

A26260- Unrestricted

## Report

## Low salt products

#### **Final report**

#### Authors

Ida Grong Aursand, Kirsti Greiff, Ulf Erikson, Lorena Gallart-Jornet, Nebojsa Perisic, Achim Kohler, Nils K Afseth, Ragni Ofstad, Ivar Storrø and Kjell Josefsen



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SINTEF Fiskeri og havbruk AS SINTEF Fisheries and Aquaculture

Address: Postboks 4762 Sluppen NO-7465 Trondheim NORWAY

Telephone:+47 40005350 Telefax:+47 93270701

fish@sintef.no www.sintef.no/fisk Enterprise /VAT No: NO 980 478 270 MVA

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## Report

### Low salt products

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Ida G. Aursand, Kirsti Greiff, Ulf Erikson, Lorena Gallart-Jornet, Nebojsa Perisic, Achim Kohler, Nils K Afseth, Ragni Ofstad, Ivar Storrø and Kjell Josefsen

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#### ABSTRACT

Salt (NaCl) is the world's most established food additive, because of its excellent preservative effects, the sensorial properties and the increased food processability. In the European countries, 75-80% of salt we consume is hidden in processed foods. The Low salt products project had comprehensive participation from Norwegian fish and meat industry and RTD-institutes and was coordinated by SINTEF Fisheries and Aquaculture. The project was running from 2008 until 2014, and has increased knowledge within salt reduction of Norwegian fish and meat products. The work was divided into three work packages focusing on food matrix and water interactions (RA1), shelf-life prediction and design of alternative preservation methods (RA2) and process- and technological improvements (RA3). Two PhD students and four MSc students have been educated within the field and several publications have been published in international journals. Within the project period, many of the industry partners have introduced new low salt products to the Norwegian market (sausages, cooked ham, etc). Furthermore, the project has given knowledge important in the development of limit values for salt in the label "nøkkelhullet". At least two spin of projects have been started (Salto and ProHealth, Norwegian Research Counil). A summary of the project results are given in this report. The project was funded by the Norwegian Research Council and industry partners.

PREPARED BY Ida G Aursand

снескер ву Marit Aursand

APPROVED BY Marit Aursand

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#### 1 Norwegian abstract

Salt (NaCl) bidrar i matvarer til økt holdbarhet, smak, funksjonelle og prosesseringsegenskaper. Salt er i dag vår viktigste kilde til natrium i kosten, men et høyt inntak av natrium øker risikoen for høyt blodtrykk, som er en viktig faktor for å utvikle hjerte-karsykdommer. Helsemyndigheten ønsker derfor å redusere inntaket av natrium i befolkningen betydelig, helst med 50 % eller mer. I Europa er 70-80 % av saltet vi får i oss såkalt "skjult salt" i industrielt framstilte næringsmidler. Prosjektet "Low salt products" ble startet i 2008 som det første store forskningsprosjektet i Norge med fokus på prosessteknologi for reduksjon av salt i industrielt prosessert mat. I prosjektet, som avsluttes i 2014, var flere store industriaktører og de største forskningsinstituttene innen matforskning samlet. Prosjektet ble koordinert av SINTEF Fiskeri og havbruk, og finansiert av Forskningsrådet samt industrideltakerne.

Prosjektet har gitt økt kunnskap om muligheter for saltreduksjon i ulike norske matvarer basert på kjøtt og fisk. Det er etablert en enkel og rask metode som kan anvendes av industrien for måling av natriuminnhold i ulike matvarer. Mer avanserte måleteknikker egnet for forskning er også blitt anvendt for å øke forståelsen av endringer i ulike produkters mikrostruktur under prosessering. For eksempel er det funnet at ulike salter (NaCl, KCl og MgSO<sub>4</sub>) gav ulik grad av denaturering av proteinene i kjøtt, og disse endringene ble koplet til makroskopiske målinger som vannbindingsevne og sensoriske egenskaper.

Ulike uorganiske salter er testet som salterstattere i blant annet fiskepudding, grillpølser, kokt skinke og margarin. Konklusjonene fra forsøkene var at å erstatte 20-30 % av natriuminnholdet i de ulike matvarene med kaliumsalt fungerte godt med hensyn til prosesseringsegenskaper og smak.

