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Sodium reduction in muscle foods

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

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Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
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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 T2 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 physiochemical 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.
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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 excepted 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.
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⁺, Mg²⁺) 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 μg/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).
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".

<table>
<thead>
<tr>
<th>Main product category</th>
<th>Sub-category</th>
<th>Guided target DK¹ Nov 2013 g salt (mg Na) per 100g</th>
<th>Targets for 2017 FSA² g salt (mg Na) per 100g</th>
<th>&quot;Keyhole&quot;³ g salt (mg Na) per 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>Bacon</td>
<td>2.4 (960)</td>
<td>2.88 (1150)</td>
<td>-</td>
</tr>
<tr>
<td>Meat products</td>
<td>Sausages</td>
<td>2.3 (920)</td>
<td>1.5 (600) (average)</td>
<td>2.0 (800) max</td>
</tr>
<tr>
<td>Meat products</td>
<td>Meat balls</td>
<td>1.6 (640)</td>
<td>1.35 (540)</td>
<td>1.7 (700) max</td>
</tr>
<tr>
<td>Meat products</td>
<td>Cooked ham</td>
<td>3.0 (1.2)</td>
<td>1.63 (650)</td>
<td>2.5 (1000) max</td>
</tr>
<tr>
<td>Fish products</td>
<td>Breaded</td>
<td>1.0 (40)</td>
<td>-</td>
<td>1.5 (600) max</td>
</tr>
<tr>
<td>Fish products</td>
<td>Marinated or smoked</td>
<td>3.0 (1.2)</td>
<td>-</td>
<td>3.0 (1200) max</td>
</tr>
<tr>
<td>Fish products</td>
<td>Canned</td>
<td>1.0 (400)</td>
<td>0.85 (340) (average)</td>
<td>1.5 (600) max</td>
</tr>
</tbody>
</table>

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.

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

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


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).
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 consist of overlapping thick and thin filaments and lighter in the H-zone, where it consist 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
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.

![Diagram showing chloride interaction in protein structure](image)

**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 bound 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](image.png)

*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...
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 H\(_2\)O) 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).

\[
I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2
\]  

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 the ensure the required texture. A gel is defined as a structure which form is 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 stages process; denaturation of individual protein molecules, aggregation (mainly hydrofobic 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 Ca\(^{2+}\)-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 (-NH₃⁺). 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).
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 person 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, aw, and taste. (2)
Lactates and salt of lactates affect the microbiology and taste, whereas 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 marked 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 marked 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.
Table 3. Summary of salt reduction and the effects thereof on physicochemical properties, taste and shelf-life in low-salt fish products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Salt reduction/ replacement</th>
<th>Whole muscle/ mince</th>
<th>Physicochemical properties/ shelf-life/taste</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-salt food</td>
<td>Low sodium mixtures</td>
<td>Fish, meat, food</td>
<td>Patents aimed to develope mixtures with low sodium content</td>
<td>Review by Toldrá &amp; Barat (2012)</td>
</tr>
<tr>
<td>Cod, ready-to-eat</td>
<td>Desalting water with NaCl/KCl</td>
<td>Whole muscle</td>
<td>Physicochemical properties/ Shelf life/taste</td>
<td>Aliño et al. (2011)</td>
</tr>
<tr>
<td>Smoked sea bass</td>
<td>Salting 100% NaCl, 50% NaCl-50% KCl, packaging, 926-1279 mg Na/100g in salted and smoked fish</td>
<td>Sea bass fillets</td>
<td>Chemical, microbial and sensory attributes</td>
<td>Fuentes et al. (2011); Fuentes et al.,(2012)</td>
</tr>
<tr>
<td>Fish gel</td>
<td>NaCl (0-1%), KCl (0-1%), CaCl2(0-1%), iota-carrageenan, kappa-carrageenan, sodium alginate.</td>
<td>Blue whiting muscle gel</td>
<td>Texture, color, WHC</td>
<td>Montero &amp; Pérez-Mateos (2002)</td>
</tr>
<tr>
<td>Surimi</td>
<td>NaCl (0, 1, 2, 3 g/100g), equal molar concentrations of KCl</td>
<td>Alaska plolock surimi</td>
<td>Protein endothermic transitions, reological properties, texture</td>
<td>Tahergorabi et al. (2012)</td>
</tr>
<tr>
<td>Cod</td>
<td>Fresh and frozen, fillet injection (0, 50, 150 and 250 g NaCl/L), additional 25 g sodium bicarbonate/L (NaHCO3)</td>
<td>Whole muscle, fillet</td>
<td>Yield, liquid retention during storage, flavor, texture</td>
<td>Åsli &amp; Mørkøre (2012)</td>
</tr>
<tr>
<td>Cod</td>
<td>Brine salting in NaCl (10 – 50 g/L) or KCl (13 – 64 g/L) solution</td>
<td>Whole muscle, fillet</td>
<td>Water uptake, drip loss and retention of low molecular components</td>
<td>Larsen &amp; Elvevoll (2008)</td>
</tr>
<tr>
<td>Low-salt foods</td>
<td>Slow and gradual reduction, replacement of Na+ with K+, ammonium, calcium, and lithium and by anion such as phosphate and glutamates.</td>
<td>Taste, sensory profile</td>
<td>Morley (2012)</td>
<td></td>
</tr>
<tr>
<td>Smoked salmon</td>
<td>NaCl or NaCl + KCl in a 2:1 ratio. Dry salting or by injection</td>
<td>Whole muscle</td>
<td>Sensory quantitative descriptive analysis. Consumer test</td>
<td>Almli &amp; Hersleth, (2013)</td>
</tr>
</tbody>
</table>
Table 3 continued. Summary of salt reduction and the effect on physicochemical properties, taste and shelf-life in low-salt meat products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Salt reduction/replacement</th>
<th>Whole muscle/mince</th>
<th>Physicochemical properties/shelf-life/taste</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frankfurter</td>
<td>Replacement of flake salt with naturally brewed soy sauce, natural flavor enhancer, and KCl.</td>
<td>Emulsion of beef and pork</td>
<td>Consumer sensory and quality impact, color, pH, emulsion stability, yield</td>
<td>McGough et al., (2012a); McGough et al. (2012b)</td>
</tr>
<tr>
<td>sausage</td>
<td>Gradually reduction of NaCl (1.6-1.1% NaCl), modified tapioca starch, wheat bran, sodium citrate, fat</td>
<td>Emulsion of beef and pork</td>
<td>Physical properties and sensory attributes</td>
<td>Ruusunen et al. (2003b)</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>Gradually reduction of salt (2.5, 2.0, 1.5% NaCl), preblended, non preblended meat</td>
<td>Emulsion of beef and pork</td>
<td>Emulsion stability, color, Kramer shear and palatability</td>
<td>Hand et al. (1987)</td>
</tr>
<tr>
<td>sausage</td>
<td>Salt (1.5-2.5% NaCl), sodium triphosphate, kappa-carrageenan, isolated scya protein</td>
<td>Emulsion of beef and pork</td>
<td>Physiochemical properties, sensory attributes</td>
<td>He &amp; Sebranek (1996)</td>
</tr>
<tr>
<td>Bologna sausage</td>
<td>Tree levels of salt, Sodium citrate, carboxymethyl cellulose, carrageenan, fat</td>
<td>Lean pork and pork back fat</td>
<td>Chemical and physical composition, sensory attributes</td>
<td>Ruusunen et al. (2003a)</td>
</tr>
<tr>
<td>Cooked sausage</td>
<td>Post-rigor pH, phosphate, salt (0.5, 1.0, 1.5, 2.0, 2.5% NaCl)</td>
<td>Beef and pork</td>
<td>WHC, firmness, sensory attributes</td>
<td>Puolanne et al. (2001)</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>Salt replacer (Ocean’s Flavor OF45, OF60) and flavor enhancer (Fonterra ⁷⁴)</td>
<td>Restructured ham of pork</td>
<td>Functional and sensory properties</td>
<td>Pietraski and Gaudette (2014)</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>Tumble salting with NaCl brine (0, 11.2, 16.5 or 22.0% NaCl)</td>
<td>Cubes of pork (seminembranosus)</td>
<td>Salt contents effect on the adhesion between pieces</td>
<td>Bombrun et al. (2014)</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>Gradually reduced NaCl content (1.1, 1.4, 1.7, 2.0, 2.3 and 2.6% NaCl)</td>
<td>Coarsely ground lean ham</td>
<td>Sensory saltiness</td>
<td>Ruusunen et al. (2001)</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>KCl, tumbling time, tumbling speed</td>
<td>Coarsely ground lean pork</td>
<td>Color, shrinkage, yield, WHC, sensory attributes</td>
<td>Lin et al. (1991)</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>Gradually reduced NaCl content (1.30, 0.74, 0.18% NaCl)</td>
<td>Coarsely ground pork</td>
<td>Chemical- and microbial properties, sensory attributes</td>
<td>Aaslyng et al. (2014)</td>
</tr>
<tr>
<td>Meat patties</td>
<td>Gradually reduced NaCl content (300, 450 and 600 mg Na/100g), phosphate and meat content</td>
<td>Coarsely ground beef and pork meat</td>
<td>Sensory attributes, cooking loss, firmness</td>
<td>Ruusunen et al. (2005)</td>
</tr>
<tr>
<td>Meat batter</td>
<td>NaCl, 0.05% CaCl₂, MgCl₂ or ZnCl₂, with or without 0.4% sodium tripolyphosphate, fat</td>
<td>Beef</td>
<td>pH, yield, texture, gel ultrastructure</td>
<td>Nayak et al. (1998)</td>
</tr>
</tbody>
</table>
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; Toldrà & 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 aw 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.
Table 4. Summary of salt reduction and the effect on physicochemical properties, taste and shelf-life in heavy salted fish and meat products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Salt reduction/replacement</th>
<th>Whole muscle/ mince</th>
<th>Physicochemical properties/ shelf-life/taste</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy salted fish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cod</td>
<td>Brine salting, pH (6.5 and 8.5), NaCl, KCl, CaCl2 and/or MgCl2</td>
<td>Whole muscle, fillet</td>
<td>WHC, protein extractability, dry matter, ion content and hardness.</td>
<td>Martínez-Alvarez et al. (2005)</td>
</tr>
<tr>
<td>Cod</td>
<td>Brine salting, pH, NaCl, KCl, CaCl2 and/or MgCl2</td>
<td>Whole muscle, fillet</td>
<td>Water loss, salt uptake, entry of chloride, WHC, water extractable protein (WEP) and hardness</td>
<td>Martínez-Alvarez and Gómez-Guillén (2013)</td>
</tr>
<tr>
<td><strong>Heavy salted meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry-cured products</td>
<td>Partial replacement of sodium chloride with KCl, CaCl2, MgCl2</td>
<td>Pork loin, pork ham, fermented sausage</td>
<td>Sensory effect and other</td>
<td>A review by Barat et al. (2012)</td>
</tr>
<tr>
<td>Dry-cured ham</td>
<td>KCl and potassium lactate instead of NaCl</td>
<td>Whole muscle, ham</td>
<td>Food-borne pathogens; Listeria monocytogenes and Salmonella</td>
<td>Stollewerk et al. (2012)</td>
</tr>
<tr>
<td>Dry-cured ham</td>
<td>Reduction of NaCl (5.5-4% NaCl as per cent edible meat)</td>
<td>Italian style dry-cured ham</td>
<td>Sensory properties, proteolysis</td>
<td>Benedini et al. (2012)</td>
</tr>
<tr>
<td>Dry-cured bacon</td>
<td>Partial substitution of NaCl with KCl</td>
<td>Pork</td>
<td>Proteolysis and sensory properties</td>
<td>Wu et al. (2014)</td>
</tr>
</tbody>
</table>
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.,
METHODOLOGY

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 (\( \Phi \)) 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 \( \Delta t \) is the time interval between the zero crossing of the voltage and current signals.

\[
Z = |Z| \exp(\Phi) = \left| \frac{v(t)}{i(t)} \right| \quad \text{Module}
\]

\[
\Phi = \frac{2\pi f \Delta t}{\text{Phase}}
\]

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.
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
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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](image)

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).
Signal = \( A_{21}e^{-t/T_{21}} + A_{22}e^{-t/T_{22}} \)  

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.](image)

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.

