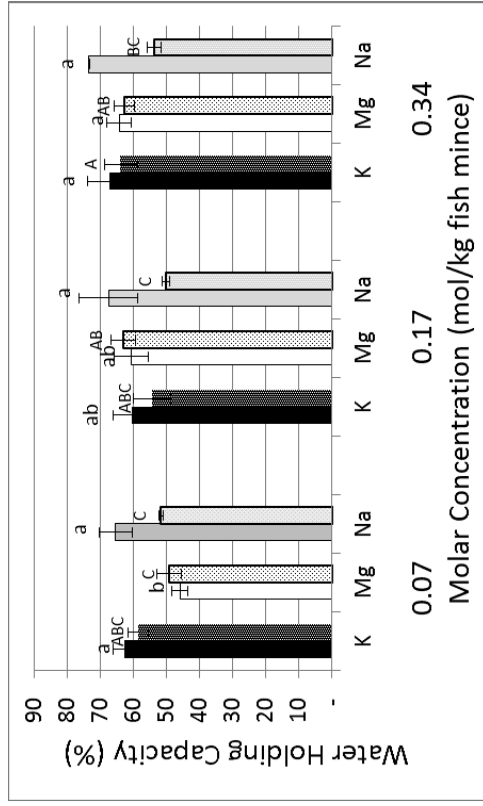
464

Sample	T21 (ms)	T22 (ms)	T21 population (%)
0.07 K	65 ± 4 ^c	407 ± 56 ^c	68 ± 3 ^c
0.07 Mg	68 ± 8 ^c	466 ± 64 ^{bc}	67 ± 6 ^c
0.07 Na	65 ± 4 ^c	402 ± 14 ^c	71 ± 2 ^{bc}
0.17 K	71 ± 2 ^{bc}	441 ± 16 ^{bc}	77 ± 3 ^{ab}
0.17 Mg	83 ± 4 ^{ab}	523 ± 35 ^{ab}	77 ± 3 ^{ab}
0.17 Na	92 ± 8 ^a	469 ± 8 ^{bc}	77 ± 1 ^{ab}
0.34 K	68 ± 4 ^c	439 ± 35 ^{bc}	80 ± 2 ^a
0.34 Mg	59 ± 2 ^c	405 ± 27 ^c	73 ± 2 ^{abc}
0.34 Na	93 ± 2 ^a	598 ± 47 ^a	80 ± 0.4 ^a
<i>P</i> - value	***	***	***

465 One – Way Anova. Mean values ± SD (n=3). Different letters indicate significant differences (p < 0.001) between samples.

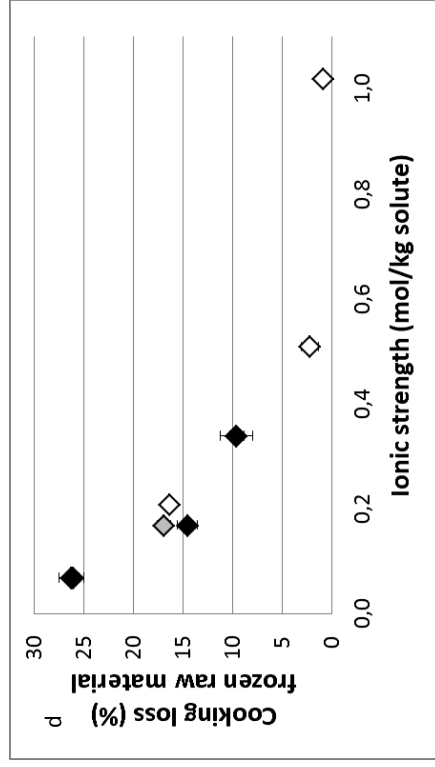
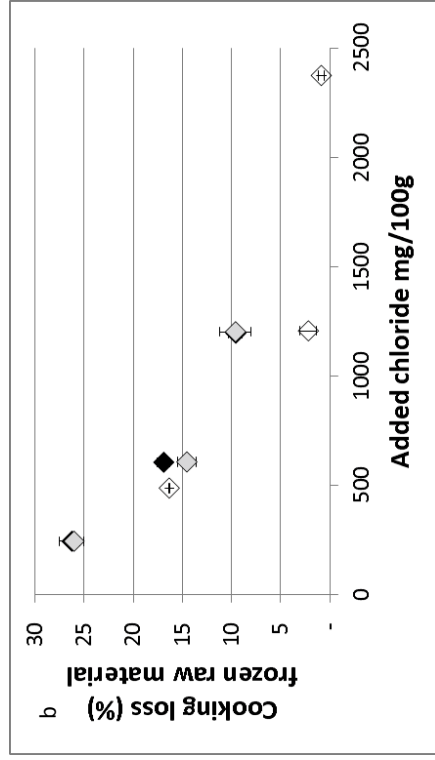
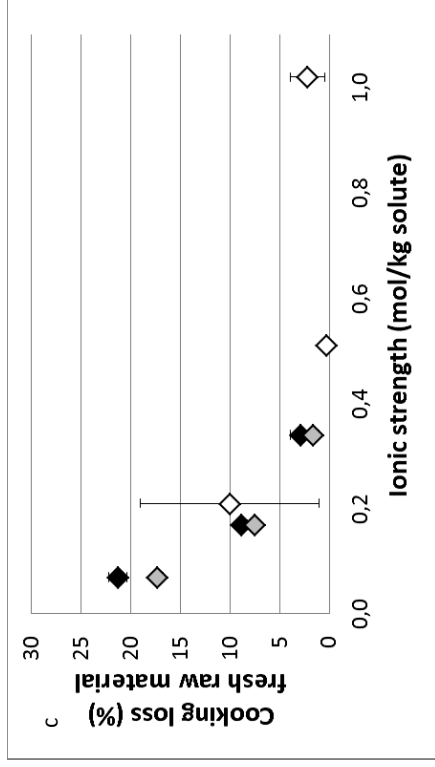
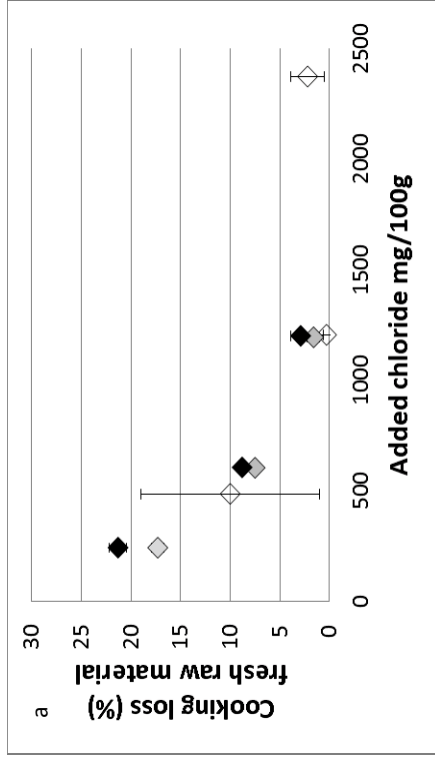
466

Fig. 1



Mean values \pm SD (n=3). Different letters (a, b or c) indicate significant differences ($p < 0.05$) between cooked haddock mince prepared from fresh fillets. Different letters (A, B or C) indicate significant differences ($p < 0.05$) between cooked haddock mince prepared from frozen fillets.

Fig. 2 a-d.



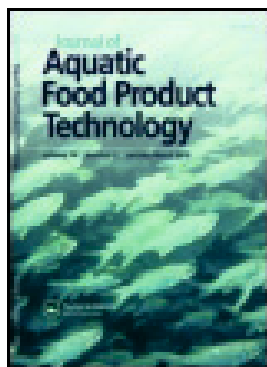
Paper IV

This article was downloaded by: [Universitetsbiblioteket I Trondheim NTNU]

On: 09 March 2015, At: 02:10

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Aquatic Food Product Technology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/wafp20>

Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins

Irina Viktorovna Andreetta-Gorelkina PhD^a, Kirsti Greiff^{ab}, Turid Rustad^a & Ida Grong Aursand PhD^b

^a Norwegian University of Science and Technology, Biotechnology, NTNU, N-7491, Trondheim, 7491 Norway

^b Stifelsen SINTEF, SINTEF Fisheries and Aquaculture, Stifelsen SINTEF, Box 4760 Sluppen, Trondheim, NO-7465 Norway

Accepted author version posted online: 06 Mar 2015.



CrossMark

[Click for updates](#)

To cite this article: Irina Viktorovna Andreetta-Gorelkina PhD, Kirsti Greiff, Turid Rustad & Ida Grong Aursand PhD (2015): Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins, Journal of Aquatic Food Product Technology, DOI: [10.1080/10498850.2013.879241](https://doi.org/10.1080/10498850.2013.879241)

To link to this article: <http://dx.doi.org/10.1080/10498850.2013.879241>

Disclaimer: This is a version of an unedited manuscript that has been accepted for publication. As a service to authors and researchers we are providing this version of the accepted manuscript (AM). Copyediting, typesetting, and review of the resulting proof will be undertaken on this manuscript before final publication of the Version of Record (VoR). During production and pre-press, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal relate to this version also.

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins

Irina Viktorovna Andreetta-Gorelkina PhD (Corresponding Author)

Email: irina.v.andreetta-gorelkina@ntnu.no

Norwegian University of Science and Technology, Biotechnology, NTNU, N-7491, Trondheim, 7491 Norway

Kirsti Greiff

Kirsti.Greiff@sintef.no

Norwegian University of Science and Technology, Biotechnology, NTNU, N-7491, Trondheim, 7491 Norway

and

Stifelsen SINTEF, SINTEF Fisheries and Aquaculture, Stifelsen SINTEF, Box 4760 Sluppen, Trondheim, NO-7465 Norway

Turid Rustad

Email: turid.rustad@ntnu.no

Norwegian University of Science and Technology, Biotechnology, NTNU, N-7491, Trondheim, 7491 Norway

Ida Grong Aursand PhD

Email: ida.g.aursand@sintef.no

Stifelsen SINTEF, SINTEF Fisheries and Aquaculture, Stifelsen SINTEF, Box 4760 Sluppen, Trondheim, NO-7465 Norway

Abstract

Due to negative health effects of high sodium intake, it is recommended to reduce the daily salt intake by around 50%. To reduce the sodium content, sodium salts can be exchanged with potassium or magnesium salts. The effect of sodium, potassium, and magnesium chlorides on extractability of proteins from fresh and frozen haddock muscle and minces was studied. Salting with KCl and MgCl₂ instead of NaCl changed protein extractability. The highest solubility of the proteins was achieved using Na⁺. However, at low concentrations, extractability in K⁺ and Mg²⁺ is on the same level as Na⁺, showing that partial substitution of NaCl with KCl or MgCl₂ is possible. Freezing affected the structure of tissue and protein properties, resulting in decreased amount of salt soluble proteins.

Key words

Fish muscle, protein solubility, salt, ion substitution, mince, sodium reduction.

1 Introduction

Salting is one of the oldest and cheapest methods of food preservation used to prolong shelf life of food by reducing water activity as well as for improvement of functional properties such as water holding, formation of matrix-structure, and gelling (Albarracín et al., 2011; Nguyen et al., 2011). The daily intake of salt in the western world is around 8 - 11 g/day (Brandsma, 2006; EFSA, 2005), which is more than twice the amount needed. Industrially prepared foods contribute 70 – 80% of the daily intake of salt. Due to negative health effects of high sodium

intake, health organizations have strongly recommended a reduction from the present levels to values of around 6 g/day (Desmond, 2006; EFSA, 2005; FSA, 2003). To achieve this, it is necessary to develop products with reduced content of sodium chloride (Ruusunen and Puolanne, 2004). This can be done by reducing the salt content (Mitchell et al., 2011; Ugawa, 2003) or by replacing part of the sodium chloride with compounds having salty taste (Floury et al., 2009a, 2009b). Both Na^+ and Cl^- affect the taste of sodium chloride; the presence of Cl^- ion stimulates the receptor cells of the tongue. Even though the size of anions affects the perception of the salty taste and stimuli of the reception system, NaCl can be exchanged with KCl , MgCl_2 , or other salts (Albarracín et al., 2011; Delwiche et al., 1999; Murphy et al., 1981; Åsli and Mørkøre, 2012). However, substitutions of Na^+ with other ions such as Mg^{2+} and K^+ often have negative effect both on the flavor and water holding capacity and texture (Åsli and Mørkøre, 2012). To achieve comparable properties in terms of structure and functional properties, exchange of cations in muscle foods should be based on equal quantity in moles. Since K^+ has a higher molar weight than Na^+ , more KCl (in grams) is needed to substitute an equal number of ions, compared to NaCl . Since KCl has a bitter taste, substitution may lead to off taste (Desmond, 2006; Rössner et al., 2009).

In manufacturing minced fish products such as fish cakes, puddings, balls, and patés, the functional properties of the muscle proteins, including water holding capacity and gelling properties, are very important, directly influencing the final product quality (Bertram et al., 2003; Martínez-Alvarez and Gómez-Guillén, 2005). The molecular size and structure, charge distribution, and ability to interact with other ingredients affect the functional properties of the food components. Environmental factors such as pH, ionic strength, composition of salting

mixtures, temperature, and shear stress are therefore important for gelling and emulsifying properties, water retention, and fat binding of proteins (Kinsella, 1982). Addition of NaCl improves the mechanical properties of restructured fish, affecting the quality of the fish products (Costa-Corredor et al., 2010; Munasinghe and Sakai, 2006; Nguyen et al., 2011; Ramírez et al., 2002); therefore, reducing the sodium chloride content may affect the functional properties and thereby change the eating quality of the products (Rössner et al., 2009).