Prosjektet har hatt fokus på industrirettet forskning, og under prosjektperioden har flere av industrideltakerne lansert nye produkter med lavere natriuminnhold. Blant annet er flere typer grillpølser og kokt skinke med lavere natriuminnhold presentert på det norske markedet, mens norskprodusert fiskemat med lavere natriuminnhold er levert som skolemat i Storbritannia. I prosjektet er to PhD-studenter og fire MSc-studenter utdannet, og flere vitenskapelige artikler er publisert i internasjonale tidsskrift.

#### 2 Background

Salt (sodium chloride, NaCl) is the world's most established food additive, because of its excellent preservative effects, the sensorial properties and the increased food processability. This combination of factors has resulted in salt being used at higher level than necessary in most of the processed foods<sup>[1-3]</sup>. In fact, in the European countries, 75-80% of salt we consume is hidden in processed foods<sup>[4]</sup> (i.e. bacon and ham, cooked sausages, cheese, canned fish, processed pudding products, pies).

Although sodium is required for normal human body functions, the body only needs around 1-3 g salt per day to function, but actual intake is three times higher (8-12 g/day) in a major part of the European countries<sup>[3,5]</sup>. Several epidemiological studies have demonstrated that high salt intake is associated with an increased risk of high blood pressure which is a significant factor for development of cardiovascular disease and strokes<sup>[6-8]</sup>. Public health and regulatory authorities such as The Norwegian Directorate for Health and Social affairs (SH dir), Food Standards Agency (FSA), French Food Safety Agency (AFSSA), World Health Organisation (WHO)<sup>[9]</sup>, have published advisory guidelines for daily salt intake, since it is recognized as an important public health concern<sup>[10]</sup>. In Norway, the authorities' goal is to reduce salt intake from 10 g/day to 5 g/day within 2020<sup>[11]</sup>.



Food manufacturers are faced with the dilemma of how to reduce the salt content of foods without losing their palatability, texture, processing yield and long shelf-life. While some ingredients can perform a few of its functions, no ingredient can perform all the functions offered by salt. Thus, alternative combinations of functional ingredients must be developed or optimised. This requires a full understanding of the technological problems associated with salt reduction.

#### 2.1 Food safety

The high salt level in fish and dry cured meat products is used for reducing the risk of microbial growth and for increasing yield by the less need of drying. Salt is an important preservative against microbial growth, since it reduces the water activity,  $a_w$ , (the availability of water molecules susceptible to microorganisms). A low salt version of a product may therefore become more vulnerable to microbial spoilage, as the water activity will increase<sup>[12]</sup>. Research has shown that it is important to examine the microbial shelf-life and safety of processed foods before NaCl levels are reduced or replaced by other ingredients<sup>[2]</sup>. Possible methods to counteract the loss of preservative function of the sodium chloride can be increasing other "hurdles" such as chemical preservatives, salt replacers, or degree of heat treatment, or by improving the hygiene of the production process, or a combination of all these.

#### 2.2 Salt alternatives

One of the biggest barriers to salt replacement is cost as salt is one of the cheapest food ingredients available. However, it is possible to lower the salt, and one of the main strategies to decrease the salt intake is reformulation of products by partially replacement of sodium such as phosphate, other mineral salts (potassium, calcium, magnesium) and flavour enhancers (amino acids such as monosodiumglutamate, yeast extracts, lactates, nucleotides or herbs and spices)<sup>[2,3]</sup>. The use of these salt replacers is limited primarily by associated non-salty taste; they are rather described as being bitter, sweet or sour<sup>[5]</sup>. However, by reaching an optimal recipe reformulation, it is possible to reduce the sodium content considerably in many of today's products.