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.

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.
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 (AgNO₃). 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 (AgNO₃) titrates with ammonium thiocyanate (NH₄SCN)

2. \[ \text{AgNO}_3 + \text{NH}_4^+ \rightarrow \text{AgSCN} + \text{NH}_4^+ + \text{NO}_3^- \], and a ferric indicator is added for determination of the end point, where a fair red-brownish color appeared

3. \[ \text{FeNH}_4\left(\text{SO}_4\right)_2 + \text{NH}_4\text{SCN} \rightarrow \text{Fe(SCN)}_3 \times \text{Fe} \left(\text{SCN}\right)^2^- \]. 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.
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),

\[
E = E_0 + S \log A \quad \text{Equation 4}
\]

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.
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 MgCl₂ due to precipitation of MgPO₄. 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⁺)

<table>
<thead>
<tr>
<th>Method</th>
<th>Application</th>
<th>Type of food</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic emission spectrophotometry with inductively coupled plasma (ICP-AES)</td>
<td>Ground state metals absorb light at specific wavelengths.</td>
<td>Low-salt infant formula Milk and milk products Wheat brain (food containing &gt; 1500 mg Na/kg)</td>
<td>AOAC,( 2008); EN, (2008); ISO,( 1996)</td>
</tr>
<tr>
<td>Flame atomic absorption spectrophotometry (FAAS)</td>
<td>Potentiometry, Na-measurement</td>
<td>Low-salt milk, bread, cheese, wine, butter and processed meats.</td>
<td>Ehling et al.,(2010); Kivikari,(1996) Paper I-V</td>
</tr>
<tr>
<td>Atomic Absorption Spectroscopy</td>
<td>Wet digestion, using a microwave oven technique</td>
<td>(broccoli, carrot, bread, salthe fillet, pork, and cheese)</td>
<td>Julshamn et al., (2005)</td>
</tr>
</tbody>
</table>

### Indirect estimation of sodium

<table>
<thead>
<tr>
<th>Method</th>
<th>Application</th>
<th>Type of food</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride analyzer stoichiometrically</td>
<td>Coulometric end-point titration by the use of a sensing electrode</td>
<td>Low-salt salmon</td>
<td>Åslí &amp; Mørkøre, (2012)</td>
</tr>
<tr>
<td>Volhart</td>
<td>Potentiometric titration method for chloride ions, indirect estimation av sodium concentration</td>
<td>Dry-cured ham</td>
<td>AOAC,(2008)</td>
</tr>
<tr>
<td>Volhart</td>
<td>Potentiometric titration method for chloride ions, indirect estimation av sodium concentration</td>
<td>Cod</td>
<td>AOAC,(2008)</td>
</tr>
<tr>
<td>Chloride analyzer</td>
<td>Potentiometric titration</td>
<td>Dry-cured ham</td>
<td>ISO, (1996); Pérez-Olmos et al. (1997); Santos-Garcés et al., (2010)</td>
</tr>
</tbody>
</table>
Table 5. continued. Overview over analytical methods used for measuring sodium and salt (NaCl) in food.

<table>
<thead>
<tr>
<th>Method</th>
<th>Application</th>
<th>Type of food</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impedance Spectroscopy, needle electrode</td>
<td>Prediction of NaCl concentration</td>
<td>Minced pork loin</td>
<td>Masot et al., (2010)</td>
</tr>
<tr>
<td>Impedance Spectroscopy, double electrode</td>
<td>Predicting salt content and $a_w$</td>
<td>Low-salt fish mince</td>
<td>Greiff et al., (2014); Paper I</td>
</tr>
<tr>
<td>Impedance Spectroscopy, needle electrode</td>
<td>NaCl content, moisture and $a_w$ during salting-smoking process.</td>
<td>Smoked Salmon, 1.67-3.62 g NaCl/100g</td>
<td>Rizo et al., (2013)</td>
</tr>
<tr>
<td>Computer tomography</td>
<td>Predicting salt content</td>
<td>Dry-cured ham</td>
<td>Vestergaard et al., (2005)</td>
</tr>
<tr>
<td>Computer tomography</td>
<td>Prediction water activity, salt and water</td>
<td>Dry-cured ham, ground pork</td>
<td>Håseth et al., (2007); Håseth et al., (2012)</td>
</tr>
<tr>
<td>LF NMR</td>
<td>Changes during brine salting</td>
<td>Salmon</td>
<td>Aursand et al. (2010); Aursand, Veliyulin, et al. (2008)</td>
</tr>
<tr>
<td>LF NMR</td>
<td>Changes during brine salting and desalting</td>
<td>Cod</td>
<td>Erikson et al., (2004)</td>
</tr>
<tr>
<td>LF NMR</td>
<td>Changes during pre-salting, dry salting and rehydration</td>
<td>Cod</td>
<td>Gudjónsdóttir et al. (2011)</td>
</tr>
<tr>
<td>LF NMR</td>
<td>Changes during brine injection, brining and freezing.</td>
<td>Wild and farmed cod</td>
<td>Gudjónsdóttir et al., (2010)</td>
</tr>
<tr>
<td>Fourier Transforming Infrared Microspectroscopy-FTIR</td>
<td>Protein structural changes</td>
<td>Beef</td>
<td>Perisic et al., (2011)</td>
</tr>
<tr>
<td>SPRITE $^{23}$Na MRI (single-point remped imaging with $T_1$ enhancerment)</td>
<td>Quantitative $^{23}$Na Magnetic Resonance Imaging</td>
<td>Model products of muscle food</td>
<td>Veliyulin et al., (2009)</td>
</tr>
<tr>
<td>$^{23}$Na MR imaging</td>
<td>Spin-echo $^{23}$Na MRI</td>
<td>Heavily salted cod and salmon</td>
<td>Aursand et al. (2010); Gallart-Jornet et al. (2007); Veliyulin and Aursand (2007)</td>
</tr>
</tbody>
</table>
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 physiochemical parameters in salted fish products with, and without sodium replacement was evaluated (Paper I).
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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 T2 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). T2 relaxation is sensitive to protein unfolding (denaturation) (Lambelet et al., 1995). Several studies have been carried out where LF NMR T2 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 T2 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 MgCl2) and contents (0.4-3.2 g/100 g salted mince), and prepared with fresh and frozen raw material.

For raw hake mince the T21 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 T21 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 T₂ 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 T₂ relaxation times increased when salt was added (Figure 19).

These results are in agreement with the results from cooked haddock mince (Paper III), where the T₂ 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 (T₂₁) and 402-598 ms (T₂₂), compared to those reported in Erikson et al. (2012); 40-60 ms (T₂₁) and 150-400 ms (T₂₂). 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 LF³H NMR T₂ relaxation data than samples where the equal amount of KCl and MgCl₂ were added (Figure 20). One explanation for the differences between samples containing pure NaCl and pure KCl can be

Figure 19. PCA score plot of LF³H NMR T₂ 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).
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 $^1$H 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).](image-url)
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When comparing the LF-NMR T₂ 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.

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, shows that the LF ¹H NMR T₂ relaxation method is sensitive to changes in the protein structure as an effect of additions of small amounts of salt. The LF ¹H NMR T₂ relaxation method can be a suitable technique to study changes in the protein structure in low-salt products. The LF-NMR T₂ 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](image_url)

*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 $b_\perp - b_\|$, using imaging, was only weakly linked to reduction in salt content, where no changes were seen in the shininess in the sensory evaluation.

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.
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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](image)

**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 matrices, both Na\(^+\) and Cl\(^-\) are of
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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).
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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 \(\%\) 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 MgCl\(_2\)) at low concentrations (0.11 and 0.28 M). The addition of higher amounts of MgCl\(_2\) (0.55 M) gave a decreased protein solubility. These results indicated that Na\(^+\) can partially be replaced with K\(^+\) and Mg\(^{2+}\) without changing the solubility of proteins. However the addition of Mg\(^{2+}\) 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 MgCl\(_2\) (in 0.6 M MgCl\(_2\)).

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
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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](https://example.com/figure26.png)

**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 ± 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.
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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, were 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
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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 physiochemical 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 physiochemical 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 Mg²⁺-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 Mg²⁺-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 Ca²⁺, 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
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A 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](image.jpg)

*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). ** 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.

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.

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**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).
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 (Na⁺: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 x 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.
RESULTS AND DISCUSSION

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 marked 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;
CONCLUSIONS AND FUTURE PERSPECTIVES

- The addition of high amounts (0.55 M) of MgCl₂ 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 MgCl₂ 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 Mg²⁺-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 Mg²⁺-ions used as salt replacer in muscle foods.
7 REFERENCES


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

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ABSTRACT: Impedance spectroscopy (IS), low-field proton nuclear magnetic resonance (LF-H 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 (R² = 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 T₂ measurements clearly distinguished samples with 0, 0.5, and 1.0–3.0% salt, based on principal component analysis (PCA). Therefore, LF-H NMR seems to be a suitable technique for studies of low-salt products.

KEYWORDS: impedance spectroscopy, low sodium, low salt, low-field NMR, T₂ 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.

For these reasons, national and international bodies have set targets for a reduction in sodium consumption. Salt is commonly employed in puddings, sausages, surimi, and surimi-based products such as fish. 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. Indeed, both salts have similar properties, and the health effects of increased potassium intake are continuously evaluated by international health authorities.