The proteins in muscle are usually divided into three main groups: proteins soluble at low ionic strength, the sarcoplasmic proteins; proteins soluble at high ionic strength, the contractile or myofibrillar proteins; and the insoluble proteins, the stroma or connective tissue proteins (Haard, 1992). Among these it is the proteins soluble at high ionic strength, myosin and actin, that are considered to be most important for the functional properties of the muscle (Jafarpour and Gorczyca, 2012; Kinsella, 1976; Stefansson and Hultin, 1994). Different buffers, salts, and pH have been used to study solubility properties of muscle proteins (Kelleher and Hultin, 1991; Munasinghe and Sakai, 2006; Xiong and Brekke, 1991). The phrase water soluble protein (WSP) is commonly used for the proteins soluble at low ionic strength, while salt soluble protein (SSP) is commonly used for the proteins soluble at high ionic strength (Hultmann and Rustad, 2002; Kinsella, 1976).

The water binding and holding behavior as well as the gelling properties of proteins in the food system have been shown to be related to the solubility properties of the proteins (Kinsella, 1982; Martínez-Alvarez et al., 2005; Nayak et al., 1996; Nguyen et al., 2011). Several studies have shown that different ions affect solubility properties of muscle proteins and functional

properties of muscle differently (Nayak et al., 1996; Nguyen et al., 2011), and this has been shown to be related to changes in water holding properties (Richardson and Jones, 1987). The effect on the water holding capacity may be attributed to preferential anion binding of chloride ions, which neutralize the positive charge of the protein at pH below the isoelectric point (Aliño et al., 2010; Nayak et al., 1996; Richardson and Jones, 1987). The composition of the soluble protein fraction in muscle depends on the pH. In brine salting, the initial pH of the brine appeared to be a more important factor in selective solubilization of the myofibrillar proteins than the actual combination of salts (Martínez-Alvarez et al., 2005). Na^+ increases the number of acidic groups resulting in an acidic shift of the iso-electric point of the proteins. Na^+ may also act as a weak acid (Lauritzen et al., 2004; Rhee and Ziprin, 2001).

Both fresh and frozen raw materials are used in the production of minced fish products. Freezing leads to changes in raw material properties including protein denaturation resulting in loss of functional properties. It is therefore important to study how freezing will affect the raw material properties and, furthermore, how this will affect the influence of different salts on the product properties.

Haddock (*Melanogrammus aeglefinus*) was chosen as raw material for the study as haddock is widely consumed in West-European countries and both fresh and frozen haddock are often used in minced products (Martínez-Alvarez et al., 2005).

In order to produce high quality minced fish products with a lower level of salt, there is a need for more knowledge on how reduction of salt content and different ions affect the functional properties and the solubility properties of fish muscle proteins.

The main aim of this study was to evaluate the effect of different types of cations and different salt concentrations on the protein solubility of haddock muscle and minces with different salts (NaCl, KCl, MgCl₂) and concentrations (0.4 – 3.2 % w/w). A second aim was to evaluate how freezing affected these properties.

2 Materials and Methods

2.1 The raw material

Two different batches of haddock (*Melanogrammus aeglefinus*) were used. In the first part of the experiment, extractions were done on fillets, and in the second part, protein extractions were done on minces.

The haddock fillets (8 fillets from 4 fish) were bought in a local fish store. The fish was stored at +4 °C from catch until used, which was within one week after catch. Half the fillets was packed in plastic bags and frozen at –28 °C for two weeks. Before analysis, they were thawed in a cold room at +4 °C for 24 hours. The rest of the fillets were used for determination of protein solubility the day after purchase.

The haddock used for the mince experiment was caught by Danish seine on Vesterålsbanken, Northern Norway. The catch was pumped on board by a vacuum pump and stored alive on board before electro stunning, bleeding, and gutting. The gutted weight was 1.8 ± 0.6 kg. The fish were iced in Styrofoam boxes and transferred to Trondheim by the coastal steamer (in a cold room). The fish were generally of very good quality. Six days after catch, one

half of the fish was packed in plastic bags and frozen at $-28\text{ }^{\circ}\text{C}$. After 67 days, the fish were thawed with drainage in a cold room ($+4\text{ }^{\circ}\text{C}$) for 67 hours. The thawed fish was hand filleted and minces were prepared as described below. The other half of the fish was hand filleted six days after catch, and minces were prepared the day after filleting. Coarsely chopped muscle was mixed together with tap water and salt in a food processor. The minces were made with three different salts: sodium chloride, potassium chloride, and magnesium chloride at three concentrations as given in section 2.2.2. The temperature of the fish was $< +4\text{ }^{\circ}\text{C}$ the entire time.

All salts and chemicals were of “pro analysis” grade.

2.2 The experimental setup

2.2.1 The first part of experiment

In this experiment, the effect of type of buffer (BisTris or phosphate), pH, and different salts (NaCl, KCl, MgCl_2) to extract the water and salt soluble proteins from fresh and frozen haddock muscle were studied. BisTris buffer was chosen because phosphate buffer cannot be used in combination with magnesium chloride, due to precipitation of MgPO_4 . All extraction of proteins was done with two molarities of salts – 0.3 M and 0.6 M.

2.2.2 The second part of experiment

In this part, the effect of pure salts (NaCl, KCl, and MgCl_2) with different concentrations on the protein solubility properties in the salted mince was studied; and the amount of water- and salt-soluble proteins in fish mince made from fresh and frozen haddock was compared.

The added salt was composed with equimolar concentrations of salts (MgCl_2 , NaCl , KCl) at three levels: 0.1, 0.3, and 0.55 moles per kg mince (corresponding to 0.4-3.2 weight % salt). The compositional model variables were: molar concentration of salt and type of salt (MgCl_2 , NaCl , KCl). The experiment was performed on both fresh and frozen / thawed fish. This was part of a larger experiment on minces salted with different salts, where the aim was to investigate the effect of cations Na^+ , K^+ , and Mg^{2+} on physicochemical properties of raw and cooked haddock mince.

The composition of the minces used for study of protein solubility is shown in Table 1:

Based on the result of the first experiment, BisTris buffer, pH 7.0 was chosen as extraction buffer, with extraction salts NaCl , KCl , MgCl_2 with concentrations of 0.3 M and 0.6 M. The salt soluble proteins were extracted from the minces using BisTris buffer, containing the same salt, which was added to the mince. In addition, all minces made from frozen raw materials were extracted with buffer containing 0.6 M KCl . KCl was chosen because it has been used for determination of extractability of salt soluble proteins in several earlier studies (Duun and Rustad, 2008; Hultmann and Rustad, 2002).

2.3 Determination of water and salt-soluble proteins

Extractions for investigation of protein solubility were performed in two steps, resulting in a water soluble and a salt soluble fraction by a modification of the methods of Licciardello (1982) as described by Hultmann and Rustad (2002). For the fillets, two different sets of buffers were used for the determination of the water and salt soluble proteins, 50 mM phosphate buffer at pH

6.0 and 7.0 and 50 mM BisTris buffer at pH 6.0 and 7.0. For the minces 50 mM BisTris buffer, pH 7.0 was used. Approximately 4 g of white muscle or mince was homogenized in 80 ml of BisTris or phosphate buffer, pH 6 or 7 at +4 °C using an Ultra Turrax and centrifuged (20 min, 9700 g, +4 °C). The supernatant was decanted through glass wool, and the volume was made up to 100 ml with the corresponding buffer. This is the water soluble fraction. The sediment was re-homogenized in 80 ml of the same buffer, with the relevant salt and re-centrifuged. The supernatant was decanted through glass wool, and the volume was made up to 100 ml with the corresponding buffer. Two parallels were extracted for each sample.

The amount of protein in the extracts was determined by the BioRad protein assay (Bradford, 1976), using bovine serum albumin as a standard (0.1 – 1.0 mg/ml).

2.4 Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to Laemmli (1970), using PhastGel® Gradient 4 – 15 gels, SDS buffer strips, and High & Low Molecular Weight Standards, by PhastSystem with programmable power and temperature conditions for separation and staining (10 mA/gel). The gels were stained with Coomassie Brilliant Blue or Silver Staining. All equipment for electrophoresis was delivered by GE Healthcare UK Ltd (Buckinghamshire, UK). The analysis was carried out according to the instructions of the manufacturer (PhastSystem™ Separation Technique File № 130 & 200). The samples were mixed with denaturing buffer (0.5M Tris-HCl, pH 6.8, 4.4% SDS, 300mM Mercaptoethanol, 10mg/ml Bromophenol Blue) in a 1:1 ratio and

boiled for 5 minutes. The high molecular weight standard contained the following proteins: rabbit muscle myosin heavy chain (220,000), bovine plasma α_2 - macroglobulin (170,000), E.coli β - galactosidase (116,000), human transferrin (76,000), and bovine liver glutamic dehydrogenase. The low molecular weight standard contained the following proteins: rabbit muscle phosphorylase b (97,000), bovine serum albumin (66,000), chicken egg white ovalbumin (45,000), bovine erythrocyte carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and bovine milk α -lactalbumin (14,400).

2.5 Determination of pH

Determination of pH was done by mixing approximately 2 g of minced sample with an equal amount of 0.15 M KCl (Duun and Rustad, 2007; Mackie, 1993). The pH was measured by a pH meter (Mettler Toledo MP 220). Mean values were calculated from six replicates.

2.6 Statistical analyses

Results are presented as mean value \pm standard deviation. The number of parallels is given for each analysis.

The two tailed Student-t test has been used to determine the significance of effects of NaCl, KCl, $MgCl_2$ on extractability of the proteins and equality of means. Unless otherwise stated, the level of significance has been set at $p = 0.05$.

Statistical analyses were carried out in addition to comparative analysis to achieve a better understanding of the possible relationships between compositional and functional properties of

the proteins in salted haddock minces. The aim was to identify significant differences in effects by simple analysis of variance (ANOVA) using the program Unscrambler X 10.1.

3 Results and discussion

3.1 Solubility properties of protein in fresh and frozen haddock muscle

No significant differences were found between the extractability of fresh tissue water soluble proteins extracted in the different buffers (Figure 1). Freezing did not result in a statistically significant difference in WSP; this is in accordance with earlier studies (Kelleher and Hultin, 1991; Lowry, 1951; Markwell, 1978). Except for phosphate buffer at pH 6.0, there was a small but not significant decrease in water soluble proteins after freezing in all the buffers. This could be due to drip loss during thawing.

Significant differences were found for the extractability of salt soluble proteins in fresh haddock fillets depending on type of buffer, ionic strength, and type of salt (Figure 2). This is in agreement with earlier studies (Martínez-Alvarez and Gómez-Guillén, 2005; Stefansson and Hultin, 1994). The amount of salt soluble proteins was significantly higher in 0.6 M solutions than in 0.3 M solutions, except for the samples extracted with MgCl_2 at pH 6.0. The ionic strength of 0.3 M and 0.6 M MgCl_2 is 0.9 and 1.8, respectively; while the ionic strength for 0.3 M and 0.6M KCl and NaCl is 0.3 and 0.6, respectively. The ionic strength of 0.3 M MgCl_2 is therefore high enough to solubilize the salt soluble proteins.

Except for samples extracted with 0.6 M NaCl in phosphate buffer at pH 7.0, the amount of SSP in BisTris buffer is higher than in phosphate buffer ($p < 0.05$).

In phosphate buffer with 0.3 M salt (both NaCl and KCl), there is a small but significant effect of pH, while no significant effect of pH was found for BisTris buffer.

For 0.6 M NaCl and 0.6 M KCl, the extractability of the salt soluble proteins in phosphate buffer was significantly higher at pH 7.0 than at pH 6.0. For BisTris buffer, no effect of pH was found for NaCl, while there was a large and significant effect of pH for KCl. When pH is further from the isoelectric point, the protein network is more open, influencing the extractability of the proteins (Kołodziejska and Sikorski, 1980). However, for both 0.3 M and 0.6 M $MgCl_2$, there is a significantly lower extractability at pH 7.0 compared to pH 6.0; this could be due to more formation of cross linkages at higher pH.

The type of ion plays a significant role both for swelling and for the formation of cross-linkages between proteins, the high electronegativity of the Mg^{2+} ion leading to a strong binding to the polar groups of proteins, thereby strengthening interactions between the proteins and reducing the pH (Kołodziejska and Sikorski, 1980; Martínez-Alvarez and Gómez-Guillén, 2005; Thorarinsdottir et al., 2002). In the second part of the experiment, a reduction in pH was found for minces salted with $MgCl_2$ (Table 2):

Potassium chloride may reduce the protein solubility in fish muscle (Thorarinsdottir et al., 2002), probably because KCl has the ability to aggregate myosin (Aliño et al., 2010; Martínez-Alvarez et al., 2005). K^+ has a lower charge density (0.026 units of charge/molecular weight)

compared to Na^+ and Mg^{2+} (0.043, 0.082). If KCl is used for salting of tissue, it may have some difficulty in penetrating inside the muscle, compared to sodium and magnesium ions (Mackie, 1993). Comparing KCl, NaCl, and LiCl, the highest extractability was found at a concentration of 0.8M for all salts, and NaCl gave the highest extractability (Aliño et al., 2010).

The extractability of salt soluble proteins was significantly lower in frozen tissue compared to fresh (Figures 2 and 3). This is in accordance with other studies on frozen fish and is due to conformational changes in the myofibrillar proteins caused by freezing and frozen storage (Mackie, 1993). In general, the molarity of the extraction buffer has less influence on extractability of salt soluble proteins from frozen fillets than for fresh fillets. The salt concentration and type of salt may therefore have less influence on physiochemical properties of mince from frozen raw material. A small but significant difference in extractability of salt soluble proteins was found between BisTris and phosphate at salt concentrations of 0.3 M. For NaCl and KCl, the amount of proteins extracted in BisTris buffer was lower than the amount extracted in phosphate buffer; while the opposite was the case for fresh fillets. Except for MgCl_2 , the increase in extractability with increase in salt concentration from 0.3 M to 0.6 M is smaller than for the fresh fillets.