Replacement of NaCl by high concentrations of KCl may have a negative influence on the flavor intensity and produce bitter tastes. Parameters such as ωw 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 it is the accurate determination of the sodium content in food. The increasing use of salt replacers such as potassium chloride 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), ion chromatography, and sodium selective electrodes. Other methods, such as the Volhart method (AOAC method 971.27) and potentiometric titration, 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. 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. However, this technique has not yet been applied to...
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 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 monitor the Na-selective electrode in fish products, and (3) assess the feasibility of employing LF NMR in low-salt tissues.

### MATERIALS AND METHODS

#### Chemicals
Ammonium chloride (NH₄Cl), ammonium hydroxide (NH₄OH), ammonium hydrogen fluoride (NH₄FHF), ammonium hydrogen fluoride (NH₄FHF) <1%, LiD₃0 mg/kg not found), chlorofluorohydrocarbons (CF₃C₁₂), ethanol (C₂H₅OH), sulfuric acid (H₂SO₄), potassium sulfate (K₂SO₄), copper sulfate (CuSO₄), hydrogen peroxide (H₂O₂), and sodium hydroxide (NaOH) (Schultra, 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 concentrations. 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 Quimica S.A.U. (Barcelona, Spain). The brines were prepared 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 boxes with ice and transported immediately to the laboratory. Upon arrival at the laboratory, two

#### 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).27 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 the voltage (V) across the electrode were monitored. The impedance of the electrode is calculated as the ratio of voltage to current, Z = V/I. The impedance spectrum is then plotted as a function of frequency.

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 Quimica 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 (Crompack 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 °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 μm 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 μL 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).

#### 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, whereas pH and conductivity of brines were determined by using a multimeter MM 40 (Cronin Instruments, S.A., Barcelona, Spain). The pH measurements of fish minces were carried out using a digital pH-meter microH 2001 (Cronin Instruments) with a puncture electrode (Cronin 5231). Water activity was assessed in brines and fish minces with a fast water activity meter (Grise FastLab, Romans sur l’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, Laborotechnik, Staufen, Germany) at 9000 rpm for 1 min. Then, samples were warmed to 90 °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 Quimica 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 (Crompack 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 °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 μm 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 μL 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).
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).

\[
Z = |Z|e^{j\phi} = \frac{|v(t)|}{|i(t)|} \quad \text{module} \\
\phi = 2\pi f_1 \Delta t \quad \text{phase}
\]

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 taken.

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.
performed in each plastic container. The penetration depth of the electrodes was constant in all analyses (1.5 mm). 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 $^1$H NMR. LF $^1$H 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 of fish mince. A set of 34 values (17 module values and 17 phase values) was obtained for each sample.

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

<table>
<thead>
<tr>
<th>S</th>
<th>C (g salt/100 g brine)</th>
<th>$a_w$</th>
<th>Conductivity* (mS/cm)</th>
<th>Na$^+$ (g/L)</th>
<th>Cl$^-$ (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>0.1</td>
<td>0.999 ± 0.000A</td>
<td>2.10 ± 0.11A</td>
<td>(0.34 ± 0.02) x $10^{-3}$A</td>
<td>1.03 ± 0.06A</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.993 ± 0.001B</td>
<td>10.27 ± 1.15B</td>
<td>(0.76 ± 0.00) x $10^{-3}$A</td>
<td>3.50 ± 0.14B</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.996 ± 0.003B</td>
<td>19.31 ± 1.24C</td>
<td>(0.89 ± 0.01) x $10^{-3}$A</td>
<td>5.78 ± 0.13C</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.990 ± 0.002C</td>
<td>31.20 ± 0.28D</td>
<td>(1.26 ± 0.02) x $10^{-3}$A</td>
<td>8.30 ± 0.26D</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.985 ± 0.001C</td>
<td>41.67 ± 1.21E</td>
<td>(1.37 ± 0.02) x $10^{-3}$A</td>
<td>10.54 ± 0.09E</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.980 ± 0.002CD</td>
<td>51.23 ± 3.61F</td>
<td>(1.69 ± 0.04) x $10^{-3}$A</td>
<td>12.64 ± 0.13aF</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.984 ± 0.002AD</td>
<td>63.30 ± 5.30G</td>
<td>(2.18 ± 0.16) x $10^{-3}$A</td>
<td>14.88 ± 0.33aG</td>
</tr>
</tbody>
</table>

$NaCl$: 0.1 0.998 ± 0.002A 2.07 ± 0.04A 0.26 ± 0.00A 1.08 ± 0.08A
|   | 0.5                    | 0.995 ± 0.002B | 9.09 ± 0.42B | 0.77 ± 0.00B | 3.74 ± 0.21B |
|   | 1.0                    | 0.991 ± 0.001B | 19.33 ± 1.26C | 1.67 ± 0.02C | 6.44 ± 0.17C |
|   | 1.5                    | 0.985 ± 0.004B | 30.17 ± 3.06D | 2.60 ± 0.03D | 9.12 ± 0.19D |
|   | 2.0                    | 0.981 ± 0.001C | 38.27 ± 0.81E | 3.45 ± 0.01E | 11.86 ± 0.21E |
|   | 2.5                    | 0.980 ± 0.002CD | 50.63 ± 3.10F | 4.23 ± 0.07F | 14.72 ± 0.31F |
|   | 3.0                    | 0.981 ± 0.001D | 63.10 ± 4.27G | 5.17 ± 0.02G | 16.52 ± 0.15G |

$Na$: 0.1 0.998 ± 0.002A 2.57 ± 0.68A 0.39 ± 0.00A 1.05 ± 0.08A
|   | 0.5                    | 0.998 ± 0.001B | 10.44 ± 1.50B | 1.60 ± 0.01B | 3.61 ± 0.21B |
|   | 1.0                    | 0.984 ± 0.004B | 19.37 ± 0.97C | 3.56 ± 0.02C | 6.72 ± 0.26C |
|   | 1.5                    | 0.980 ± 0.002B | 28.37 ± 1.51D | 5.53 ± 0.03D | 10.01 ± 0.19D |
|   | 2.0                    | 0.981 ± 0.002C | 39.87 ± 2.00E | 7.65 ± 0.06E | 12.64 ± 0.05E |
|   | 2.5                    | 0.983 ± 0.001CD | 50.80 ± 3.10F | 9.45 ± 0.19F | 16.20 ± 0.24F |
|   | 3.0                    | 0.984 ± 0.002BC | 59.10 ± 3.05G | 11.07 ± 0.06G | 18.78 ± 0.26G |

$S$ = 22.45*** 0.616 0.0000 0.0000 0.0000
$C = 80.43*** 589.11*** 14371.42*** 10525.71***

$S$ $\times$ $C$ 7.90*** 0.294 5104.166*** 69.82***

*Mean values ± SD (n = 5). ANOVA F ratio for each of the two factors (S and C) and its interaction in the physicochemical parameters. Different lowercase 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). **p values: ***, p < 0.001; **, p < 0.01; *, p < 0.05; ns, nonsignificant.

For the biexponential fitting, the populations sum to 100%. Three populations are the relaxation time components, and $A_{11}$ 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 $T_{11}$ populations were calculated as $A_{11} / (A_{11} + A_{22})$.

For the biexponential fitting, the populations sum to 100%. Three parallel samples from each fish mince (n = 2) were averaged. (3) 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 analyses were carried out using the software.
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 to 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.

The correlation can be explained by the conductance of an aqueous solution as a function of the ionic 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 (< 0.001 but not for the type of salt (< 0.05) (ANOVA data not shown).

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

The correlation can be explained by the conductance of an aqueous solution as a function of the ionic 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 (< 0.001 but not for the type of salt (< 0.05) (ANOVA data not shown).

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

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**Table 2. Physicochemical Parameters of Fish Mince Prepared with Different Salts (NaCl, NaCl:KCl, and NaCl:KCl) and Contents (g/100 g)**

<table>
<thead>
<tr>
<th>Salt</th>
<th>pH</th>
<th>Module (mΩ cm²)</th>
<th>Phase (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>7.9</td>
<td>19.2 ± 1.0</td>
<td>12.0 ± 0.3</td>
</tr>
<tr>
<td>NaCl:KCl 1:1</td>
<td>7.8</td>
<td>18.5 ± 0.8</td>
<td>11.5 ± 0.2</td>
</tr>
<tr>
<td>NaCl:KCl 1:2</td>
<td>7.7</td>
<td>18.0 ± 0.6</td>
<td>11.0 ± 0.1</td>
</tr>
<tr>
<td>NaCl:KCl 1:3</td>
<td>7.6</td>
<td>17.5 ± 0.4</td>
<td>10.5 ± 0.0</td>
</tr>
<tr>
<td>NaCl:KCl 1:4</td>
<td>7.5</td>
<td>17.0 ± 0.2</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td>NaCl:KCl 1:5</td>
<td>7.4</td>
<td>16.5 ± 0.0</td>
<td>9.5 ± 0.0</td>
</tr>
</tbody>
</table>

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**References**

the mince led to a reduction in moisture, from about 80.2% (minces 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 $w_0$ decreased from 0.992 (mince without additions) to 0.974 (Na,K) and 0.969 (Na). The moisture and $w_0$ 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 $w_0$, compared to the brines, where the $w_0$ 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 $w_0$ 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 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, 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. 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.

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 study 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 fish samples than in solutions. A possible explanation for this behavior is the salting-in effects on muscle proteins. At salt contents lower than 0.5 M, the swelling of myofibrils starts and reaches a maximum at 0.8–1 M. 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. 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 ($T_{12}$) and 150–400 ms ($T_{12}$), similar to those of the present research. The mean $T_{12}$ and $T_{12}$ 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). 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.

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 $T_{12}$, whereas the $T_{12}$

![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).](Image)
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). *p values: **, p < 0.001; *, p < 0.01; *, p < 0.05; ns: non significant.

<table>
<thead>
<tr>
<th>S</th>
<th>C (g salt100 g fish mince)</th>
<th>(T_{21}^a) (ms)</th>
<th>(T_{22}^a) (ms)</th>
<th>(T_{21}^{pop}) (%)</th>
<th>(T_{22}^{pop}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fish mince</td>
<td>0</td>
<td>54 ± 1</td>
<td>219 ± 7</td>
<td>86 ± 3</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>NaCl/KCl</td>
<td>0.5</td>
<td>61 ± 1A</td>
<td>226 ± 10A</td>
<td>87 ± 2A</td>
<td>11 ± 2A</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>67 ± 1B</td>
<td>266 ± 46B</td>
<td>96 ± 1BD</td>
<td>4 ± 1AC</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>71 ± 1C</td>
<td>496 ± 42aD</td>
<td>99 ± 0BC</td>
<td>1 ± 0AC</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>68 ± 2A BC</td>
<td>342 ± 13aC</td>
<td>98 ± 0aCD</td>
<td>2 ± 0aAB</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5</td>
<td>59 ± 1A</td>
<td>215 ± 10aA</td>
<td>85 ± 1A</td>
<td>15 ± 1D</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>69 ± 1B C</td>
<td>366 ± 48B</td>
<td>98 ± 1B</td>
<td>2 ± 1C</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>78 ± 6B</td>
<td>427 ± 34BD</td>
<td>99 ± 0aCD</td>
<td>1 ± 0aAB</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>68 ± 0aBCD</td>
<td>423 ± 54BC</td>
<td>99 ± 0aD</td>
<td>1 ± 0a</td>
</tr>
</tbody>
</table>

Figure 5. PCA score plot of LF \(^1\)H NMR \(T_1\) 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.

Table 3. Biexponential Fitting of LF \(^1\)H NMR \(T_1\) Relaxation Data Obtained in Fish Mince and Fish Mince Prepared with Different Salts (S: NaCl and NaCl:KCl) and Concentrations (C)

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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 0.5, 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 \(^1\)H NMR can be a suitable tool for indirect studies of structural changes in low-salt systems. 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 (R²), 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

<table>
<thead>
<tr>
<th>LV</th>
<th>R²</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>a_w</td>
<td>0.886</td>
<td>0.003</td>
</tr>
<tr>
<td>Na (mg/100 g)</td>
<td>0.812</td>
<td>0.003</td>
</tr>
<tr>
<td>K (mg/100 g)</td>
<td>0.480</td>
<td>0.276</td>
</tr>
<tr>
<td>NaCl (g/100 g)</td>
<td>0.844</td>
<td>0.240</td>
</tr>
<tr>
<td>KCl (g/100 g)</td>
<td>0.425</td>
<td>0.070</td>
</tr>
<tr>
<td>solute content (g/100 g)</td>
<td>0.393</td>
<td>0.068</td>
</tr>
<tr>
<td>solute content in liquid phase (g/100 g)</td>
<td>0.950</td>
<td>0.021</td>
</tr>
<tr>
<td>solute content in liquid phase (g/100 g)</td>
<td>0.953</td>
<td>0.203</td>
</tr>
</tbody>
</table>

1LV, number of latent variables; R², coefficient of determination; RMSEP, root mean square error of prediction.

**Acknowledgments**

We thank our co-workers at UPV, Isabel Fernández-Segovia, Arantza Rico, 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**


(10) 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.


**Associated Content**

Supporting Information

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

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**Notes**

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


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ABSTRACT

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

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

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

END PAGE 2
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 down to 2 g/day [2-4].

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

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

It is essential to characterize and understand the sensory effects of sodium reduction in foods. 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 ingredient and process
variables [12]. For measurement of flavor no instruments which exist today can replace the human senses and describe the sensory perception. For appearance and texture attributes, the situation is different, as there are several examples of studies where instrumental data representing appearance and texture attributes, show a strong correlation with data from sensory trained panels [13-16].

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

The effect of reduced salt/sodium content on cooked ham has previously been investigated by different authors [5, 20, 21], but nobody, as far as we know, have used the combination of sensorial Descriptive Analysis (DA) and multimodal machine vision system to
investigate the changes in the sensorial properties in cooked ham with effect of reduced salt/sodium content. In this paper, we apply a novel dual-polarization multimodal machine vision system that, to our knowledge, is new to meat imaging and imaging of other muscle foods such as fish [22].

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

Materials and Methods

Samples and Chemicals

**Cooked ham preparation.** The preparation of ham Eight different formulas of cooked hams were manufactured by Espeland AS, Ålgård, Norway 26-28 February 2013. Each of the formulas contained 20 kg of fresh coarsely grounded (perforated disc with hole size 30 mm), lean pork meat (*M. semimembranosus, M. adductor, M. semitendinosus, M. biceps femoris* and *M. psoas major*), obtained from a local meat processing company (PrimaGruppen, Norway) four days after slaughtering. The brine solution for each formulation was prepared by dissolving 200 g commercial phosphate mixture (57% P₂O₅)(A. B. Corneliussen AS, Norway), 150 g gelatin (Gelita Sweden AB, Sweden), 60 g carrageenan (CEAMSA, Pontverda, Spain), 450 g Na-lactate (Purac Biochem, Gorichem, The Netherlands) and 150 g maltodextrin (Avebe U.A., The Netherlands) in 2.5 L tap water (t =2- 4 °C). All sample except from the reference ham (Na 100) were added 3.3 g sodium nitrite (Merck, KGaA Darmstadt, Germany), were the commercial food salt containing nitrite salt (Hoff Norske Potetindustrier, Gjøvik, Norway) was added to the reference ham (Na100). Sodium chloride (Hoff Norske Potetindustrier, Gjøvik, Norway) and potassium chloride (Culinar Lyckeby,
Sweden) were added to the brine to achieve the expected Na\(^+\) and K\(^+\) content as shown in Table 1. The mole ratio of sodium (Na\(^+\)) and potassium (K\(^+\)) was kept constant (Na\(^+\): K\(^+\) = 3:1) in all formulas containing potassium (NaK), were the contribution of all sodium and potassium in all ingredients were taken into account. The salt (NaCl) content (g/100g) was calculated as: added Na\(^+\) (including natural sodium content in the meat) \(\times 2.5\) (recalculation factor for NaCl from Na\(^+\)), according on the provision of food information to consumers [23]. The reference cooked ham did not contain potassium, only sodium (Na 100), and the amount of sodium in the formulation was at same level as in a commercially cooked ham, 1.24 g Na\(^+\)/100g, corresponding to 3.1% salt. The amount of total salt content in the formulation was gradually decreased to 20% of the amount of the salt in the reference cooked ham, corresponding to 1.3% salt (NaK20). The brine was added to the pork meat in a tumbler (Fatosa, Barcelona, Spain) without vacuum and massaged for 2 \(\times\) 25 min, stored 20 h at 2°C, and then tumbled for 15 more min. The meat matrix was stuffed in to a plastic casing (160 mm in diameter, Viscofan S. A., Navarra, Spain). Two parallel production batches of the hams with formulation NaK 100 and NaK 60 were prepared to control the reproducibility of the production. Seven hams (3.0 kg) from each formulation were produced. The hams were pressed in molds and kept at 0-2°C for 20 h before cooking. The hams were cooked in a cooking chamber (Fessmann, Germany) until they reached a core temperature of 78 °C, chilled down in tap water and kept in a cold-storage chamber at 0-2°C. Two of the hams per formula were sliced in 20 slices (16 ± 1 g) 12 days after production, and alternate of the slices were subjected to sensorial evaluation and analysis of machine vision, respectively. Sensorial evaluation and machine vision analyses were carried out directly after slicing. Two of the hams per formula were sliced and packed in modified atmosphere (30% CO\(_2\) and 70% N\(_2\)) and stored at 4°C for bacterial analyzes. The remaining cooked hams were stored at 4°C for
15 days after production before chemical analysis. In addition, one slice (approximately 100 g) was used for further analysis.

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

**Chemicals.** Ammonium chloride (NH₄Cl), ammonium hydroxide (NH₄OH) and ammonium hydrogen fluoride (NH₄HF < 1%, LD₅₀ mg/kg not found) of analytical-reagent grade (Thermo Fisher Scientific, USA). Chemicals of food grade: sodium tripoly phosphate (Na₅P₃O₁₀) (A. B. Corneliussen AS, Norway), Na-lactate (C₃H₅NaO₃) (Purac Biochem, Gorichem, The Netherlands) and sodium nitrite (NNaO₂)(Merck, KGaA Darmstadt, Germany).

**Imaging Setup and Image Acquisition.**

The imaging system consisted of a ColorRanger multimodal line-scan camera (SICK IVP AB, Linköping, Sweden), two high-intensity white LED linear array lights (Banner Engineering, Minneapolis, MN, USA), with polarizers on the LED arrays and in front of the camera lens. Additionally, a nematic liquid crystal industrial-grade polarization rotator (ARCoptix SA, Neuchâtel, Switzerland) was placed in front of the polarizer on the camera lens. The polarization rotator is controlled by the image acquisition PC and enables effectively to rotate 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.

The experiment sought to separately image the subsurface color and the surface color of the ham slices, in order to separately image the light that was scattered in the subsurface.
Subsurface imaging revealed the bulk color of the ham near the surface, whereas surface imaging revealed the surface roughness, shininess and other effects such as "mother-of-pearl" appearance commonly seen in some hams. To separate the subsurface from the surface image, two images – $I_\parallel$ LED camera polarizers oriented parallel to each other were acquired, and $I_\perp$ with the polarizers oriented perpendicular to each other. 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 Fig. 1.

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

**Supplementary Image Acquisition.** Image acquisition of the hams used for the sensory evaluation was done with a polycarbonate window in the imaging setup, which unfortunately affected the polarization of the imaged light. This resulted in suboptimal images. Therefore, only the blue channel images ($b_\parallel$ and $b_\perp$) were used for analysis and a supplementary image acquisition was done. Here, the polycarbonate window was replaced with an optical grade AR-coated BK7 glass window. Replicated formulas of cooked ham were manufactured for Na100, NaK80 and NaK60, respectively, which were used for validation of the multimodal imaging acquisition system. The formulation, production process, and storage were similar to the cooked ham preparation as described above.
example set of images is seen in Fig. 2. The images in Fig. 2 are included in order to provide the reader with a visual understanding of the possibilities of using multi-modal imaging of cooked ham with dual polarization states.

Chemical analysis

Water activity ($a_W$) was determined in cooked hams with a fast water activity-meter (GBX Fast/lab, Romans sur Isère Cedex, France). The pH measurements on light and dark muscle, in brine and cooked ham were carried out using a digital pH-meter WTW pH3110 (Weilheim, Germany) with a puncture electrode (WTW A 120513078, Weilheim, Germany). The moisture content was determined by drying three parallel samples of 5 g of minced cooked ham at 105 °C for 24 hours [24]. Water holding capacity (WHC) was determined on minced cooked ham by low-speed centrifugation as described by Eide, Børresen [25] with a centrifugation force of 210 g. The WHC is expressed as the percentage of water retained in the mince after centrifugation for 5 min. The analyses were run in quadruplicate. Analyses sodium contents in cooked hams were measured in an extract of the sample, using 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). For preparing the extract, 7.5g of ham was homogenized in ultra-pure water using an Ultra-turrax T-25 D (IKA, Labortechnik, Staufen, Germany) at 9000 rpm for 1 min. Then, samples were warmed up to 90°C for 30 min, cooled down to room temperature, transferred to a volumetric flask and diluted to 200 mL with ultra-pure water. Finally, samples were filtered through a cellulose filter paper (Whatman nº 1, Whatman International Ltd., Maidstone, UK). The Na-selective electrode method was a modification of the Kivikari [26] method. In this study 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.