Both for fresh and frozen tissue, the type of buffer influences the amount of salt soluble proteins extracted in 0.3 M NaCl and KCl. When extraction of proteins was performed with 0.6 M NaCl and KCl, both the type of buffer and pH influenced amount of salt soluble proteins.

The molecular weight distribution of the extracted proteins was analyzed using SDS gel electrophoresis. The molecular weight distribution of water soluble proteins from fresh and

frozen haddock extracted in 50 mM phosphate or BisTris buffer, pH 6.0 and 7.0, were similar (results not shown).

The results for the salt soluble proteins show clear bands for myosin heavy chain (MHC) for samples extracted with NaCl, both for BisTris and phosphate buffers (Figures 4 a and b.)

Very few bands are visible for salt soluble proteins, extracted by 0.3 M KCl in BisTris buffer at pH 7.0, and there is no visible band for myosin heavy chain. This might be due to the ability of KCl to aggregate myosin (Aliño et al., 2010), as mentioned above. For proteins extracted by 0.3 M magnesium chloride, visible MHC bands are found, but this is not the case for extracts made in 0.6 M MgCl₂. The disappearance of MHC bands in extracts made in 0.6 M MgCl₂ is probably due to cross linkage and aggregation, due to the high electronegativity of the magnesium ions. Only weak bands for myosin light chains are visible for samples extracted in KCl and MgCl₂, while clear bands are visible for samples extracted in NaCl. For the extracts made from frozen samples, weak bands for MHC are seen for the extracts made with 0.3 M and 0.6 M NaCl and 0.3 M KCl. No bands for actin are visible; but for samples extracted with MgCl₂, bands for myosin light chains are visible. The disappearance of the myosin heavy bands is caused by conformational changes and aggregation of myosin during frozen storage (Mackie, 1993). The molecular weight of the separated proteins was affected by the state of the raw material (fresh/frozen), and no effect was seen due to the type of buffer and pH. The results of SDS gel electrophoresis confirm that using BisTris buffer to extract the salt soluble proteins has no adverse effect on the qualitative characteristics of the extracted proteins. It is therefore possible

to substitute phosphate buffer with BisTris for determination of the extractability of water- and salt- soluble proteins.

3.2 Solubility properties of proteins in salted mince

No significant differences were found in amount of extracted water soluble proteins for the minces salted with different salts. In the minces made from frozen and thawed raw material, the amount of water soluble proteins was approximately on the same level as in the fresh mince (results not shown). For mince made from fresh haddock, the extractability of salt soluble proteins in buffer containing 0.6 M of the corresponding salt (Figure 5) was around 5 % w/w for all three salts except for mince salted with 0.55 M MgCl_2 . As discussed earlier, the drop in extractability in minces with 0.55 M MgCl_2 could be due to formation of cross linkages between proteins (Barat et al., 2002; Martínez-Alvarez and Gómez-Guillén, 2005, 2006; Tahergorabi et al., 2012). Except for mince salted with 0.55 M MgCl_2 , significant differences were found between amount of salt soluble proteins extracted in buffers with salt concentrations of 0.3 M and 0.6 M for all the three salts. An increase in SSP extractability with increasing salt concentration (from 0.11 to 0.55 M in the mince) was found for minces extracted with 0.6 M NaCl. No significant differences were found for minces extracted with 0.6 M KCl. The highest extractability of SSP was found for minces with 0.55 M NaCl. The extractability in 0.3 M NaCl decreases with increasing salt concentration in the mince, while the opposite was found for KCl. This could be due to differences in charge density of the ions (Kinsella, 1982; Perisik et al., 2011). This and salt concentration are the main factors for secondary structural changes in

proteins (Barat et al., 2002; Martínez-Alvarez and Gómez-Guillén, 2005, 2006; Tahergorabi et al., 2012).

The range of molarities used in this study was from 0.11 to 0.55 (Table 1). This is low compared to studies on brine salted fish (Duerr and Dyer, 2011; Dyer et al., 1950; Thorarinsdottir et al., 2004), but the concentrations chosen are close to the ones used in the industry for minced fish products. While high salt concentrations led to denaturation of proteins, at low concentrations \approx 0.3 – 0.5 M NaCl, up to 95% of fish muscle proteins may be extracted (Costa-Corredor et al., 2010).

Figure 5 shows extractability of myofibrillar proteins from the fresh salted minces, which were extracted with the salts used in the mince. To enable comparison of extractability in one standard solution, all the minces made from frozen and thawed fillets were also extracted in 0.6 M KCl. The results show that the amount of extracted proteins depends not only on the salt added into the raw material, but also on the salt in the extraction buffer. There is a small but significant difference between amount of SSP in mince salted with 0.11 M NaCl and extracted in KCl and amount of SSP in mince salted with 0.11 M KCl and extracted with KCl (1.0 vs 0.8). For all three concentrations of $MgCl_2$, a significant difference was found between extractability in $MgCl_2$ and KCl. At $MgCl_2$ concentrations of 0.11 M and 0.55 M in the mince, the amount of SSP extracted in 0.6 M $MgCl_2$ is higher than the amount extracted in 0.6 M KCl (1.13 % vs 0.38 % w/w and 0.40 % vs 0.16 % w/w correspondingly); but at a $MgCl_2$ concentration of 0.28 M, KCl gives a higher yield of proteins compared to $MgCl_2$.

The largest differences between extraction in 0.3 and 0.6 molar salts were found in minces made from fresh raw material containing 0.28 M NaCl, 0.55 M NaCl, 0.11 M KCl, 0.11 M, and 0.28 M MgCl₂. For the other minces, no significant differences were found. For minces made from frozen raw material containing 0.28 M salt, small, but significant differences were found between amount extracted in buffers with 0.3 M and 0.6 M salt (Figure 5).

For minces made from frozen tissue, no significant differences were found between amount of SSP extracted in buffer with 0.6 M salt corresponding to the salt used in the mince and buffer with 0.6 M KCl; the only exception was minces containing 0.55 M NaCl (Figure 5).

The ionic strength of minces with MgCl₂ is approximately three times higher than in minces salted with NaCl and KCl. Reducing the amount of magnesium chloride to obtain the same ionic strength may lead both to change in taste but also to reduced water activity and therefore reduction of shelf life. The fish puddings made from salted minces with MgCl₂ were less hard and had a lower water holding capacity compared to puddings made from minces salted with NaCl and KCl (Aliño et al., 2010).

The composition of salt soluble proteins in the salted minces studied by SDS page electrophoresis (Figures 6 and 7) shows that minces salted with NaCl have the clearest bands for MHC. For minces salted with 0.11 M and 0.55 M KCl, more intense bands for myosin heavy chain were seen in extracts made with 0.3 M KCl; while very weak bands were seen for minces with 0.55 M KCl, when extracted by 0.6 KCl (Figure 6). For samples extracted with NaCl, clear bands for actin and myosin light chains are visible; while for samples extracted with KCl, clear bands for actin are visible, but the myosin light chains are weak. For samples extracted with

MgCl₂, bands for actin are not visible. As discussed earlier, this is in agreement with the ability of KCl to aggregate myosin. For minces salted with MgCl₂, MHC bands were not visible. As earlier mentioned in section 3.1, the high electronegativity of the Mg²⁺ ion may lead to the strong binding to the polar groups of proteins and lead to cross linkages and myosin aggregation.

Only minor differences were observed between SSP extracted by KCl and extracted by the corresponding salts as found with SDS-gel electrophoresis (Figure 7). Thus to compare protein extractability in minces and fillets salted with different salts, it is possible to use one extraction agent for determination of the salt soluble proteins for all the studied salts.

Conclusion

The type of buffer and pH did not influence amount of water soluble proteins in fresh or frozen haddock fillets. Significant differences were found for the extractability of salt soluble proteins in fresh haddock fillets depending on type of buffer, ionic strength, and type of salt. Except for extracts in MgCl₂, the amount of salt soluble proteins was significantly higher in buffers with 0.6M salt than in buffers with 0.3 M salt. The type of buffer influences the extractability of the salt soluble proteins, extracted by 0.3 M NaCl and KCl; while pH matters for phosphate buffer only. When extraction of proteins was performed with 0.6 M NaCl and KCl, both the type of buffer and pH affected extractability. The extractability of salt soluble proteins was significantly lower in frozen tissue compared to fresh. For frozen raw materials, the highest extractability was found in 0.6M MgCl₂, but the molarity of the buffer had less influence on the extractability of salt soluble proteins from frozen raw materials. Extractability of SSP for fresh and frozen fillets

is the same or better in BisTris buffer compared to phosphate buffer, showing that BisTris buffer can be used as an extraction buffer for salt soluble proteins.

For minces salted with NaCl, an increase in extractability of salt soluble proteins at 0.6 M NaCl was found with increasing salt concentration in the mince,

while no difference was found for minces salted with KCl and extracted with 0.6 M KCl. For minces salted with MgCl₂, the extractability in minces with 0.55 M MgCl₂ was significantly lower than for minces with 0.11 M and 0.28 M MgCl₂.

Extractability of salt soluble proteins in minces made from frozen raw material was significantly lower than in minces made from fresh raw material. For frozen raw material, use of MgCl₂ increased solubility properties of the proteins, except for the highest concentration (0.55 M).

All the effects (molarity of extracting salt, kind of salt, storage conditions) performed and analyzed by ANOVA test show insignificance of interactions. Effect of conditions presents significant difference at 95% confidence level for the salt soluble proteins, extracted by 0.6M salts only. This may mean that the nature of the salt at low concentrations does not significantly influence the extractability of the proteins.

Based on these results, it seems possible to exchange part of the NaCl in minced fish products with KCl or MgCl₂ without affecting protein solubility. However, this should be further investigated.

References:

- Albarracín, W., Sánchez, I.C., Grau, R. and Barat, J.M. 2011. Salt in food processing: usage and reduction: a review. *Int. J. Food Sci. & Technol.* 46 (7): 1329-1336.
- Aliño, M., Grau, R., Fuentes, A. and Barat, J.M. 2010. Characterisation of pile salting with sodium replaced mixtures of salts in dry-cured loin manufacture. *J. Food Eng.* 97 (3): 434-439.
- Anderson, M.L. and Ravesi, E.M. 1968. Relation between protein extractability and free fatty acid production in cod muscle aged in ice. *J. Fish. Res Bd. Can.* 10 (25): 2059-2069.
- Barat, J.M., Rodríguez-Barona, A.A. and Fito, P. 2002. Influence of increasing brine concentration in the cod-salting process. *J. Food Sci.*, 67 (5): 1922-1925.
- Bertram, H.C., Karlsson, A.H. and Andersen, H.J. 2003. The significance of cooling rate on water dynamics in porcine muscle from heterozygote carriers and non-carriers of the halothane gene--a low-field NMR relaxation study. *J. Meat Sci.*, 65 (4): 1281-1291.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *J. Anal. Biochem.*, (72): 248-254.
- Brandsma, I. 2006. Reducing sodium, a European perspective. *J. Food Technol.*, 60 (3): 24-29.
- Costa-Corredor, A., Muñoz, I., Arnau, J. and Gou, P. 2010. Ion uptakes and diffusivities in pork meat brine-salted with NaCl and K-lactate. *J. LWT - Food Sci. & Technol.*, 3: 1-8.
- Delwiche, J.F., Halpern, B.P. and DeSimone, J.A. 1999. Anion size of sodium salts and simple taste reaction times. *J. Physiol. & Behavior*, (66): 27-32.

- Desmond, E. 2006. Reducing salt: A challenge for the meat industry. *J. Meat Sci.*, 74 (1): 188-196.
- Duerr, J.D. and Dyer, W.J. 2011. Proteins in fish muscle. IV. Denaturation of salt. *J. Fish. Res Bd. Can.*, 8 (5): 325-331.
- Duun, A.S. and Rustad, T. 2007. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *J. Food Chem.*, 105: 1067-1075.
- Duun, A.S. and Rustad, T. 2008. Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at -1.4 and - 3.6 °C. *J. Food Chem.*, 106: 122-131.
- Dyer, W.J., French, H.V. and Snow, J.M. 1950. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish *J. Fish. Res Bd. Can.*, 7 (10): 585-593.
- EFSA 2005. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the commission related to the tolerable upper intake level of sodium (Request No EFSA -Q-2003-018). *J. EFSA.*, 209: 1-26.
- Floury, J., Camier, B., Rousseau, F., Lopez, C., Tissier, J.-P. and Famelart, M.-H. 2009a. Reducing salt level in food: Part 1. Factors affecting the manufacture of model cheese systems and their structure–texture relationships. *LWT, Food Sci. & Technol.*, 42: 1611-1620.
- Floury, J., Rouaud, O., Le Poullennec, M. and Famelart, M.-H. 2009b. Reducing salt level in food: Part 2. Modelling salt diffusion in model cheese systems with regards to their composition. *LWT, Food Sci. & Technol.*, 42: 1621-1628.
- FSA 2003. Salt and health. Norwich : 134.