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

**Microbial analysis**

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

**Sensory evaluation**

Descriptive Analysis (DA) was conducted by a trained sensory panel according to Generic Descriptive Analysis as described by [12]. All assessors (n=9) were selected and trained in accordance with ISO 8586-1 (ISO, 1993) and the test was done in a sensory
laboratory designed in accordance with ISO 8589 (ISO, 2007). Each assessor evaluated all samples using EyeQuestion for direct recording of data (v3.8.7, Logic8, Elst(Gld), The Netherlands). A list of 21 attributes was generated from a brainstorming session with the assessors. This list included attributes representing appearance (color-hue, color intensity, whiteness, color evenness, shiny, marbling, cohesiveness, visible moist on surface), odor (sour, pork meat, metal), taste/flavor (sourness, sweetness, saltiness, bitterness, pork meat, metal, after flavor) and texture (hardness, juiciness, tenderness). Attributes were evaluated using a continuous, non-structured scale ranging from no intensity (1) on the left to high intensity (9) on the right. The assessors had previous experience with analyses of meat products including ham and were calibrated on selected attributes in a pre-test. DA was performed during 6 sessions on one day, in which 8 different hams were served in two replicates (totally 16 samples). The hams were sliced by a machine to a weight of 16 ± 1 g, and each assessor got one slice served per sample. Appearance attributes were evaluated on the surface of the ham slice, while the texture was evaluated biting over a rolled slice. The serving order was randomized across all sessions. All samples were expectorated and unsalted crackers and lukewarm water was available for rinsing.

**Data analysis**

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

PanelCheck 1.3.2 (www.panelcheck.com) was used to evaluate the panel performance in the pre-test. For determination of sensory attributes discriminating between samples, a two-
way ANOVA with product as a fixed factor, panelist as a random factor and product x panelist as an interaction factor was performed. Tukey’s Multiple Comparisons Test was applied to determine which products that was significantly different. The significance level was defined to $p<0.05$. (The ANOVA was run by SAS 9.2, SAS Institute, Inc., Cary, NS, USA).

**Results and Discussion**

**Chemical analysis**

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

As expected, a decrease in WHC was observed in hams were the salt content was reduced by 60% (NaK40) and 80% (NaK20) (on molar basis), corresponding to a measured salt content of 1.73 and 1.38% salt, respectively. These results are in accordance with Hamm (1972) and Offer & Knight (1988) who found that sodium chloride increases the water-binding of meat [28, 29]. Albarracin, Sánchez [30] explained the increasing in WHC in meat by increasing salt content where the anions (Cl⁻) preferentially bind to protein molecules. The moisture content in hams added a mixture of sodium and potassium salt without reduction of the total salt, 2.70% salt (NaK100) was significant lower than in cooked ham containing 1.38% salt (NaK20).
The pH in the raw material was within the range of 5.5-5.8. These pH values are similar to typical ultimate pH values in Norwegian pork [31]. The pH of the hams increased from pH 6.15 ± 0.02 to 6.25 ± 0.01 with decreasing sodium content. The pH in hams with the lowest levels of sodium, 1.73% salt (NaK40) and 1.38% salt (NaK20), were significant higher than the reference containing 3.38% salt (Na100) and the cooked ham with the mixture of sodium and potassium salt (NaK100). These findings are in accordance with Puolanne, Ruusunen [32], who found an average decrease, about 0.1 pH-units/%-units of salt, in cooked sausages.

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

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

Compared to the reference ham (Na100), a replacement of Na⁺-ions by 25% of K⁺-ions gave no significant changes in WHC, moisture, pH or expressible moisture. This replacement results in a total sodium content of approximately 1.1 g/100g cooked ham. This finding is in accordance with Zanardi, Ghidini [34] who found no effects on pH and water activity in reduced sodium when replacing (Na⁺) by a mixture of KCl, CaCl₂ and MgCl₂ in Cacciatore salami compared to the traditional recipe.

**Microbial stability**

The total aerobic microbial count is a common indicator of shelf-life. The total aerobic counts were relatively low during the entire storage period and varied between 1-3 log cfu/g.
There were no significant changes in total aerobic microbial counts presumably due to the addition of salt or storage time in our cooked hams (data not shown).

Descriptive sensory profile

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

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

To summarize, a reduction to 40% (NaK60) of total salt, corresponding to 2.04% salt, was possible without significantly influencing the salty taste. The changes in saltiness in this experiment correspond to another low-salt ham study, Ruusunen, Särkkä-Tirkkonen [5] found that hams with 1.4% salt were less salty than those containing 1.7 - 2.6% salt. The reduced saltiness can be explained by the reduced content of Cl\(^-\) anions in the cooked ham since Cl\(^-\) anions have an effect on the receptor cells and consequently on the perception of salt taste [35]. There were no significant differences in metal flavor and metal odor except for a higher value for metal flavor for one of the parallels of cooked ham containing the highest amount of potassium chloride (NaK100_1) compared to the cooked ham containing the lowest salt content (NaK20). These results indicate that a three to one replacement of Na\(^+\) ions with K\(^+\) ions might be an acceptable level, regarding the issue of bitter (no significant differences
Previous studies have shown that KCl may induce a bitter taste at high concentrations. In dry-cured loins [9] and fermented sausages [10], the maximum level was 40% replacement of sodium with potassium. In a study of cooked ham they showed that 2.0% NaCl had a higher score than a 50% replacement with KCl [11]. Another study found that it was possible to replace one-third of NaCl with KCl without altering the sensory properties of smoked salmon [36]. Those results show that the maximum level of KCl replacement may be dependent on product type.

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

Compared to the reference ham (Na100), a reduction down to 60% (NaK40) of total salt, corresponding to 1.73% salt, had no effect on tenderness or hardness in the cooked hams. However, a reduction of total salt content by 80% and replacement of Na\(^+\) ions by K\(^+\) ions of ¼ (NaK20), corresponding to a salt content of 1.38%, gave a more tender and less hardness on cooked ham. These results were as expected and in accordance with Ruusunen, Vainionpää [39], who found that added salt increased the firmness in Bologna type sausages added 1.10, 1.35 to 1.60% salt, respectively and Sofos [40] found that a reduction in salt content of more than 20% (<2.0% NaCl) in Frankfurters resulted in softer and less firm texture. It is important to keep in mind that the effects of proteins may differ in cooked ham where small pieces of
lean meat are connected together whereas sausages have it an emulsified fat protein network. In both cases the solubility and swelling of the myosin are nevertheless important. In cooked ham, molecular bonds are able to form inside the exudate matrix (gel cohesion) and between the exudate and the muscle [20], and when the salt concentration is reduced, the protein extractability is limited due to the solubility of the myosin [28, 29].

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

**Multimodal machine vision system**

The main imaging experiment, using the multimodal machine vision system, was done using the same hams as with the chemical and sensorial measurements. Machine vision was used to objectively study changes in color and texture in the surface. The change in lightness, as a function of reducing the salt content, is clearly seen in Fig. 4a.

The intensity of the subsurface backscattered reflected blue light $b_1$ shows a clear increase as the salt content is reduced. This is confirmed by sensorial evaluation, indicating an increase in whiteness and decrease in color-hue with decreased salt content. A possible explanation for this behavior might be the decreased swelling of the salt soluble proteins and a weaker heat induced gel in cooked ham with 1.38% salt (NaK 20) as discussed in above in section 3.3. Due to this theory, the surface of the cooked ham with less salt might be rougher, and the interaction of light will change, as has been shown previously [13]. Another explanation for the increased whiteness due to decreased salt content may be that salt affects the formation of heat stable nitric oxide myoglobin, the "cured meat color" [41].
A reduction in surface shininess, as measured by $b_l-b_{1.1}$, using imaging, was only weakly linked to reduction in salt content, Fig. 4b. Despite the challenges in the imaging setup in the main experiment, the improvements from the supplementary experiment in Fig. 2 show that imaging with two polarization orientations can provide images that potentially can quantify subtle changes in visual appearance.

Since we used only simple features, namely the $(r_g, g_g, b_g)$ and $(r_4, g_4, b_4)$ values computed as the mean over each slice, we see greater potential in dual-polarization imaging if more advanced features such as those described in the literature [13-15, 17, 19] would be applied to dual-polarization images using the multimodal machine vision system described in this paper. We would also suggest that automatic segmentation between the dark and light portions of the mean would help improve the quantification of the visual appearance characteristics in a way that human sensorial evaluators do with their combined vision and decision process.

**Conclusions**

Compared to the reference ham (3.4% salt), a replacement of 25% Na⁺-ions by K⁺-ions gave no significant changes in WHC, moisture, pH, expressed moisture, the sensory profile attributes or the surface lightness and shininess. However, a reduction of salt content by 60% and 80% on molar basis or more (down to 1.7-1.4% salt) led to a decrease in WHC and an increase in expressible moisture. The salt reduction had highest influence on the sensory attributes salty taste, after taste, tenderness, hardness and color hue. To summarize, a reduction to 40% of total salt, corresponding to 2.04% salt, was possible without significantly influencing the salty taste. Further reduction down to 1.4% salt led to increased softness and reduced harness in the cooked hams. When reduction of salt by more than 60% (down to
1.7% salt) together with a replacement of 25% Na\textsuperscript{+}-ions by K\textsuperscript{+}-ions, the low-salt ham manufacture procedure has to be modified. The multimodal machine vision system showed changes in lightness, as a function of reduced salt content.

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 authors thank Marte Schei and Anne Blikra for their contribution in production and analyzing of the hams, MSc Aleksander Eilertsen and research scientist Morten Bondø for their assistance in setting up the electronics and programming of the machine vision system.


8. Olson D. Salt for processing probably can be cut by only one quarter. The National Provisioner. 1982;7-10.


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

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

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

Figure 4a Mean subsurface reflectance $b_\perp$ for the hams with varying salt content.

Figure 4b Mean surface reflectance $b_\parallel - b_\perp$ for the hams with varying salt content.
Table 1. Sample ID, description of the sample, estimated sodium, potassium and NaCl content in the preparation of the eight cooked hams.

Each formulation varied in its amount of total salt. The reference cooked ham contained nitrite salt (Na 100 Reference). In the rest of the formulations (NaK100, NaK80, NaK60, NaK40 and NaK20) the amount of total salt content was gradually decreased to 20% of the amount of 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 sodium and potassium from the raw material and the ingredients was added when calculating the mole ratio.