- Haard, N.F. 1992. Control of chemical composition and food quality attributes of cultured fish. *Food Research Int.*, 25 (4): 289-307.
- Hultmann, L. and Rustad, T. 2002. Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *J. Aquat. Food Prod. Tech.*, (11): 105-123.
- Jafarpour, A. and Gorczyca, E.M. 2012. Contribution of sarcoplasmic proteins to myofibrillar proteins gelation. *J. Food Sci.*, 77 (2): 73-81.
- Kelleher, S.D. and Hultin, H.O. 1991. Lithium chloride as a preferred extractant of fish muscle proteins. *J. Food Sci.*, 2 (56): 315-317.
- Kinsella, J.E. 1976. Functional properties of proteins in foods: a survey, *crit.rev. J. Food Sci. Nutr.*, 7: 219.
- Kinsella, J.E. 1982. Protein structure and functional properties: emulsification and flavor binding effects. In *food protein deterioration: mechanisms and functionality*. Am. Chem. Society. Washington.
- Kołodziejska, I. and Sikorski, Z.E. 1980. Inorganic salts and extractability of fresh and frozen fish proteins. *Int. J. Refrigeration*, 3 (3): 151-155.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227: 680-685.
- Lauritzen, K., Akse, L., Gundersen, B. and Olsen, R.L. 2004. Effects of calcium, magnesium, and pH during salt curing of cod (*Gadus morhua L.*). *J. Sci. Food & Agr.*, 84: 683-692.

- Licciardello, J.J., Ravesi, E.M., Lundstrom, R.C., Wilhelm, K.A., Correia, F.F., & Allsup, M.G. 1982. Time-temperature tolerance and physical-chemical quality tests for frozen red hake. *J. Food Quality*, 5: 215-234.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Mackie, I.M. 1993. The effects of freezing on flesh proteins. *J. Food Rev. Int.*, 9 (4): 575-610.
- Markwell, M.K., Haas, S.M., Bieber, L.L., Tolbert, N.E. 1978. Modification of Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *J. Anal. Biochem.*, 87: 206-210.
- Martínez-Alvarez, O., Borderias, A.J. and Gómez-Guillén, M.C. 2005. Sodium replacement in the cod (*Gadus morhua*) muscle salting process. *J. Food Chem.*, 93: 125-133.
- Martínez-Alvarez, O. and Gómez-Guillén, M.C. 2005. The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*). *J. Food Chem.*, 92: 71-77.
- Martínez-Alvarez, O. and Gómez-Guillén, M.C. 2006. Effect of brine salting at different pHs on the functional properties of cod muscle after subsequent dry salting. *J. Food Chem.*, 94: 123-129.
- Mitchell, M., Brunton, N.P. and Wilkinson, M.G. 2011. Current salt reduction strategies and their effect on sensory acceptability: a study with reduced salt ready meals. *Eur Food Res Technol*, 232: 529-539.

- Munasinghe, D.M.S. and Sakai, T. 2006. Sodium chloride (0.8 M) as a better protein extractant for fish meat quality assessments. *J. Food Sci.*, 68 (3): 1059-1062.
- Murphy, C., Cardello, A.V. and Brand, J.G. 1981. Tastes of fifteen halide salts following water and NaCl: Anion and cation effects. *J. Physiol. & Behavior*, 26 (6): 1083-1095.
- Nayak, R., Kenney, B.P. and Slider, S. 1996. Protein extractability of turkey breast and thigh muscle with varying sodium chloride solutions as affected by calcium, magnesium and zinc chloride. *J. Food Sci.*, 61 (6): 1149-1154.
- Nguyen, M.V., Thorarinsdottir, K.A., Gudmundsdottir, A., Thorkelsson, G. and Arason, S. 2011. The effects of salt concentration on conformational changes in cod (*Gadus morhua*) proteins during brine salting. *J. Food Chem.*, 125 (3): 1013-1019.
- Perisik, N., Afseth, N.K., Ofstad, R. and Kohler, A. 2011. Monitoring protein structural changes and hydration in bovine meat tissue due to salt substitutes by Fourier Transform Infrared (FTIR) microspectroscopy. *J. Agr. Food Chem.*, 59: 10052-10061.
- Ramírez, J., Uresti, R., Téllez, S. and Vázquez, M. 2002. Using Salt and Microbial Transglutaminase as Binding Agents in Restructured Fish Products Resembling Hams. *J. Food Sci.*, 67 (5): 1778-1784.
- Rhee, K.S. and Ziprin, Y.A. 2001. Pro-oxidative effects of NaCl in microbial growth - controlled and uncontrolled beef and chicken. *Meat Sci.*, 57: 105-112.
- Richardson, R.I. and Jones, J.M. 1987. The effects of salt concentration and pH upon water-binding, water-holding and protein extractability of turkey meat. *Int. J. Food Sci. Tech.*, 22 (6): 683-692.

- Ruusunen, M. and Puolanne, E. 2004. Reducing sodium intake from meat products. Meat Sci., Helsinki, Finland, 50-th Int. Congr. Meat Sci. & Tech., (ICoMST).
- Rössner, S., Verkleij, T., Goldbohm, R.A. and Jong, G.A.H. 2009. Sodium reduction in meat products: an opportunity for industry. Parallel session 9: Meat in nutrition. The 55th Int. Congress Meat Sci. & Tech. (ICoMST), Copenhagen, Denmark,
- Stefansson, G. and Hultin, H.O. 1994. On the solubility of cod muscle protein in water. J. Agr. & Food Chem., 42 (12): 2656-2664.
- Tahergorabi, R., Beamer, S.K., Matak, K.E. and Jaczynski, J. 2012. Salt substitution in surimi seafoof and its effects on instrumental quality attributes. LWT - Food Sci. Technol., 48: 175-181.
- Thorarinsdottir, K.A., Arason, S., Bogason, S.G. and Kristbergsson, K. 2004. The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). Int. J. Food Sci. Technol., 39: 79-89.
- Thorarinsdottir, K.A., Arason, S., Geirsdottir, M., Bogason, S.G. and Kristbergsson, K. 2002. Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. Food Chem., 77 (3): 377-385.
- Ugawa, S. 2003. Identification of sour-taste receptor genes. Anat. Sci. Int., 78 (4): 205-210.
- Xiong, Y.L. and Brekke, C.J. 1991. Gelation properties of chicken myofibrils treated with calcium and magnesium chlorides. J. Muscle Foods, 2 (1): 21-36.
- Åsli, M. and Mørkøre, T. 2012. Brines added sodium bicarbonate improve liquid retention and sensory attributes of lightly salted Atlantic cod. LWT - Food Sci. Technol., 46: 196-202.

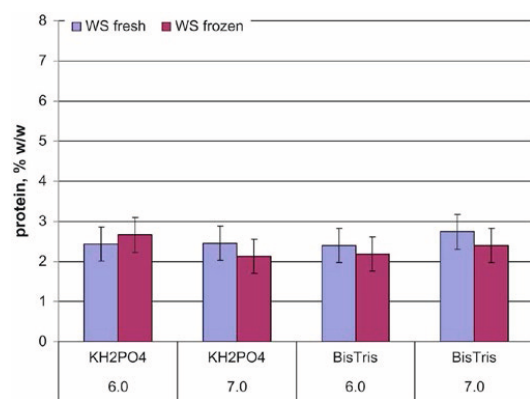
Table 1. Ionic strength, molar concentration, and salt concentration in the salted minces (I and M) * was calculated corresponding to the mass of mince without added water.

Sample	I *	M *	Amount of salt, g	Salt %
NaCl	0.11	0.11	3.0	0.4
NaCl	0.28	0.28	7.5	1.0
NaCl	0.55	0.55	15.0	2.0
KCl	0.11	0.11	3.8	0.5
KCl	0.28	0.28	9.6	1.3
KCl	0.55	0.55	19.2	2.5
MgCl ₂	0.33	0.11	10.4	0.7
MgCl ₂	0.83	0.28	26.1	1.6
MgCl ₂	1.66	0.55	52.2	3.2

Table 2. pH in minces made from fresh and frozen-thawed haddock fillets; values are given as means \pm STDEV, n=6.

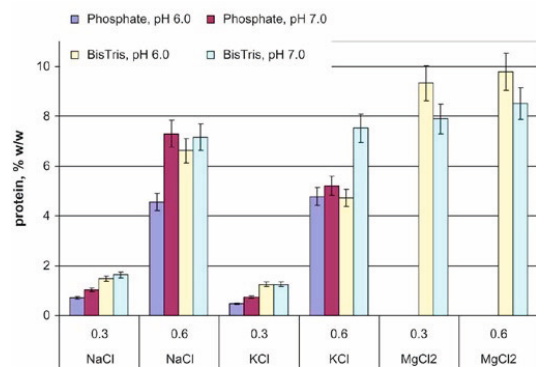
Salt concentration in minces:	NaCl			KCl			MgCl ₂			Unsalted minces
	0.1 M	0.3 M	0.55 M	0.1 M	0.3 M	0.55 M	0.1 M	0.3 M	0.55 M	
Fresh	6.51	6.46	6.34	6.63	6.54	6.49	6.09	5.71	5.52	7.0
Frozen	6.48	6.44	6.24	6.55	6.52	6.48	6.16	5.97	5.63	7.2

Figure 1. Extractability of water soluble proteins in fresh and frozen haddock fillets (% wet weight) in 0.05M BisTris and phosphate buffer, pH 6.0 and 7.0. Values are given as mean \pm stdev, n=6.



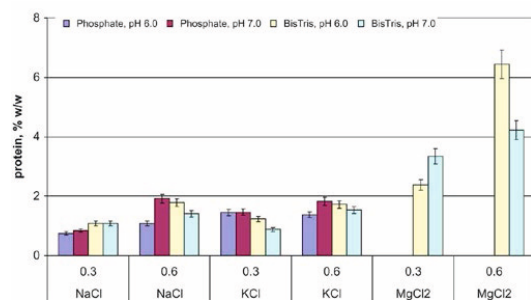
Accepted Manuscript

Figure 2. Extractability of salt soluble proteins (% of wet weight) in fresh haddock fillets in 0.3 M and 0.6 M NaCl, KCl, MgCl₂ in phosphate and BisTris buffers at pH 6.0 and pH 7.0. Values are given as mean (n=2) ± uncertainty of the method (7.5 %).



Accepted Manuscript

Figure 3. Extractability of salt soluble proteins (% of wet weight) in frozen haddock fillets in 0.3 M and 0.6 M NaCl, KCl, MgCl₂ in phosphate and BisTris buffers at pH 6.0 and pH 7.0. Values are given as mean (n=2) ± uncertainty of the method (7.5 %).



Accepted Manuscript

Figure 4 (a,b). Composition of salt soluble proteins extracted in BisTris and phosphate buffers with 0.3 M and 0.6 M NaCl, KCl, MgCl₂, pH 7.0, analyzed by SDS PAGE. Molecular weight standards are presented as: LMW – low and HMW – high.

4 a wells:

1-0.3M NaCl in BisTris; 2-0.6M NaCl in phosphate; 3-0.6M NaCl in BisTris; 4-0.3M KCl in phosphate; 5-0.3M KCl in BisTris; 6-0.6M KCl in phosphate; 7-0.6M KCl in BisTris; 8-0.3M MgCl₂ in BisTris; 9-0.6M MgCl₂ in BisTris.

4 b wells:

1-0.3M NaCl in BisTris; 2-0.6M NaCl in phosphate; 3-0.6M NaCl in BisTris; 4-0.3M KCl in phosphate; 5-0.3M KCl in BisTris; 6-0.6M KCl in phosphate; 7-0.6M KCl in BisTris; 8-0.3M MgCl₂ in BisTris; 9-0.6M MgCl₂ in BisTris.

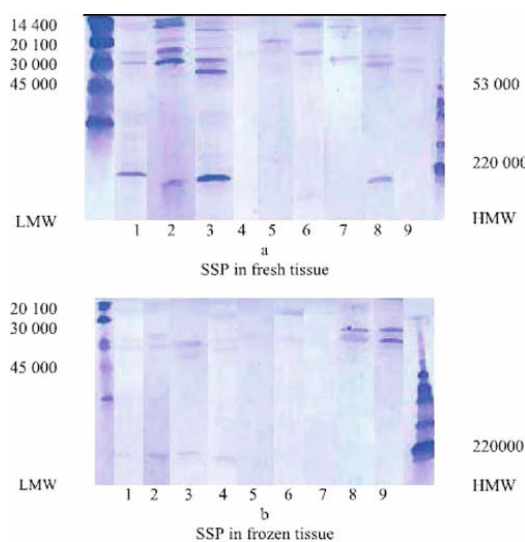


Figure 5. Extractability of salt soluble proteins in salted minces made from fresh and frozen haddock. Extraction was done in 0.3 M and 0.6 M salt corresponding to the salt in the mince. Results are given in % w/w of muscle in the sample. Values are given as mean (n=2) ± uncertainty of the method (7.5 %).

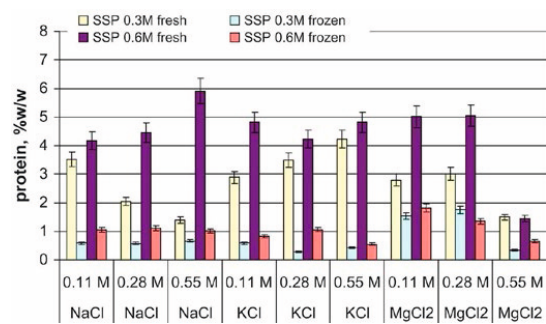
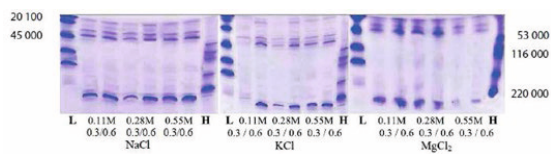


Figure 6. Composition of salt soluble proteins from minces made from fresh haddock, salted with NaCl, KCl, MgCl₂ in 3 different concentrations (0.11M, 0.28M, 0.55M), extracted in BisTris buffer, pH 7.0 by 0.3 M and 0.6 M salts, analyzed by SDS-PAGE. Molecular weight standards are presented as: L (LMW) – low and H (HMW) – high.