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

* Produced twice to control the reproducibility of the production

** 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⁺)
Table 2. Water holding capacity (WHC), moisture, pH, sodium, salt and expressible moisture of cooked ham prepared with different levels 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 (NaK100, NaK80, NaK60, NaK40 and NaK20) the amount of salt was gradually decreased to 20% of the amount of salt in the reference ham. Mean values ± SD (n=3)

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

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 significantly different.
<table>
<thead>
<tr>
<th>Group</th>
<th>Colour hue</th>
<th>Cohesiveness</th>
<th>Whiteness</th>
<th>Shiny</th>
<th>Salty taste</th>
<th>Metal flavour</th>
<th>Metal odour</th>
<th>After taste</th>
<th>Tenderness</th>
<th>Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na 100</td>
<td>6.67&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>6.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.71</td>
<td>6.18&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>4.74&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.94&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.78&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>NaK 100_1</td>
<td>6.84&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.21</td>
<td>6.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.21&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>6.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.42&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3.76</td>
<td>6.58&lt;sup&gt;ab&lt;/sup&gt;</td>
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p-value: 0.0010 0.0013 n.s. n.s. <0.0001 0.0065 0.0098 <0.0001 0.0001 <0.0001

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 significantly different. Non-significant: n.s.
\[ I_\parallel - I_\perp \] (Surface)

\[ I_\perp \] (Subsurface)

\[ I_\parallel \] (Subsurface + Surface)

Fig. 1
Fig. 2

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

$I_{\parallel}$ (Subsurface)

$I_{\perp}$ (Subsurface + Surface)
Fig. 4a

Subsurface reflectance $b_{\perp}$

Na100   NaK100          NaK80  NaK60        NaK40

Group 'JHB
Surface reflectance $b_\parallel - b_\perp$

Fig. 4b
Paper III
Sodium reduction in minced fish products by adding Magnesium and Potassium Chloride; effect on physicochemical properties.

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Running title header: Sodium reduction in minced fish.

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Abstract

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

Key words: haddock; low sodium; potassium chloride; magnesium chloride; low field NMR
1. Introduction

A high intake of sodium (Na) is associated with an increased risk of high blood pressure, which is a significant risk factor in the development of cardiovascular disease (CVD) and stroke (Cook et al., 2007; He & MacGregor, 2002). The daily Na-intake in Europe and USA is estimated to 4-5 g/day (The Norwegian Directorate of Health, 2011), and salt (NaCl) added to food products during production, preparation and at the table is the main source of this Na.

Guidelines published by public health and regulatory authorities (FSA, 2004; The Norwegian Directorate of Health, 2011; WHO, 2006) recommend a reduction of Na intake to 2 g/day or less. Salt is commonly employed in fish processing, due to its preservative effects, taste, positive technological effects and low cost (Fuentes, Ferández-Segovia, Serra, & Barat, 2010; Martínez-Alvarez & Gómez-Guillén, 2006).

The development of safe high quality low-sodium fish products is of interest, especially considering the good nutritional characteristics of fish. It is often possible to reduce the addition of salt in "traditional" recipes to some extent, but further reduction then requires the addition of salt substitutes to maintain palatability, texture, processing yield and shelf-life (Kilcast & Angus, 2007). Salts contribute to formation of a viscous protein paste, that creates a dense protein network, a gel, by solubilising the myofibrillar proteins (Kilcast & Angus, 2007). Several authors have reported that when NaCl is partially replaced with other salts like KCl, MgCl₂ or CaCl₂ this will affect the enzyme activity, protein matrix and texture (Andreetta-Gorelkina, Greiff, Rustad, & Aursand, 2014; Barat, Pérez-Esteve, Arito, & Toldrà, 2012; Martinez-Alvarez & Gómez-Guillén, 2013). Partial substitution of NaCl by KCl is one of the best alternatives for reducing sodium content (Aliño, Fuentes, Fernández-Segovia, & Barat, 2011; Fuentes et al., 2010; Toldrà & Barat, 2012). MgCl₂ is used in low concentrations in commercial "low-sodium" salts on the market (Barat et al., 2012). Most of
the studies on the effect of salt and "low-sodium" salts on physicochemical properties have employed meat and meat batters (Barat et al., 2012; Nayak, Kenney, Slider, Head, & Killefer, 1998; Offer & Trinick, 1983; Ruusunen & Puolanne, 2005; Toldrá & Barat, 2012) or heavily salted cod (Aliño et al., 2011; Martínez-Alvarez, Borderías, & Gómez-Guillén, 2005; Martínez-Alvarez & Gómez-Guillén, 2005). In a study of salt substitution in surimi gels, Tahergorabi, Beamer, Matak, and Jaczynski (2012) found that hardness increased when NaCl or KCl was added to the gel, but there are limited results available on cooked fish mince. Low-field \(^1\)H NMR (LF-NMR) has been used to indirectly determine the effect of salting by monitoring the changes in proton relaxation behavior as a result of the salting process (reviewed by Erikson, Standal, Aursand, Veliyulin, & Aursand, 2012), However, none of these studies dealt with salt replacers such as MgCl\(_2\) and, it was of interest to explore the LF-NMR method further as a tool for measuring proton relaxation behavior in low-salt applications.

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

2. Materials and methods

2.1 Raw material

Haddock (Melanogrammus aeglefinus) was caught by Danish seine on Vesterålsbanken North-Norway in March 2011. The catch was pumped on board by a vacuum pump, stored alive on-board before electrostunning, bleeding and gutting. The fish were iced in styrofoam boxes and shipped to Trondheim (cold storage). The core temperature in the fish was <4°C during the experiment. Six days after catch the fish were evaluated, filleted and skinned. The
fish were considered of very good quality. Gutted mean weight was $1.8 \pm 0.6$ kg. Half of the fishes were packed in plastic bags and frozen at $-28^\circ$C, the other half were filleted and minced the day after filleting. After 67 days, the frozen fish were thawed with drainage in a cold room ($+4^\circ$C) for 67 h. The thawed fish were filleted and the minces prepared in the same manner as with the fresh fish.

2.2 Experimental design

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

2.3 Process and heat treatment

The fillets were coarsely ground (perforated disc, hole size 5 mm), (Hobart A 200 N, Hobart manufacturing, Braunton, UK) and mixed (45 sec) with salt before ice water was added. The mixture was homogenized (1.5 min) in a food processor (BRAUN Multiquick 7, Braun GmbH, Kronberg, Germany), interrupted at one interval to scrape the sides of the bowl. Two parallel minces from each sample were produced and blended into one batch. The fish mince was divided in three plastic trays (~460 g in each tray) and cooked in a convection oven (Rational SCC 61, Rational AG, Landsberg a. Lech, Germany) ($100^\circ$C, 100 % RH) to a core temperature of $80^\circ$C. After cooling the minces were packed in plastic bags and stored cool.
(4°C) for 24 h before further analysis. The minces (n=3) were subjected to physicochemical analyses and LF-NMR.

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

2.4 Analytical methods

The pH of the raw and cooked minces was measured directly with a pH-meter (WTW PH3110, Weilheim, WTW, Germany) equipped with a glass electrode (SenTix Sp, A, Weilheim, WTW, Germany). Moisture content was determined by drying 5 g cooked fish mince at 105 °C for 24 h to constant weight according to AOAC method (AOAC, 1997).

WHC of ground fillets and cooked mince was determined by low-speed centrifugation (210 x g) as described by Eide, Børresen, and Strøm (1982). The analyses were run in quadruplicate and WHC expressed as the percentage of water retained in the mince after centrifugation for 5 min. Cooking loss was determined by weighing the mince before cooking and 24 h after cooking and chilling. The cooked mince was stored on a grid for 1 min before weighing. Cooking loss was calculated as percent weight difference between the heated and unheated minces (n=3).

The sodium (Na⁺) and chloride (Cl⁻) content of cooked minces were determined in mince extracts. Sodium was determined at ambient temperature with a Na-selective electrode (Ross® Sodium Ion Selective Electrode, Thermo Fisher Scientific, USA) and a Dual Star pH/ISE meter (Thermo Fisher scientific, Waltman, MA, USA), under constant stirring (Kivikari, 1996) as modified by Greiff et al. (2014). The analytical uncertainty of the method was determined by analysing three extracts from each of three replicates of the same cooked mince.
mince. This showed very low variation (1-2%). For the rest of the minces, only one extract from each of three replicates of the same formulation and were analysed. The Cl– content was determined by end point titration of halides according to the titration application issued by Radiometer Analytical SAS (TTEP01-04AFD/2001-05A). The titration end-point was assessed potentiometrically using an automatic titrator (TitraLab980) coupled with a silver electrode (M295Ag) and a reference electrode (REF 921) (all equipment from Radiometer Analytical ASA, Copenhagen, Denmark).

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

2.5 Proton relaxation behavior

**LF ¹H NMR samples preparation and analysis.** LF-NMR measurements were made on all cooked minces. Three subsamples (about 1 × 1 × 3 cm, approx. 2-3 g) from each mince were placed in NMR tubes (diameter 10 mm). The tubes were immediately placed on ice for about 30 min before equilibration to 1°C in a water bath (Julabo Labortechnik GmbH, Seelbach, Germany) and analysis on a minispec mq 20 (Bruker Optik GmbH, Rheinstetten, 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 probe temperature was controlled at 4°C by blowing compressed air through the
sample holder. Proton transversal ($T_2$) relaxation was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (CPMG) (Carr & Purcell, 1954; Meiboom & Gill, 1958). The $T_2$ measurements were performed with a time delay between the 90° and 180° pulses ($t$) of 150 μs. Data from 6000 echoes were acquired from 16 scan repetitions. The repetition time between two succeeding scans was set to 4 s. All even echoes were sampled.

**Low-field NMR data processing.** The NMR transverse relaxation data were analysed using two different calculation methods. (1) 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 1000 data points were used for the principal component analysis (PCA) (Jolliffe, 1997) using an in-house program written in Visual Basic. Each row (n) represented a single mince sample and each column (m) represented a signal amplitude from an echo in the CPMG echo train. Four principal components (k) were used. The input matrix was not mean-centred. (2) Biexponential analysis of $T_2$ relaxation data was performed by using MatLab (The Mathworks Inc., Natric, MA) to fit the following equation to the experimental CPMG curves, as reported by Erikson, Veliyulin, Singstad, and Aursand (2004) and Lambelet, Renevey, Kaabi, and Raemy (1995):

$$Signal = A_{21}e^{-t/T_{21}} + A_{22}e^{-t/T_{22}}$$

(Eq. 1)

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

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

3. Results and discussion

3.1 Physicochemical Parameters

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

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

The pH of the raw mince after addition of salt depended on the type and amount of salt added (Table 2). Compared to fresh ground haddock, pH increased slightly in mince with 0.07 mol
KCl /kg, was fairly similar in raw mince with 0.07 mol NaCl /kg and decreased significantly in mince with 0.07 mol MgCl₂/kg. Upon further addition of salt, pH in the minces decreased for all types of salts, most for minces added MgCl₂. In raw mince added 0.34 mol/kg, pH was one unit lower than in freshly ground haddock.

A decrease of pH in fish flesh and animal meat with increasing addition of salt (NaCl) has frequently been observed (Abbas Bakhiet & Khougali, 2011; Leroi & Joffraud, 2000; Puolanne, Ruusunen, & Vainionpää, 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 rate of decrease depended on the type of salt due, among others, to the charge of the ions, their size and hydration number. For the salts relevant here, the rate of pH decrease with increasing salt concentration was lowest for KCl and highest for MgCl₂. This is accordance with the rate sequence observed here. The pH in all minces increased slightly (0.05-0.23 pH units) after cooking (Table 2) as also observed for cooked sausages with NaCl (Puolanne et al., 2001).

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

The WHC in the cooked fish minces added different amounts of salts are shown in Fig.1. WHC tended to increase slightly with increasing addition of the different salts, but only the mince added 0.07 mol MgCl₂/kg had WHC significantly lower than the rest of the minces. Neither ionic strength nor pH appears to explain this result. It may be related to the ability of divalent Mg²⁺-ion to form cross-bridges between peptide chains (Aliño et al., 2009), but that this effect of Mg²⁺ is masked by the increased ionic strength when the addition of MgCl₂ is
increased. Addition of KCl and NaCl gave similar WHC, in agreement with Larsen & Elvevoll’s (2008) claim that KCl can replace NaCl without affecting the technological quality parameters. Minces added NaCl had slightly higher WHC than the corresponding minces added KCl, possibly because NaCl can lead to more muscle swelling than KCl due to cation effects in accordance with the Hofmeister series (Damodaran, 2008). The moisture content of cooked mince added 0.34 mol MgCl₂/kg was significantly lower than in all the other minces. Weinberg, Regenstein, & Baker, (1984), observed poor moisture retention in cod containing MgCl₂.

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

The LF-NMR T₂ relaxation method was used to study the proton relaxation behaviors in the cooked minces. 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. The two major ones have relaxation times of 40-60 ms (T₂₁) and 150-400 ms (T₂₂) (see Erikson et al., 2012). In the present study, the relaxation times were slightly higher; 64-93 ms (T₂₁) and 402-598 ms (T₂₂). A possible explanation for the higher relaxation times is changes in the protein matrix due to the added salt and water and the cooking. The interpretation of such data have been controversial, but it is 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, 2011; Halle, 2006). Several studies have shown that these processes are linked to the morphology of the sample, which in turn can be affected by
processing such as salting (Aursand, Erikson, & Veliyulin, 2010; Erikson et al., 2012; Gudjonsson et al., 2010).

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

3.4 Breaking force

The breaking force increased with increasing Na$^+$ content as also observed for cooked pork sausages within the same range of NaCl contents (Puolanne et al., 2001). However, the hardness of cod frankfurter decreased with increasing NaCl content (Cardoso, Mendes, & Nunes, 2009). The disagreement may be related to differences in protein extractability at different ionic strengths between fish species, as discussed by Jafarpour & Gorczyca (2012). The 0.34 Mg mince was softer than 0.34 K and 0.34 Na minces. Nayak et al. (1998) found that low levels of MgCl$_2$ decreased the hardness on beef batter, possibly due to the amount of proteins solubilised by the salt. A significant decrease in salt soluble proteins in raw haddock
mince added 0.34 mol/kg MgCl₂ compared to 0.34 mol/kg KCl or NaCl was observed by Andreetta-Gorelkina et al. (2014).

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

As for minces made from fresh raw material, increased addition of NaCl or KCl had no effect on WHC in cooked minces made from frozen raw material (Fig.1), but WHC in minces prepared from frozen raw material and added NaCl decreased significantly compared to the corresponding minces prepared from fresh raw material and added NaCl (p-value < 0.05 for 0.07 and 0.34 Na, p-value < 0.10 for 0.17 Na). This effect was much less pronounced in minces added KCl and not present in minces added MgCl₂. The use of frozen raw material increased the cooking loss compared to fresh raw material (Fig.2 a-d). As for fresh raw material (Section 3.3), the cooking loss decreased with increasing concentration of chloride ions and increased ionic strength (Fig.2 b,d). However, the results fit best when the cooking loss seen as an effect of ionic strength (Fig. 2).

4. Conclusion

Replacing NaCl with the same molar concentration of KCl had no or only small effects on pH, moisture, breaking force WHC and cooking loss in cooked minces made from fresh raw material. However, replacing NaCl with an equal molar concentration of MgCl₂ affected several of the physicochemical properties and in particular WHC, cooking loss and pH. Cooking loss decreased with increased salt content, and the results indicate that the concentration of ionic strength was more important than the type of cations. Mince prepared with frozen raw material had a much higher cooking loss than the corresponding minces prepared with fresh material, particularly at low salt concentrations.
5. Acknowledgements

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

<table>
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<td>MgCl₂</td>
<td>0.07</td>
<td>0.7</td>
<td>0.21</td>
</tr>
<tr>
<td>0.07 Na</td>
<td>NaCl</td>
<td>0.07</td>
<td>0.4</td>
<td>0.07</td>
</tr>
<tr>
<td>0.17 K</td>
<td>KCl</td>
<td>0.17</td>
<td>1.3</td>
<td>0.17</td>
</tr>
<tr>
<td>0.17 Mg</td>
<td>MgCl₂</td>
<td>0.17</td>
<td>1.6</td>
<td>0.51</td>
</tr>
<tr>
<td>0.17 Na</td>
<td>NaCl</td>
<td>0.17</td>
<td>3.2</td>
<td>0.17</td>
</tr>
<tr>
<td>0.34 K</td>
<td>KCl</td>
<td>0.34</td>
<td>2.5</td>
<td>0.34</td>
</tr>
<tr>
<td>0.34 Mg</td>
<td>MgCl₂</td>
<td>0.34</td>
<td>3.2</td>
<td>1.02</td>
</tr>
<tr>
<td>0.34 Na</td>
<td>NaCl</td>
<td>0.34</td>
<td>2.0</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Table 2 Moisture, pH and breaking force in haddock mince (from fresh raw material) with different salts at different concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture cooked mince (%)</th>
<th>pH raw mince</th>
<th>pH cooked mince</th>
<th>Breaking force cooked mince (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07 K</td>
<td>86.3 ± 0.1^d</td>
<td>6.63 ± 0.02^e</td>
<td>6.80 ± 0.07^e</td>
<td>192 ± 7^e</td>
</tr>
<tr>
<td>0.07 Mg</td>
<td>86.6 ± 0.2^ed</td>
<td>6.09 ± 0.01^e</td>
<td>6.14 ± 0.09^d</td>
<td>235 ± 7^bc</td>
</tr>
<tr>
<td>0.07 Na</td>
<td>86.2 ± 0.2^d</td>
<td>6.51 ± 0.03^bc</td>
<td>6.65 ± 0.02^b</td>
<td>250 ± 10^bc</td>
</tr>
<tr>
<td>0.17 K</td>
<td>86.6 ± 0.1^lst</td>
<td>6.54 ± 0.00^f</td>
<td>6.67 ± 0.02^f</td>
<td>217 ± 16^e</td>
</tr>
<tr>
<td>0.17 Mg</td>
<td>87.3 ± 0.1^a</td>
<td>5.71 ± 0.02^f</td>
<td>5.94 ± 0.03^c</td>
<td>230 ± 8^c</td>
</tr>
<tr>
<td>0.17 Na</td>
<td>86.9 ± 0.0^lbc</td>
<td>6.46 ± 0.01^c</td>
<td>6.56 ± 0.05^bc</td>
<td>214 ± 10^c</td>
</tr>
<tr>
<td>0.34 K</td>
<td>86.9 ± 0.3^lbc</td>
<td>6.49 ± 0.03^b</td>
<td>6.61 ± 0.01^bc</td>
<td>290 ± 54^ag</td>
</tr>
<tr>
<td>0.34 Mg</td>
<td>85.4 ± 0.2^c</td>
<td>5.52 ± 0.01^d</td>
<td>5.73 ± 0.08^c</td>
<td>202 ± 3^c</td>
</tr>
<tr>
<td>0.34 Na</td>
<td>87.1 ± 0.3^h</td>
<td>6.33 ± 0.02^g</td>
<td>6.50 ± 0.01^c</td>
<td>313 ± 15^a</td>
</tr>
</tbody>
</table>

*p-value*** *** *** ***

Mean value ± SD (n=3). Different letters (a-g) indicate significant differences (p < 0.05).
Table 3
Biexponential fitting of LF \textsuperscript{1}H NMR T\textsubscript{2} relaxation data obtained in cooked haddock mince (from fresh raw material) with different salts and concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T21 (ms)</th>
<th>T22 (ms)</th>
<th>T21 population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07 K</td>
<td>65 ± 4\textsuperscript{c}</td>
<td>407 ± 56\textsuperscript{c}</td>
<td>68 ± 3\textsuperscript{c}</td>
</tr>
<tr>
<td>0.07 Mg</td>
<td>68 ± 2\textsuperscript{c}</td>
<td>466 ± 64\textsuperscript{bc}</td>
<td>67 ± 6\textsuperscript{c}</td>
</tr>
<tr>
<td>0.07 Na</td>
<td>65 ± 4\textsuperscript{c}</td>
<td>402 ± 14\textsuperscript{c}</td>
<td>71 ± 2\textsuperscript{bc}</td>
</tr>
<tr>
<td>0.17 K</td>
<td>71 ± 2\textsuperscript{bc}</td>
<td>441 ± 16\textsuperscript{bc}</td>
<td>77 ± 3\textsuperscript{ab}</td>
</tr>
<tr>
<td>0.17 Mg</td>
<td>83 ± 4\textsuperscript{ab}</td>
<td>523 ± 35\textsuperscript{ab}</td>
<td>77 ± 3\textsuperscript{ab}</td>
</tr>
<tr>
<td>0.17 Na</td>
<td>92 ± 8\textsuperscript{a}</td>
<td>469 ± 8\textsuperscript{bc}</td>
<td>77 ± 1\textsuperscript{ab}</td>
</tr>
<tr>
<td>0.34 K</td>
<td>68 ± 4\textsuperscript{c}</td>
<td>439 ± 35\textsuperscript{bc}</td>
<td>80 ± 2\textsuperscript{a}</td>
</tr>
<tr>
<td>0.34 Mg</td>
<td>59 ± 2\textsuperscript{c}</td>
<td>405 ± 27\textsuperscript{c}</td>
<td>73 ± 2\textsuperscript{abc}</td>
</tr>
<tr>
<td>0.34 Na</td>
<td>93 ± 2\textsuperscript{a}</td>
<td>598 ± 47\textsuperscript{a}</td>
<td>80 ± 0.4\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\(P\)-value: *** *** ***