Accepted Manuscript

Figure 7. Composition of salt soluble proteins from frozen minces using SDS-PAGE, made from frozen raw material, salted with NaCl, KCl, MgCl₂ in 3 different concentrations (0.11M, 0.28M, 0.55M), extracted by the 0.6 M corresponding salt and 0.6 M KCl for comparison, in BisTris buffer, pH 7.0. Molecular weight standards are presented as: L (LMW) – low and H (HMW) – high. Numbered:

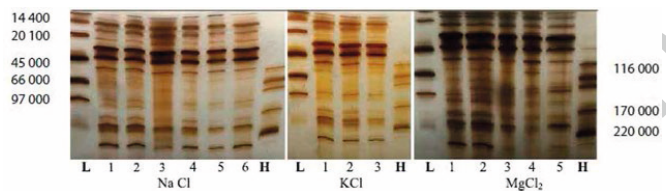
Well NaCl 1,3,5 salted with 0.11 M, 0.28 M and 0.55 M NaCl, extracted with 0.6 M NaCl;

Well NaCl 2,4,6 salted with 0.11 M, 0.28 M and 0.55 M NaCl, extracted with 0.6 M KCl;

Well KCl 1,2,3 salted with 0.11 M, 0.28 M and 0.55 M KCl, extracted with 0.6 M KCl;

Well MgCl₂ 1,3,5 salted with 0.11 M, 0.28 M and 0.55 M MgCl₂, extracted with 0.6 M MgCl₂;

Well MgCl₂ 2,4,6 salted with 0.11 M, 0.28 M and 0.55 M MgCl₂, extracted with 0.6 M KCl.



Paper V



Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Novel utilization of milk-based ingredients in salt reduced fish pudding

Kirsti Greiff^{a, b, *}, Charlotte Jatteau Staurem^b, Berit Nordvi^c, Turid Rustad^b^a SINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway^b Department of Biotechnology, Norwegian University of Science and Technology, Sem Sælands v. 6/8, N-7491, Trondheim, Norway^c TINE SA R&D, Bedriftsveien 7, N-0950, Oslo, Norway

ARTICLE INFO

Article history:

Received 10 December 2014
 Received in revised form
 25 February 2015
 Accepted 17 March 2015
 Available online 25 March 2015

Keywords:

Sodium reduction
 Salt substitute
 Milk mineral
 Fish pudding
 Physicochemical properties

ABSTRACT

Both the food industry and the health authorities have increased their focus on salt reduction in food, due to the known negative health effect of high sodium intake. For this reason, there is great interest in developing products with reduced salt content without affecting properties related to sensory parameters, texture, yield and shelf-life. Whey and milk based permeates with favorable combinations of milk minerals and lactose can be used as natural ingredients in for instance meat and fish products and work as salt replacers. The aim of this study was to investigate how two different types of milk minerals; low mineral permeate, based on whey obtained in cheese production, and high mineral permeate, based on milk, affect the quality of comminuted fish products, using fish pudding as a model. Results showed that low mineral permeate can improve textural and water-holding properties of puddings at salt concentrations down to 0.8%, while it does not affect salt flavor. High mineral permeate contributes to changes in the textural and water-holding properties, and also increases salt flavor. Based on investigated factors, high mineral permeate is regarded as a promising salt replacer allowing for considerable salt reduction in fish puddings.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Salt (NaCl) is a well-established and widely used food additive due to its contribution to several desired properties, in addition to being inexpensive. A high intake of sodium is associated with greater risk of high blood pressure, which is a major cause of cardiovascular disease and stroke (Cook et al., 2007; He, Burnier, & MacGregor, 2011). Based on this, public health and regulatory authorities (FSA, 2004; The Norwegian Directorate of Health, 2011; WHO, 2006) have published advisory guidelines for reduction of salt intakes down to 5 g/day or lower. However, the daily salt intake per person in most European countries is twice as high as the maximal recommended salt intake.

Salt is commonly employed in fish processing, due to its excellent preservative effects, taste as well as positive technological effects (Fuentes, Fernandez-Segovia, Barat, & Serra, 2010; Martínez-Alvarez & Gómez-Guillén, 2006). Favorable health effects from

intake of fish combined with the need to lower salt intake in the population has led to the desire of producing fish-based products with high fish and low salt content. Whey and milk-based permeates with favorable combinations of milk minerals and lactose can be used as natural ingredients in for instance meat and fish products and work as salt replacers. The food industry employs various milk-based ingredients in form of powders such as whey, whey permeate, lactose, skimmed milk and whole milk, which contributes to textural properties, water-holding and desirable milk, sweet and umami flavor. In a product with a high percentage of milk and/or cream such as fish pudding, the flavor of milk and umami are desired. The lactose in the milk-based ingredients contribute to a browning Maillard reaction as well, this is desirable in products such as fish puddings (BeMiller & Whistler, 1996). Challenges connected to the use of high level of whey permeate and milk-based permeate are mainly connected to their contribution of flavors that might be unexpected in some foods.

In the manufacture of minced fish products such as fish puddings and pâtés, the functional properties of the milk proteins and the fish muscle proteins including water holding capacity and gelling properties are important influencing the product quality (Martínez-Alvarez & Gómez-Guillén, 2005). Fish muscle proteins

* Corresponding author. SINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway. Tel.: +47 47 90 38 91; fax: +47 93 27 07 01.
 E-mail address: kirsti.greiff@sintef.no (K. Greiff).

can form stable gels when heated and the ionic strength as well as type of salt is important for the behavior of the proteins during heating. To form gels, the myofibrillar proteins must be solubilized (Jafarpour & Gorczyca, 2012). Both water binding and holding properties as well the gelling properties have been shown to be related to the solubility properties of the proteins in the food (Kinsella, 1976; Nayak, Kenney, & Slider, 1996; Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2011).

In addition to potentially allowing a salt reduction, the use of whey permeate in food for human consumption is favorable due to the fact that whey is a by-product and large amounts of whey is discarded without exploitation of its potential as a food ingredient. Whey and milk based permeates contain high amounts of minerals such as Ca^{2+} , Na^+ and K^+ and due to this it would be valuable to increase the knowledge of using whey permeate as a salt replacer. Various authors have reported that when NaCl is partially replaced with other salts like KCl, MgCl_2 , CaCl_2 this will affect the enzyme activity, protein matrix and texture (Andreetta-Gorelkina, Greiff, Rustad, & Aursand, 2014; Barat, Pérez-Esteve, Aritoy, & Toldra, 2012; Martínez-Alvarez & Gómez-Guillén, 2013).

The aim of this study was to investigate the possibility of using milk minerals to reduce the salt content in products based on haddock (*Melanogrammus aeglefinus*) mince. Two different powders were tested; low mineral whey permeate (LM), which is based on whey obtained in cheese production, and high mineral milk permeate (HM), which is based on milk. In order to investigate the outcome of their addition in fish-based products, the effect on the flavor of fish puddings, and also their effect on other physical properties such as texture, color, water-holding properties and protein solubility were investigated.

2. Material and methods

2.1. Chemical compounds

Ammonium Chloride (PubChem CID:25517), Ammonium Hydroxide (PubChem CID:14923), Ammonium Hydrogen Fluoride (NH_4FHF < 1%, LD_{50} mg/kg not found), sodium chloride (PubChem CID:5234), potassium chloride (PubChem CID:4873), potassium dihydrogen phosphate (PubChem CID:516951), (Thermo Fisher Scientific, USA or ACS, ISO, Merck, MA, USA). All the chemicals were of analytical-reagent grades.

2.2. Materials

Haddock (*Melanogrammus aeglefinus*) fillets (Laks- & Vildtcentralen AS, Oslo, Norway) were stored at 4 °C until production of the fish mince (1 day), and put on ice during production. Two different permeate powders were used in this study; low mineral whey permeate (LM) with high lactose and low mineral content, and high mineral milk permeate (HM) with low lactose and high mineral content. LM is constituted by 85 g lactose/100 g, 7 g salt/100 g, 3 g NPN (non-protein nitrogen)/100 g and 1 g lipids/100 g and HM by 47 g lactose/100 g, 37 g salt/100 g, 5 g NPN (non-protein nitrogen)/100 g and <1 g lipids/100 g. Additional ingredients used in the minces were skimmed milk (TINE SA, Oslo, Norway), potato flour (HOFF SA, Gjøvik, Norway) and Jozo salt (iodine free, Akzo Nobel Salt, Göteborg, Sweden).

2.3. Sample preparation

Preparation of fish puddings was performed at TINE R&D (Oslo, Norway). Each recipe was produced in batches of 5 kg. The minces were prepared in a bowl cutter (KILIA, Neunmünster, Germany). Ingredients were added during mincing in the following order:

haddock fillet, salt, spices and permeate powders, milk (1/2), potato flour and milk (1/2). Controls were produced without addition of permeate powder. Mincing was ended when the mince temperature had reached 15 °C (after ~4 min). Mince was transferred to aluminium moulds (lightly sprayed with cooking spray to avoid sticking) to give puddings of approximately 200 g, and were cooked in water bath at 110 °C for 45 min (preliminary experiment) or 50 min (main experiment). The puddings were vacuum-packed (98% vacuum, 0% gas, time 1.6, Tecnovac, Confezionatrici Packaging Machines, Grassobio, Italy) and stored at 4 °C until further analyses.

2.4. Preliminary experiment

The composition of fish puddings in the preliminary experiment are given in Table 1. Two salt levels (0.5 and 1.0 g/100 g) and five permeate powder levels (control), 1.5 g LM/100 g, 2.9 g LM/100 g, 1.5 g HM/100 g, 2.9 g HM/100 g were used in the preliminary experiment. The calculated salt concentration in the minces is based on the sodium content in all the added ingredients (total sodium content \times 2.54). The potassium (K^+) content was calculated to be between 0.25 and 0.77 g/100 g. Three of the recipes were produced in duplicate batches to estimate reproducibility, giving a total of 13 batches. The minces had a content of 52.3 ± 1.0 g haddock fillet/100 g, 43.4 ± 0.8 g skimmed milk/100 g, 2.0 g potato flour/100 g, 0.1 g mace/100 g and 0.1 g white pepper/100 g.

2.5. Main experiment

The composition of fish puddings in the main experiment are given in Table 2. Based on the results from the preliminary experiment, three salt levels (0.6, 0.8 and 1.0 g/100 g) and three permeate powder levels (control, 2.9 g LM/100 g and 2.2 g HM/100 g) were used in the main experiment. The calculated salt concentration in the minces is based on the sodium content in all the added ingredients (total sodium content \times 2.54). The potassium content in the control, LM and HM mince were calculated to be 0.25, 0.30 and 0.64 g/100 g, respectively. Three of the recipes were produced in duplicate batches, giving a total of 12 batches. The minces had a content of 52.7 ± 0.7 g/100 g haddock fillet, 43.6 ± 0.8 g/100 g skimmed milk, 2.0 g/100 g potato flour, 0.1 g/100 g mace and 0.1 g/100 g white pepper.

2.6. Physicochemical analyses

2.6.1. Cooking loss

Cooking loss (%) was determined by weighing the mince in pre-weighed moulds before cooking, and weighing them again after cooking and cooling.

2.6.2. Water holding capacity/water content

Water-holding capacity (WHC) of the puddings was determined using a low-speed centrifugation method as described by Eide, Børresen, and Strøm (1982). Analyses were run in quadruplicate. The WHC is expressed as percentage of water retained in the pudding cubes after centrifugation at $210 \times g$ for 5 min.

Total water content in haddock fillet, fish minces and puddings was determined by drying approximately 2 g of sample ($n = 2$) at 105 °C for 24 h. Results were mean of 2 determinations and were expressed as g water/100 g of sample.

2.6.3. Extraction of water and salt soluble proteins

Proteins were extracted from the minces by a modification of the methods of Anderson and Ravesi (1968) and Licciardello et al. (1982), as previously described by Hultmann and Rustad (2002).

Table 1

Composition of fish puddings in the preliminary experiment. Salt level is indicated by LS = low salt (0.5 g/100 g) and HS = high salt (1.0 g/100 g), permeate addition by LM = low mineral whey permeate and HM = high mineral milk permeate (1.5 g/100 g or 2 = 2.9 g/100 g).

Sample	Salt ^a (g/100 g)	K:Na ratio	Fat (g/100 g)	Carbohydrates (g/100 g)	Protein (g/100 g)
LS/Control	0.5	1.3	0.15	3.7	10.3
LS/LM1.5	0.5	1.4	0.16	4.9	10.2
LS/LM2.9	0.5	1.5	0.18	6.1	10.1
LS/HM1.5	0.5	2.5	0.15	4.4	10.3
LS/HM2.9	0.5	4.1	0.15	5	10.2
HS/Control	1	0.6	0.15	3.7	10.3
HS/LM1.5	1	0.7	0.16	4.9	10.2
HS/LM2.9	1	0.7	0.17	6.1	10.1
HS/HM1.5	1	1.1	0.15	4.4	10.2
HS/HM2.9	1	1.7	0.15	5.0	10.1

^a The salt content (g/100 g) is calculated by using the formula: Added sodium (Na⁺) including natural Na⁺ content in the meat × 2.54 (recalculation factor for NaCl from Na⁺).

Analyses were run as duplicates. Mince (6.5 g) was homogenized using an Ultra-Turrax disperser (IKA, Labortechnik, Staufen, Germany) for 10 s in 0.05 mol/l phosphate buffer (80 ml, pH 7.0). Next, they were centrifuged at 10,400 × g for 20 min at 4 °C. The supernatant was decanted and the volume was adjusted to 100 ml with phosphate buffer. This constituted the water soluble fraction. The sediment was homogenized in 0.05 mol/l phosphate buffer with 0.6 mol/l KCl (80 ml, pH 7.0) for 10 s, and centrifuged as above. The supernatant was decanted and the volume was adjusted to 100 ml with phosphate buffer with 0.6 mol/l KCl. This constituted the salt soluble fraction.