One-Way Anova. Mean values ± SD (n=3). Different letters indicate significant differences (p < 0.001) between samples.
Fig. 1

Mean values ± 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.
Reduction of salt in haddock mince: Effect of different salts on the solubility of proteins

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

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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 (Albarracin 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, MgCl₂, 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 Mg²⁺ 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; Ramirez 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; Martinez-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 (Lauritzsen 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 °C. After 67 days, the fish were
thawed with drainage in a cold room (+4°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 °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, MgCl₂) 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 MgPO₄. 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 MgCl₂) 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 α2- 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 ± 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, MgCl2 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 MgCl₂, 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 Mg²⁺ 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 MgCl₂ (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; Martinez-Alvarez et al., 2005). K⁺ has a lower charge density (0.026 units of charge/molecular weight)
compared to Na⁺ and Mg²⁺ (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 MgCl₂, 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 MgCl₂. As discussed earlier, the drop in extractability in minces with 0.55 M MgCl₂ 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 MgCl₂, 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; Thorarinsdóttir 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 ≈ 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 MgCl₂, a significant difference was found between extractability in MgCl₂ and KCl. At MgCl₂ concentrations of 0.11 M and 0.55 M in the mince, the amount of SSP extracted in 0.6 M MgCl₂ 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 MgCl₂ concentration of 0.28 M, KCl gives a higher yield of proteins compared to MgCl₂.
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:


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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>I *</th>
<th>M *</th>
<th>Amount of salt, g</th>
<th>Salt %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.11</td>
<td>0.11</td>
<td>3.0</td>
<td>0.4</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.28</td>
<td>0.28</td>
<td>7.5</td>
<td>1.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.55</td>
<td>0.55</td>
<td>15.0</td>
<td>2.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.11</td>
<td>0.11</td>
<td>3.8</td>
<td>0.5</td>
</tr>
<tr>
<td>KCl</td>
<td>0.28</td>
<td>0.28</td>
<td>9.6</td>
<td>1.3</td>
</tr>
<tr>
<td>KCl</td>
<td>0.55</td>
<td>0.55</td>
<td>19.2</td>
<td>2.5</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.33</td>
<td>0.11</td>
<td>10.4</td>
<td>0.7</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.83</td>
<td>0.28</td>
<td>26.1</td>
<td>1.6</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.66</td>
<td>0.55</td>
<td>52.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Table 2. pH in minces made from fresh and frozen-thawed haddock fillets; values are given as means ± STDEV, n=6.

<table>
<thead>
<tr>
<th>Salt concentration in minces:</th>
<th>NaCl</th>
<th>KCl</th>
<th>MgCl₂</th>
<th>Unsalted minces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>0.3 M</td>
<td>0.55 M</td>
<td>0.1 M</td>
<td>0.3 M</td>
</tr>
<tr>
<td>Fresh</td>
<td>6.51</td>
<td>6.46</td>
<td>6.34</td>
<td>6.63</td>
</tr>
<tr>
<td>Frozen</td>
<td>6.48</td>
<td>6.44</td>
<td>6.24</td>
<td>6.55</td>
</tr>
</tbody>
</table>
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 ± stdev, n=6.
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 %).
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%).
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.
Figure 7. Composition of salt soluble proteins from frozen minces using SDS-PAGE, made from frozen raw material, salted with NaCl, KCl, MgCl$_2$ 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 MgCl$_2$ 1,3,5 salted with 0.11 M, 0.28 M and 0.55 M MgCl$_2$, extracted with 0.6 M MgCl$_2$;
Well MgCl$_2$ 2,4,6 salted with 0.11 M, 0.28 M and 0.55 M MgCl$_2$, extracted with 0.6 M KCl.
Paper V
Novel utilization of milk-based ingredients in salt reduced fish pudding

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A B S T R A C T

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, high mineral permeate can improve textural and water-holding properties of puddings at salt concentrations down to 0.83%, 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.

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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 intake of 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; Martinez-Alvarez & Gomez-Guillen, 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 contribute 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 patés, 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 (Martinez-Alvarez & Gomez-Guillen, 2005). Fish muscle proteins...
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 as 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 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++, 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, MgCl2, CaCl2 this will affect the enzyme activity, protein matrix and texture (Andreiretta-Gorelikina, Greiff, Rustad, & Aursand, 2014; Barat, Pérez-Esteve, Artiyo, & 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 (NH4HF < 1%, LIO, 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- & Vildcentralen AS, Oslo, Norway) were stored at 4 °C until production of the haddock (Melanogrammus aeglefinus) mince. 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 83 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 cooker (KIELA, Neumünster, 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 1.5, 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 × 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 × 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 preweighed 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 × 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 Liacciardello et al. (1982), as previously described by Hultmann and Rustad (2002).
Analyses were run as duplicates. Minces (6.5 g) were 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 rpm for 10 s in 0.05 mol/l phosphate buffer (80 ml, pH 7.0). 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 measured 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). 75 g of pudding was homogenised in deionized water (18.2 MΩ, 100–150 ml) using an Ultra-Turrax 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⁺).

### Table 2

Composition of fish minces in the main 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 g/100 g).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Salt (g/100 g)</th>
<th>K/Na ratio</th>
<th>Fat (g/100 g)</th>
<th>Carbohydrates (g/100 g)</th>
<th>Protein (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS/Control</td>
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<td>LS/HM</td>
<td>0.6</td>
<td>2.1</td>
<td>0.15</td>
<td>4.7</td>
<td>10.2</td>
</tr>
<tr>
<td>IS/Control</td>
<td>0.8</td>
<td>0.8</td>
<td>0.15</td>
<td>3.7</td>
<td>10.3</td>
</tr>
<tr>
<td>IS/LM</td>
<td>0.8</td>
<td>0.9</td>
<td>0.17</td>
<td>6.1</td>
<td>10.1</td>
</tr>
<tr>
<td>IS/HM</td>
<td>0.8</td>
<td>1.6</td>
<td>0.15</td>
<td>4.7</td>
<td>10.2</td>
</tr>
<tr>
<td>HS/Control</td>
<td>1</td>
<td>0.6</td>
<td>0.15</td>
<td>3.7</td>
<td>10.3</td>
</tr>
<tr>
<td>HS/LM</td>
<td>1</td>
<td>0.7</td>
<td>0.17</td>
<td>6.1</td>
<td>10.1</td>
</tr>
<tr>
<td>HS/HM</td>
<td>1</td>
<td>1.3</td>
<td>0.15</td>
<td>4.7</td>
<td>10.2</td>
</tr>
</tbody>
</table>

* 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⁺).
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 – ((L* − a* − b*)/2) (Benjakul, Visessanguan, & Srivilai, 2001; Dungnan & Taulenghob, 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 Andreella, Corellina et al. (2014). Statistical analyses were performed using the software Mininab® (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. Z-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/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 (WHC) 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 KC1 (SSP). The results are expressed as g protein/100 g of muscle in sample a, 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.](image-url)

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cooking loss (g/100 g)</th>
<th>WHC (%)</th>
<th>Moisture (g/100 g)</th>
<th>Salt (g/100 g)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>W 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS/Control</td>
<td>6.8 ± 0.8</td>
<td>83.9 ± 3.3**</td>
<td>82.3 ± 0.3**</td>
<td>0.45 ± 0.00*</td>
<td>74.9 ± 0.9**</td>
<td>-5.9 ± 0.09**</td>
<td>11.7 ± 0.07**</td>
<td>71.7 ± 0.85**</td>
</tr>
<tr>
<td>LS/LM1.5</td>
<td>6.5 ± 0.2</td>
<td>82.5 ± 3.1**</td>
<td>81.1 ± 0.5**</td>
<td>0.48 ± 0.02*</td>
<td>74.2 ± 0.7**</td>
<td>-6.0 ± 0.11**</td>
<td>11.7 ± 0.10**</td>
<td>71.5 ± 0.73**</td>
</tr>
<tr>
<td>LS/LM2.9</td>
<td>6.5 ± 1.3</td>
<td>82.5 ± 3.1**</td>
<td>81.0 ± 0.2**</td>
<td>0.40 ± 0.02*</td>
<td>73.9 ± 0.3**</td>
<td>-6.4 ± 0.13**</td>
<td>11.5 ± 0.12**</td>
<td>70.8 ± 0.48**</td>
</tr>
<tr>
<td>HS/Control</td>
<td>6.8 ± 0.06</td>
<td>91.7 ± 0.3*</td>
<td>81.7 ± 0.2**</td>
<td>0.46 ± 0.03*</td>
<td>73.1 ± 0.3**</td>
<td>-6.2 ± 0.09**</td>
<td>10.4 ± 0.2**</td>
<td>70.6 ± 0.24**</td>
</tr>
<tr>
<td>HS/LM1.5</td>
<td>5.9 ± 0.9</td>
<td>93.9 ± 0.7**</td>
<td>81.2 ± 0.3**</td>
<td>0.40 ± 0.00*</td>
<td>72.1 ± 0.2*</td>
<td>-6.4 ± 0.03**</td>
<td>9.6 ± 0.2**</td>
<td>69.8 ± 0.2**</td>
</tr>
<tr>
<td>HS/LM2.9</td>
<td>5.6 ± 0.0</td>
<td>92.8 ± 1.3**</td>
<td>81.5 ± 0.3**</td>
<td>0.57 ± 0.00*</td>
<td>72.1 ± 0.2**</td>
<td>-6.3 ± 0.05**</td>
<td>9.9 ± 0.11**</td>
<td>69.7 ± 0.20**</td>
</tr>
<tr>
<td>HS/HS2.9</td>
<td>5.5 ± 0.2</td>
<td>94.4 ± 1.4**</td>
<td>81.0 ± 0.2**</td>
<td>1.04 ± 0.07**</td>
<td>70.4 ± 0.08**</td>
<td>-6.6 ± 0.08**</td>
<td>9.3 ± 0.17**</td>
<td>68.3 ± 0.33**</td>
</tr>
</tbody>
</table>

4.83*** 0.64 ns

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

**T ratio is given for salt level.
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. Andreotta-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 5.0% 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...
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, salt content and color) of 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 all salt levels. Those added HM 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, R² = 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 replicates evaluated by each panelist, 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 (Martinez-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 (Almi & 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 HM 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 < 0.001), elasticity (p < 0.000) and cohesiveness (p < 0.001) and a decreasing breaking force with increasing

![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.](image-url)
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. 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.)](image)

![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).](image)
descriptive profile in sausages and Atanli 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 texture 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.

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References


As犯罪分子,高矿化度 whey permeate 是一个有希望的盐替代品。