The Bio-Rad protein assay (Bradford, 1976) was used to determine the amount of proteins in the extracts, using bovine serum albumin (1 mg/ml) as a standard. The extractable proteins were expressed in g protein/100 g of muscle in the sample.

2.6.4. Determination of sodium and salt content

Sodium concentration in puddings was determined using a Sodium Selective Electrode (Orion ROSS Sure-Flow Sodium, Thermo Fisher Scientific, MA, USA). The sample preparation and measurement method is developed by Kivikari (1996) and modified by Greiff et al. (2014). 7.5 g of pudding was homogenised in deionized water (18.2 MΩ, 100–150 ml) using an Ultra-Turax disperser (IKA, Labortechnik, Staufen, Germany) at low to intermediate speed for 20 s. The samples were heated at 90 °C for 30 min, cooled to room temperature, diluted to 250 ml with deionized water. Finally, samples were filtered through a cellulose filter paper (Whatman no. 4, Whatman International Ltd., Maidstone, UK) and the filtrate was used for the measurements.

Measurements were performed based on direct measurement at room temperature. A calibration curve was made by using four

standards of dried, analytical grade NaCl (Sodium chloride for analysis, ACS, ISO, Merck, MA, USA) and deionized water. Sodium Ionic Strength Adjuster (ISA, Thermo Fisher Scientific, MA, USA) was added to all solutions to ensure that samples and standard had similar ionic strengths. The salt content (g/100 g) was calculated by using the formula: measured sodium (Na⁺) × 2.54 (recalculation factor for NaCl from Na⁺). For each pudding (n = 2), three replicates were prepared and measured, and average values were calculated. Results were means of six determinations from each sample and were expressed as g NaCl/100 g pudding.

2.6.5. Texture analysis

Texture analyses were performed in the rheology laboratory at NTNU with a Texture Analyzer TA XT.plus using Exponent Software (Stable Micro System, Surrey, UK) for data processing. The measurements were performed with a weighing cell of 5 kg and a cylindrical probe of 50 mm (P50). Texture Profile Analysis (TPA) was run on room tempered fish pudding cubes of approx. 18 × 18 × 18 mm. The analysis settings were as follows; pre-test speed: 1.00 mm/s, test speed: 2.00 mm/s, post-test speed: 2.00 mm/s, target mode: distance, distance: 15 mm, time: 5.0 s, trigger type: auto (force), trigger force: 5.0 g. Force (N) was collected at first break in the continuous time/force curve. For each pudding (n = 2), five replicates were prepared and measured, and average values were calculated.

2.6.6. Color

The color analyses were performed using a Chroma Meter CR-400 (Konika Minolta, Osaka, Japan) calibrated with a white calibration plate (CH00). The CIE L*a*b* color system was chosen for the measurements. Color was measured on a cross-section (n = 3) of the puddings. The measurements were taken on the most

Table 2

Composition of fish minces in the main experiment. Salt level is indicated by LS = low salt (0.6 g/100 g), IS = intermediate salt (0.8 g/100 g) and HS = high salt (1.0 g/100 g), permeate addition by LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.2 g/100 g).

Sample	Salt ^a (g/100 g)	K:Na ratio	Fat (g/100 g)	Carbohydrates (g/100 g)	Protein (g/100 g)
LS/Control	0.6	1.1	0.15	3.7	10.3
LS/LM	0.6	1.2	0.18	6.1	10.1
LS/HM	0.6	2.1	0.15	4.7	10.2
IS/Control	0.8	0.8	0.15	3.7	10.3
IS/LM	0.8	0.9	0.17	6.1	10.1
IS/HM	0.8	1.6	0.15	4.7	10.2
HS/Control	1	0.6	0.15	3.7	10.3
HS/LM	1	0.7	0.17	6.1	10.1
HS/HM	1	1.3	0.15	4.7	10.2

^a The salt content (g/100 g) is calculated by using the formula: Added sodium (Na⁺) including natural Na⁺ content in the meat × 2.54 (recalculation factor for NaCl from Na⁺).

Table 3

Cooking loss, WHC, moisture, salt content and color of puddings in the preliminary experiment. Salt level is indicated by LS = low salt (0.5 g/100 g) and HS = high salt (1.0 g/100 g), permeate addition by LM = low mineral whey permeate and HM = high mineral milk permeate (1.5 g/100 g or 2.9 g/100 g). Significant difference with regard to salt level and permeate powder within the salt levels is indicated with lower-case and upper-case letters, respectively.

Sample	Cooking loss (g/100 g)	WHC (%)	Moisture (g/100 g)	Salt (g/100 g)	L*	a*	b*	WI
LS/Control	6.8 ± 0.8	83.9 ± 3.3 ^{Aa}	82.3 ± 0.3 ^{Aa}	0.45 ± 0.00 ^{Aa}	74.9 ± 0.9 ^{Aa}	-5.9 ± 0.09 ^{Aa}	11.7 ± 0.07 ^{Aa}	71.7 ± 0.8 ^{Aa}
LS/LM1.5	6.7 ± 1.2	82.2 ± 4.1 ^{Aa}	81.1 ± 0.5 ^{Ba}	0.48 ± 0.02 ^{Aa}	74.2 ± 0.7 ^{ABa}	-6.0 ± 0.11 ^{Aa}	11.7 ± 0.15 ^{Aa}	71.06 ± 0.7 ^{ABa}
LS/LM2.9	6.5 ± 1.3	82.5 ± 2.3 ^{Aa}	81.0 ± 0.2 ^{Ba}	0.49 ± 0.02 ^{Aa}	73.9 ± 0.5 ^{ABa}	-6.4 ± 0.13 ^{Ba}	11.5 ± 0.12 ^{Aa}	70.8 ± 0.4 ^{ABa}
LS/HM1.5	6.8 ± 0.06	91.7 ± 0.3 ^{Ba}	81.7 ± 0.2 ^{ABa}	0.46 ± 0.03 ^{Aa}	73.1 ± 0.3 ^{BCa}	-6.2 ± 0.09 ^{Ba}	10.4 ± 0.2 ^{Ba}	70.6 ± 0.2 ^{ABa}
LS/HM2.9	5.9 ± 0.9	93.9 ± 0.7 ^{Ba}	81.2 ± 0.3 ^{Ba}	0.49 ± 0.00 ^{Aa}	72.1 ± 0.2 ^{Ca}	-6.4 ± 0.03 ^{Ba}	9.6 ± 0.2 ^{Ca}	69.8 ± 0.2 ^{Ba}
HS/Control	6.1 ± 0.6	89.5 ± 0.2 ^{Ab}	82.3 ± 0.3 ^{Aa}	0.91 ± 0.00 ^{Ab}	73.2 ± 0.3 ^{ABb}	-6.1 ± 0.12 ^{Ab}	10.4 ± 0.10 ^{Ab}	70.7 ± 0.2 ^{Ab}
HS/LM1.5	5.9 ± 0.9	91.5 ± 0.9 ^{ABb}	81.5 ± 0.2 ^{Ba}	0.97 ± 0.04 ^{Ab}	73.5 ± 0.3 ^{Ab}	-6.5 ± 0.11 ^{BCb}	10.4 ± 0.2 ^{ABb}	70.8 ± 0.3 ^{Ab}
HS/LM2.9	5.8 ± 0.4	93.2 ± 2.7 ^{Bb}	80.9 ± 0.3 ^{Ca}	0.96 ± 0.02 ^{Ab}	72.3 ± 0.4 ^{BCb}	-6.7 ± 0.07 ^{Cb}	10.4 ± 0.08 ^{Ab}	69.7 ± 0.4 ^{BBb}
HS/HM1.5	6.7 ± 0.5	92.8 ± 1.1 ^{ABb}	81.5 ± 0.3 ^{Ba}	0.97 ± 0.007 ^{Ab}	72.1 ± 0.2 ^{Cb}	-6.3 ± 0.05 ^{ABb}	9.9 ± 0.11 ^{Bb}	69.7 ± 0.2 ^{BBb}
HS/HM2.9	6.5 ± 0.2	94.4 ± 1.4 ^{Bb}	81.0 ± 0.0 ^{Ca}	1.04 ± 0.07 ^{Ab}	70.4 ± 0.4 ^{Db}	-6.4 ± 0.08 ^{Bb}	9.3 ± 0.17 ^{Cb}	68.3 ± 0.3 ^{Cb}
F ratio								
Permeate (LS)	0.89 ^{ns}	16.46 ^{***}	6.95 ^{**}	1.46 ^{ns}	7.62 ^{**}	14.89 ^{***}	129.94 ^{***}	3.79 [*]
Permeate (HS)	2.75 ^{ns}	5.98 ^{***}	21.74 ^{***}	1.09 ^{ns}	47.55 ^{***}	16.72 ^{***}	45.64 ^{***}	36.31 ^{**}
Salt ^b	1.90 ^{ns}	-4.83 ^{***}	0.64 ^{ns}	-22.14 ^{***}	4.36 ^{***}	3.72 ^{**}	4.84 ^{***}	4.02 ^{***}

^a p values: ***, p < 0.001; **, p < 0.01; *, p < 0.05; ns, non significant.

^b T ratio is given for salt level.

representative parts of the samples, and areas with a darker crust than the rest of the surface or larger air holes were avoided. The following equation to calculate whiteness index (WI): $WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Benjakul, Visessanguan, & Srivilai, 2001; Duangmal & Taluengphol, 2010; Rawdkuen & Benjakul, 2008; Xiong et al., 2009). For each pudding (n = 2), three replicates were measured, and average L*, a* and b* values were calculated.

2.7. Descriptive analysis

A descriptive quantitative sensory analysis (DA) of fish puddings made in the main experiment was performed at TINE R&D in Stavanger 8 days after production of the puddings. The panel consisted of six assessors (one man and five women). The evaluation included 14 different attributes (color, firmness, cohesiveness, elasticity, chewiness, coarseness, solubility, flavor intensity, fish taste, salt taste, bouillon/umami taste, harsh taste, metallic taste and after-taste), and the vocabulary was in accordance with ISO Standard 5492 (ISO, 1992). Each attribute was evaluated on a scale from 1 to 9. Before the session, the panel members participated in a calibration session by evaluating the test extremities in order to agree on the use of attributes and scales. The puddings were served in a room where the temperature was held at around 14 °C, and the temperature measured in randomly selected puddings was 10–12 °C. The samples were served in a randomized order (n = 2). The software EyeQuestion (Logic8, Wageningen, Netherlands) was used for analysis setup and data collection.

2.8. Statistical analysis

The uncertainty of the results is presented as standard deviation except for the amount of extractable proteins, where it is presented as the uncertainty of the method (7.5%) as reported by Andreetta-Gorelkina et al. (2014).

Statistical analyses were performed using the software Minitab[®] (v.17.1.0, Coventry, UK). One-way ANOVA was run to investigate whether there were significant differences based on salt level and on addition of LM and HM within each salt level. Replicates of the recipes were incorporated with the rest of the data in the ANOVA. Where significant differences were found, the Tukey test (least significant difference) was chosen as a post hoc test. 2-sample t-tests were used to find differences between two different groups.

Results from the sensory analysis were evaluated using general linear model (GLM) with assessor and recipe as factors. The level of significance was set at p < 0.05.

3. Results and discussion

3.1. Preliminary experiment

Physicochemical properties (cooking loss, WHC, moisture, salt content and color) of the fish puddings from the preliminary experiment are shown in Table 3. Salt concentration and permeate addition did not lead to any differences in cooking loss. The highest salt level (1.0 g salt/100 g) showed improved WHC, which was also observed for puddings added HM. The moisture difference among the puddings can be explained by the addition of permeate powder leading to an increase in relative dry matter. Lower lightness (L*), redness (a*), yellowness (b*) and whiteness (WI) was obtained for puddings with 1.0 g/100 g salt. Addition of HM affected color similarly. The observed differences could be an effect of different gel structures affecting the way light is scattered.

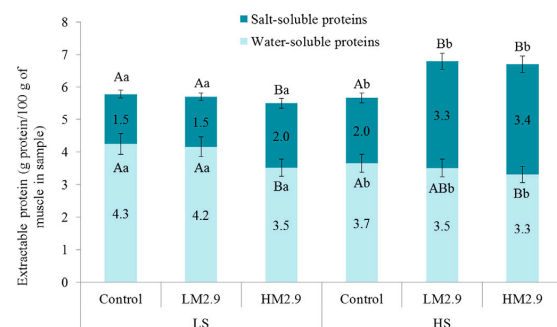


Fig. 1. Extractable SSP and WSP in fish mince (n = 2) extracted in 0.05 mol/l phosphate buffer (pH 7.0) without (WSP) and with 0.6 mol/l KCl (SSP). The results are expressed as g protein/100 g of muscle in sample ± the uncertainty of the method (7.5%). LS = low salt (0.5 g/100 g), HS = high salt (1.0 g/100 g), LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.9 g/100 g). Significant difference with regard to salt level and permeate powder within the salt levels is indicated with lower-case and upper-case letters, respectively.

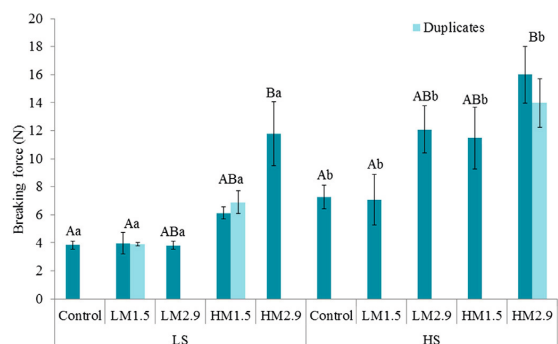


Fig. 2. Breaking force (N) of room tempered fish pudding from preliminary experiment. Salt level is indicated by LS = low salt (0.5 g/100 g) and HS = high salt (1.0 g/100 g), and permeate addition by LM = low mineral whey permeate and HM = high mineral milk permeate. Significant difference with regard to salt level and permeate powder within the salt levels is indicated with lower- and upper-case letters, respectively.

Fig. 1 shows the amount of extractable proteins in the minces. The amount of SSP was higher in the minces with 1.0 g salt/100 g, showing that the extractability of SSP increases with increasing ionic strength. Both LM and HM showed an increase in SSP compared with the control at 1.0 g salt/100 g (and at 0.5 g salt/100 g for HM). Less WSP being extracted from minces with 1.0 g salt/100 g could be due to a greater amount of dissolved SSP in the mince, potentially trapping the WSP, or to a more swollen network.

The amount of total protein in haddock (*Melanogrammus aeglefinus*) fillets is about 17 g/100 g, (The Norwegian Seafood Export Council, 1993) meaning that less than half of the proteins were extracted. Since only a small part of proteins in fish are insoluble stromal proteins (3–10 g/100 of total protein (Haard, 1992)), the low percentage of extracted proteins must be due to the extraction conditions or the physical state of the proteins in solution. Andreetta-Gorelkina et al. (2014) reported values of around 2.5% of wet weight for water soluble proteins and around 5% for salt soluble proteins for fresh haddock fillets. For mince made from fresh haddock, the values for salt soluble proteins were between 4.1 and 5.9% of wet weight depending on the amount and type of salt added.

Significant differences in breaking force based on both salt level and powder addition were found for the puddings, see Fig. 2. There was a high similarity among the duplicates, showing a high degree of reproducibility in production of the puddings, excluding uncertainties related to cooking or production order. Both HM levels led to a higher breaking force than the control at both salt levels. A higher breaking force due to LM was obtained only at 2.9 g LM/100 g and 1.0 g salt/100 g. Since the breaking force is a parameter that reflects hardness, one can therefore conclude that LM does not result in as hard puddings as HM.

A relation was found between amount of extractable proteins and WHC (Fig. 3), showing increasing WHC with increasing SSP ($p = 0.028$) and decreasing WSP ($p = 0.000$). Breaking force was also correlated with amount of extractable proteins, with increasing breaking force with increasing SSP ($p = 0.010$) and decreasing WSP ($p = 0.001$). This is in accordance with earlier studies (Jafarpour & Gorczyca, 2012). The strong correlation

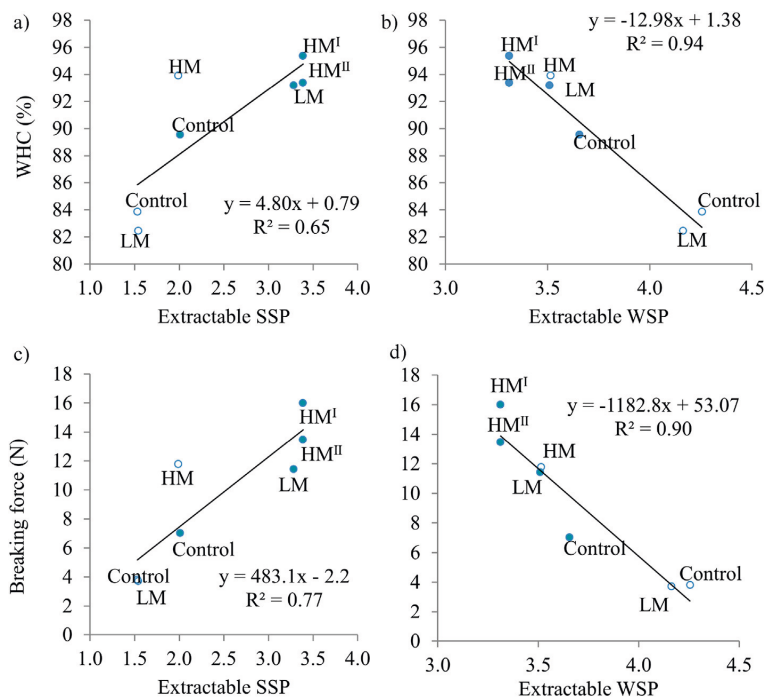


Fig. 3. Linear relation between extractable proteins in minces (g protein/100 g muscle in sample) and physical properties of puddings. Extractable SSP vs. WHC (a), extractable WSP vs. WHC (b), extractable SSP vs. breaking force (c) and extractable WSP vs. breaking force (d). ○ = LS (0.5 g salt/100 g), ● = HS (1.0 g salt/100 g), LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.9 g/100 g), ^I and ^{II} represent duplicate batches.

Table 4

Cooking loss, WHC, moisture, salt content and color of puddings in the main experiment. Salt level is indicated by LS = low salt (0.6 g/100 g), IS = intermediate salt (0.8 g/100 g) and HS = high salt (1.0 g/100 g), and permeate addition by LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.2 g/100 g). Significant difference with regard to salt level and permeate powder within the salt levels is indicated with lower-case and upper-case letters, respectively.

Sample	Cooking loss (g/100 g)	WHC (%)	Moisture (g/100 g)	Salt (g/100 g)	L*	a*	b*	W I
LS/Control	7.1 ± 1.1 ^{Aa}	79.6 ± 1.5 ^{Aa}	82.7 ± 0.2 ^{Aa}	0.67 ± 0.00 ^{Aa}	78.6 ± 0.4 ^{Aa}	-6.4 ± 0.2 ^{Aa}	12.9 ± 0.4 ^{Ba}	74.3 ± 0.4 ^{Aa}
LS/LM	7.3 ± 1.2 ^{Aa}	86.0 ± 2.1 ^{Ba}	80.6 ± 0.2 ^{Ca}	0.64 ± 0.00 ^{Ba}	78.3 ± 0.3 ^{Aa}	-6.5 ± 0.2 ^{ABa}	13.4 ± 0.4 ^{Aa}	73.7 ± 0.2 ^{Ba}
LS/HM	6.0 ± 0.4 ^{Ba}	92.9 ± 0.3 ^{Ca}	81.5 ± 0.3 ^{Ba}	0.74 ± 0.00 ^{Ca}	76.9 ± 0.4 ^{Ba}	-6.7 ± 0.2 ^{Ba}	10.9 ± 0.4 ^{Ca}	73.6 ± 0.4 ^{Ba}
IS/Control	7.1 ± 1.8 ^{Aa}	74.2 ± 2.5 ^{Aa}	83.0 ± 0.2 ^{Aa}	0.92 ± 0.00 ^{Ab}	76.8 ± 0.4 ^{Aa}	-6.3 ± 0.2 ^{Ab}	11.9 ± 0.3 ^{Ba}	73.2 ± 0.4 ^{Aa}
IS/LM	7.09 ± 0.7 ^{Aa}	89.7 ± 0.8 ^{Ba}	80.9 ± 0.2 ^{Ba}	0.87 ± 0.02 ^{Bb}	78.1 ± 0.5 ^{Ba}	-7.0 ± 0.2 ^{Cb}	12.9 ± 0.4 ^{Aa}	73.7 ± 0.5 ^{Aa}
IS/HM	7.0 ± 1.0 ^{Aa}	92.3 ± 2.5 ^{Ba}	81.0 ± 0.2 ^{Ba}	0.96 ± 0.00 ^{Ab}	77.2 ± 0.4 ^{Aa}	-6.8 ± 0.2 ^{Bb}	11.2 ± 0.3 ^{Ca}	73.8 ± 0.5 ^{Aa}
HS/Control	7.2 ± 0.2 ^{Aa}	88.4 ± 1.9 ^{Aa}	82.6 ± 0.4 ^{Aa}	1.08 ± 0.00 ^{Ac}	77.8 ± 0.4 ^{Ba}	-6.7 ± 0.2 ^{Ab}	11.7 ± 0.6 ^{Ba}	74.0 ± 0.5 ^{Aa}
HS/LM	7.0 ± 1.0 ^{Aa}	92.5 ± 0.5 ^{Ba}	80.7 ± 0.5 ^{Ba}	1.06 ± 0.02 ^{Ac}	78.7 ± 0.14 ^{Aa}	-7.1 ± 0.10 ^{Bb}	12.8 ± 0.5 ^{Aa}	74.1 ± 0.3 ^{Aa}
HS/HM	6.6 ± 0.9 ^{Aa}	92.7 ± 1.9 ^{Ba}	81.4 ± 0.6 ^{Ba}	1.20 ± 0.03 ^{Bc}	75.6 ± 0.5 ^{Ca}	-7.0 ± 0.2 ^{Bb}	10.7 ± 0.3 ^{Ca}	72.5 ± 0.5 ^{Ba}
F ratio								
Permeate (LS)	8.28 ^{**}	87.28 ^{***}	47.01 ^{***}	355.23 ^{***}	77.85 ^{***}	8.28 ^{**}	157.23 ^{***}	8.58 ^{**}
Permeate (IS)	0.15 ^{ns}	99.00 ^{***}	48.20 ^{***}	33.40 ^{**}	26.41 ^{***}	36.20 ^{***}	72.71 ^{***}	3.68 [*]
Permeate (HS)	0.99 ^{ns}	10.63 ^{**}	22.64 ^{***}	48.32 ^{**}	137.14 ^{***}	13.92 ^{***}	41.67 ^{***}	41.18 ^{***}
Salt	1.78 ^{ns}	1.46 ^{ns}	0.77 ^{ns}	134.96 ^{***}	0.52 ^{ns}	10.93 ^{***}	2.22 ^{ns}	0.96 ^{ns}

p values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, nonsignificant.

between WSP and both WHC and breaking force might be explained by the high salt levels leading to a greater amount of dissolved SSP in the mince, potentially retaining more WSP. If a greater amount of WSP is trapped in the gel matrix, these proteins could lead to increased amounts of bound water due to osmotic forces, leading to an increased WHC.

3.2. Main experiment

Physicochemical properties (cooking loss, WHC, moisture, sodium and color) of the fish puddings from the main experiment are shown in Table 4. As in the preliminary experiment, no differences in cooking loss were observed. The investigated salt levels did not have any effect on WHC, however, both LM and HM showed an improvement in WHC at all three salt levels.

Overall, the breaking force was lower in the main experiment (Fig. 4) than in the preliminary experiment, but the difference among the recipes appears to be the same. Puddings added HM showed a higher breaking force than the 1.0 g salt/100 g control at

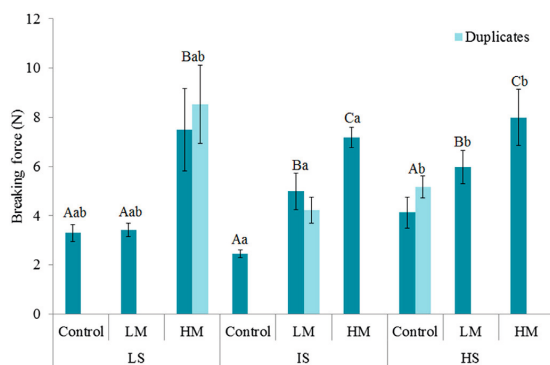


Fig. 4. Breaking force (N) of room tempered fish pudding from main experiment. Salt level is indicated by LS = low salt (0.6 g/100 g), IS = intermediate salt (0.8 g/100 g) and HS = high salt (1.0 g/100 g), and permeate addition by LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.2 g/100 g). Significant difference with regard to salt level and permeate powder within the salt levels is indicated with lower-case and upper-case letters, respectively.

all salt levels. Those added LM obtained a breaking force equal to the 1.0 g salt/100 g control at 0.8 g salt/100 g and higher at 1.0 g salt/100 g, indicating that LM can contribute to an improved texture above a certain salt level. A significant linear relation was found between breaking force and WHC ($p = 0.003$, $R^2 = 0.75$), which reinforces the findings in the preliminary experiment that showed a relation between breaking force, WHC and soluble proteins.

Mean intensity scores of significant sensory description obtained from DA are shown in Fig. 5. There were no significant differences between the two replicas evaluated by each panellist, and analyses of the results were therefore performed on the replica means. Significant differences ($p < 0.05$) were found between samples for five texture attributes, i. e. firmness, cohesiveness, elasticity, chewiness, coarseness, and three flavor attributes, i. e. flavor intensity, fish and salt taste. The firmness was higher in the HS control than in the LS control. The texture attributes firmness and elasticity were significantly higher compared to the control samples in the fish pudding added milk minerals (LM or HM), however the added salt content had to be 0.8 g/100 g or higher before the LM had significant influence on these parameters. The elasticity in the puddings increased significantly by adding HM compared to the control, from score 2.9 to 6.1 for LS, score 2.2 to 6.0 for IS and score 3.5 to 6.4 for HS, respectively, and by adding LM in the IS pudding (from score 2.2–4.8). The puddings added HM, had significantly higher cohesiveness and chewiness than the control at the salt levels LS and IS (0.6–0.8 g salt/100 g). A greater hardness has been observed for salt substitution with K^+ -ions in previous studies on dry salted cod fillets (Martínez-Alvarez et al., 2013). One explanation for the textural changes in the puddings containing HM might be the high mineral content, a.o. K^+ - ions. Textural changes have been observed for salt substitution with K^+ ions in previous studies on salt replacement in smoked salmon (Almli & Hersleth, 2013). The textural parameters in the sensory analysis show correspondence with the instrumental texture analysis, where pudding added HM at all salt levels showed a larger breaking force and a lower adhesiveness (results not shown) compared with the HS control. Puddings added LM at 0.8 g salt/100 g and 1.0 g salt/100 g showed larger breaking force than the respective control. Strong linear correlation between the instrumentally measured breaking force of the puddings and their textural sensory parameters was found, with an increasing breaking force with increasing firmness ($p = 0.000$), elasticity ($p = 0.000$) and cohesiveness ($p = 0.001$) and a decreasing breaking force with increasing

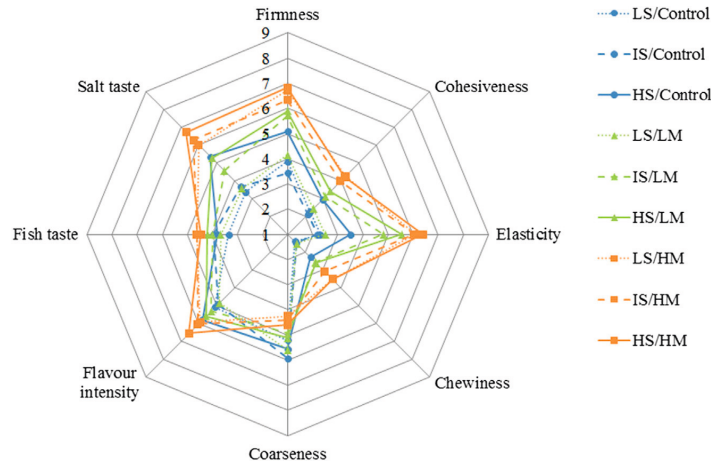


Fig. 5. Descriptive sensory profile of the 9 samples of fish pudding: control (blue), added LM = low mineral whey permeate (2.9 g/100 g) (green) and added HM = high mineral milk permeate (2.2 g/100 g) (orange) with LS = 0.6 g salt/100 g (dotted lines), IS = 0.8 g salt/100 g (discontinuous lines) and HS = 1.0 g salt/100 g (continuous lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

coarseness ($p = 0.002$). This also indicates that these textural parameters are interdependent. Several studies have reported a correlation between instrumental and sensory textural parameters (Cardoso, Mendes, & Nunes, 2009; Lawless & Heymann, 2010; Meullenet, Lyon, Carpenter, & Lyon, 1998), with the strongest correlation being observed for parameters related to hardness and springiness. Some of the puddings had a high K:Na ratio (HM puddings), and a negative effect on the taste attributes could be expected, but the DA shows no differences for odor attributes; harsh or metallic taste, typically related to sodium substitutes (Desmond, 2006). An interaction plot for salt taste of the puddings are shown in Fig. 6 and gives a good indication on which parameter

has the largest effect on salt taste. HM leads to an increase in perceived saltiness (Fig. 6, upper right), while LM has little or no effect on salt taste compared to the controls. An increase in salt level (Fig. 6, lower left) has less effect for the perceived saltiness; with puddings added HM clearly dominating in saltiness at all salt levels.

Results from DA indicate that milk mineral containing high amount of minerals (HM) will change the texture quality and salty taste in salt reduced fish puddings. This results is in accordance with Paulsen, Nys, Kvarberg, and Hersleth (2014), who found that sodium reduction up to 40% combined with milk minerals substitution was possible without observing significant differences in the

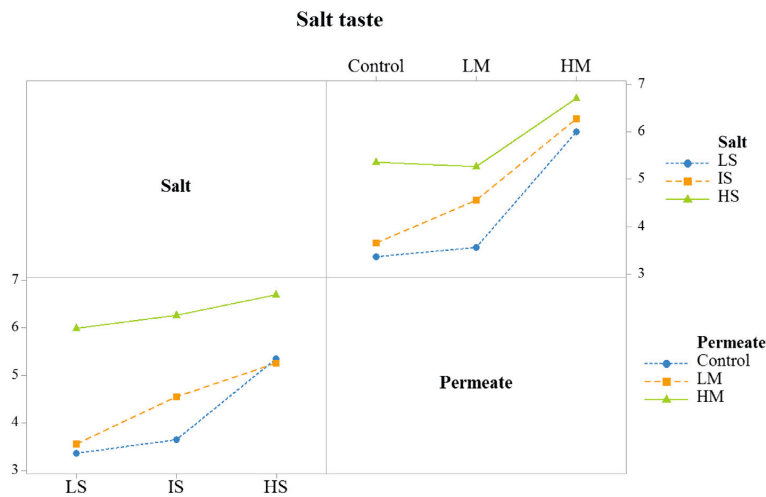


Fig. 6. Interaction plot of salt taste where increasing score indicates increasing salt intensity of the puddings. The left plot shows the effect of increasing salt level on perceived saltiness and the right plot shows the effect of permeate addition on perceived saltiness. Salt level is indicated by LS = low salt (0.6 g salt/100 g), IS = intermediate salt (0.8 g/100 g) and HS = high salt (1.0 g/100 g), and permeate addition with LM = low mineral whey permeate (2.9 g/100 g) and HM = high mineral milk permeate (2.2 g/100 g).

descriptive profile in sausages and Almlí and Hersleth (2013) who showed that one-third replacement of NaCl by KCl was possible without altering the sensory properties in smoked salmon.

4. Conclusions

Low mineral whey permeate (LM) improved textural and water-holding properties of puddings at salt concentrations down to 0.8%, while it does not affect salt flavor. High mineral milk permeate (HM) contributed to change in the textural and water-holding properties, and also increased salt flavor. Based on investigated factors, high mineral permeate is regarded as a promising salt replacer allowing for considerable salt reduction in fish puddings containing lean haddock.

Acknowledgments

The work is financed by the SALTO project no. 210431/O10 and Low salt project no. 185063/O10. Supported by the Norwegian industry and the Research Council of Norway. The technical assistance of Marie Steinslien and Hilde Kraggerud are highly appreciated.

References

- Almlí, V., & Hersleth, M. (2013). Salt replacement and injection salting in smoked salmon evaluated from descriptive and hedonic sensory perspectives. *Aquaculture International*, 21(5), 1091–1108. <http://dx.doi.org/10.1007/s10499-012-9615-4>.
- Anderson, M. L., & Ravesi, E. M. (1968). Relation between protein extractability and free fatty acid production in cod muscle aged in ice. *Journal of the Fisheries Research Board of Canada*, 25(10), 2059–2069. <http://dx.doi.org/10.1139/f68-183>.
- Andreetta-Gorelkina, I. V., Greiff, K., Rustad, T., & Aursand, I. G. (2014). Reduction of salt in haddock mince: effect of different salts on the solubility of proteins. *Journal of Aquatic Food Product Technology*, 26.
- Barat, J. M., Pérez-Estevé, E., Aritoy, M.-C., & Toldra, F. (2012). Partial replacement of sodium in meat and fish products by using magnesium salts. A review. *Plant Soil*. <http://dx.doi.org/10.1007/s11104-012-1461-7>.
- BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed.). New York: Marcer Dekker, Inc.
- Benjakul, S., Visessanguan, W., & Srivilai, C. (2001). Porcine plasma protein as proteinase inhibitor in bigeye snapper (*Priacanthus tayenus*) muscle and surimi. *Journal of the Science of Food and Agriculture*, 81(10), 1039–1046. <http://dx.doi.org/10.1002/jsfa.887>.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. [http://dx.doi.org/10.1016/0003-2697\(76\)90527-3](http://dx.doi.org/10.1016/0003-2697(76)90527-3).
- Cardoso, C. M., Mendes, R., & Nunes, M. L. (2009). Instrumental texture and sensory characteristic of cod frankfurter sausages. *International Journal of Food Properties*, 12, 625–643.
- Cook, N. R., Cutler, J. A., Obarzanek, E., Buring, J. E., Rexrode, K. M., Kumanyika, S. K., et al. (2007). Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). *BMJ*, 334(7599), 885–888.
- Desmond, E. (2006). Reducing salt: a challenge for the meat industry. *Meat Science*, 74(1), 188–196.
- Duangmal, K., & Taluengphol, A. (2010). Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and colour of red tilapia surimi gel. *International Journal of Food Science & Technology*, 45(1), 48–55. <http://dx.doi.org/10.1111/j.1365-2621.2009.02102.x>.
- Eide, O., Børresen, T., & Strøm, T. (1982). Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from small fatty fish species. *Journal of Food Science*, 47(2), 347–349. <http://dx.doi.org/10.1111/j.1365-2621.1982.tb10078.x>.
- FSA. (2004). *Nutrition statement. Common statement of representatives of national food safety agencies and institutions involved in nutrition in the European countries and Norway*. UK: Food Standards Agency.
- Fuentes, A., Ferandez-Segovia, I., Barat, J. M., & Serra, J. A. (2010). Physicochemical characterization of some smoked and marinated fish products. *Journal of Food Processing and Preservation*, 34(1), 83–103. <http://dx.doi.org/10.1111/j.1745-4549.2008.00350.x>.
- Greiff, K., Fuentes, A., Aursand, I. G., Erikson, U., Masot, R., Alcañiz, M., et al. (2014). Innovative nondestructive measurements of water activity and the content of salts in low-salt hake minces. *Journal of Agricultural and Food Chemistry*, 62(12), 2496–2505. <http://dx.doi.org/10.1021/jf405527t>.
- Haard, N. F. (1992). Control of chemical composition and food quality attributes of cultured fish. *Food Research International*, 25(4), 289–307. [http://dx.doi.org/10.1016/0963-9969\(92\)90126-P](http://dx.doi.org/10.1016/0963-9969(92)90126-P).
- He, F. J., Burnier, M., & MacGregor, G. A. (2011). Nutrition in cardiovascular disease: salt in hypertension and heart failure. *European Heart Journal*, 32(24), 3073–3080. <http://dx.doi.org/10.1093/eurheartj/ehr194>.
- Hultmann, L., & Rustad, T. (2002). Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology*, 11(3–4), 105–123. http://dx.doi.org/10.1300/J030v11n03_09. Sensory analysis - Vocabulary (1992).
- Jafarpour, A., & Gorczyca, E. M. (2012). Contribution of sarcoplasmic proteins to myofibrillar proteins gelation. *Journal of Food Science*, 77(2), R73–R81. <http://dx.doi.org/10.1111/j.1750-3841.2011.02521.x>.
- Kinsella, J. E. (1976). Functional properties of food proteins: a survey. *Critical Reviews in Food Science and Nutrition*, 7, 219–280.
- Kivikari, R. (1996). Analysis of sodium in meat products using an Na-selective electrode. Paper presented at the Proc. of Meat Day Seminar.
- Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food – Principles and practices* (2nd ed.). New York: Springer.
- Licciardello, J., Ravesi, E., Lundstrom, R., Wilhelm, K., Correia, F., & Allsup, M. (1982). Time-temperature tolerance and physical-chemical quality tests for frozen red hake. *Journal of Food Quality*, 5(3), 215–234.
- Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2005). The effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*Gadus morhua*). *Food Chemistry*, 92(1), 71–77.
- Martínez-Alvarez, O., & Gómez-Guillén, M. C. (2006). Effect of brine salting at different pHs on the functional properties of cod muscle proteins after subsequent dry salting. *Food Chemistry*, 94(1), 123–129.
- Martínez-Alvarez, O., & Gómez-Guillén, C. (2013). Influence of mono- and divalent salts on water loss and properties of dry salted cod filets. *LWT – Food Science and Technology*, 53(2), 387–394. <http://dx.doi.org/10.1016/j.lwt.2013.04.013>.
- Meulenen, J.-F., Lyon, B. G., Carpenter, J. A., & Lyon, C. E. (1998). Relationship between sensory and instrumental texture profile attributes. *Journal of Sensory Studies*, 13(1), 77–93. <http://dx.doi.org/10.1111/j.1745-459X.1998.tb00076.x>.
- Nayak, R., Kenney, P. B., & Slider, S. (1996). Protein extractability of turkey breast and thigh muscle with varying sodium chloride solutions as affected by calcium, magnesium and zinc chloride. *Journal of Food Science*, 61(6), 1149–1154.
- Nguyen, M. V., Thorarindottir, K. A., Gudmundsdottir, A., Thorkelsson, G., & Arason, S. (2011). The effects of salt concentration on conformational changes in cod (*Gadus morhua*) proteins during brine salting. *Food Chemistry*, 125(3), 1013–1019. <http://dx.doi.org/10.1016/j.foodchem.2010.09.109>.
- Paulsen, M. T., Nys, A., Kvarberg, R., & Hersleth, M. (2014). Effects of NaCl substitution on the sensory properties of sausages: temporal aspects. *Meat Science*, 98(2), 164–170. <http://dx.doi.org/10.1016/j.meatsci.2014.05.020>.
- Rawdkuen, S., & Benjakul, S. (2008). Whey protein concentrate: autolysis inhibition and effects on the gel properties of surimi prepared from tropical fish. *Food Chemistry*, 106(3), 1077–1084. <http://dx.doi.org/10.1016/j.foodchem.2007.07.028>.
- The Norwegian Directorate of Health. (2011). Strategy for the reduction of salt intake in the population. In Department for National Public Health (Ed.), *Recommendation from the National Nutrition Council*. <http://helsedirektoratet.no/folkehelse/ertering/strategier-og-satsninger/Documents/saltstrategi.pdf>.
- The Norwegian Seafood Export Council, the Directorate for Fisheries, The Fresh Fish Sales Association, The Information Council for Fish and the Norwegian National Council for Nutrition. (1993). *Facts about fish*. Tromsø.
- WHO. (2006). *Reducing salt intake in populations*. Report of a WHO Forum and Technical Meeting. Paris: World Health Organization.
- Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., et al. (2009). Effects of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (*Ctenopharyngodon idella*). *Food Chemistry*, 116(2), 413–418. <http://dx.doi.org/10.1016/j.foodchem.2009.02.056>.