#### 2.3 Role of salt in muscle food

Salt imparts a number of functional properties in meat and fish products. It activates proteins to increase hydration and water-binding capacity and improves texture in processed meat and fish, viscosity of meat batters, and it facilitates the incorporation of fat to form stable batters<sup>[2]</sup>. Increasing the water holding capacity of muscle food reduces cooking loss thereby increasing the tenderness and juiciness of the muscle foods. It is also assumed that the matrix components (proteins, carbohydrates and salts) affect the structuring of the water molecules in the matrix. Structuring of the water components may be a key factor in maintaining shelf-life as well as texture and flavour in the low salt products. A high salt content in dry cured meat is likely to reduce the activity of proteases, which are necessary for the development of proper taste and texture. Reducing salt levels in such products may affect the texture and flavour.

#### 2.4 Alternative processing techniques

Salting has been commonly used separately or in combination with other processes (air-drying, smoking marinating and fermentation). Fish can be salted in different ways - dry, wet or pickle, brining and mixed salting. The injection-salting technology is gradually acquiring popularity due to its time reduction and the higher processing yields obtained when compared to conventional salt-curing techniques. It has been claimed to cause uniform distribution of salt and to lower salt concentrations. Other methods for alternative processing techniques such as use of pre-rigor meat/fish, and high

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pressure technologies have been proposed<sup>(13,14)</sup>. The use of ultrasound to speed the salting process<sup>(15)</sup> and vacuum pulsing<sup>(16)</sup> has also been tested. Both the osmotic effect of other ions than salt and viscosity of brine on diffusion of sodium and water loss have been studied<sup>[17]</sup>. This study clearly shows that the barriers to diffusion that form in the liquid surrounding the product play a key role in the control of mass transfer. The freshness of fish also influences the rate of the salt uptake<sup>[18,19]</sup>, and the salt uptake is dependent upon the fish species<sup>[20]</sup>, pointing towards different technological solution for optimal salting of different products.

#### **3** Organization of the "Low salt products" project

The "Low salt products" project running from 2008 until 2014 was established as a co-operation between food manufacturers, retailers, and RTD institutes. Within this consortium a significant part of the Norwegian food industry and RTD partners were gathered. The project has created a base of knowhow, educated personnel, and technological solutions for salt reduction of Norwegian fish and meat products. It was coordinated by SINTEF Fisheries and Aquaculture and funded by the Norwegian Research Council (80%) and industry participants (20%) with a total budget of 14 million NOK. The project has had focus on identifying and running case studies with industrial relevance, and several experiments have been run in industrial pilot labs. Model products such as cooked sausage and fish mince relevant for several industry partners have been chosen for the different studies.

#### **RTD** partners

- SINTEF Fisheries and Aquaculture
- NOFIMA
- SINTEF Materials and Chemistry
- Polytechnical University of Valencia, Spain

#### **Industry partners**

- Mills
- Nortura
- Berggren
- Brødrene Remø
- Orkla Foods Norway (previously Stabburet)
- Finsbråten
- SPIS-Grilstad

#### 4 Project objective

The main objective of the project was to strengthen the knowledge and competence in industry and research institutes on low salt products with emphasis on food quality, shelf-life and economy.

#### The sub objectives were as follows:

- 1. To measure effects of reducing salt content on the food matrix structure in relation to food quality emphasizing texture and sensorial properties.
- 2. To develop methods for shelf-life prediction in products with reduced salt and strategies (e.g. hurdle technology, salt substitutes) to maintain food shelf-life.
- 3. To identify industrial process and technological changes needed to ensure acceptable quality and shelf-life of low-salt products.
- 4. To develop decision models to facilitate optimal choice of profitable production technology.
- 5. Knowledge based production of second generation low salt products.

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#### 5 Impact on Norwegian food research, food industry and the society in general

The project was the first large scale Norwegian research project with focus on salt reduction in food, and during the project period, "Low salt products" has contributed to a strong focus on salt reduction in food produced in Norway. Many of the industrial partners in the project have developed their salt reduction strategies during the project running, using result from the project as an important information source. The RTD-partners have increased their knowledge considerably, and are now in a strong position in the EU in the field of salt reduction.

Some examples of direct impact of the project:

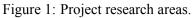
- The project has contributed with knowledge that has led to new products with reduced salt content on the Norwegian market. Some examples are sausages with reduced salt by Finsbråten and Nortura and cooked ham with reduced sodium content produced by Espeland.
- The project has contributed to reputation building for the industry partners
- The project has given knowledge important in the development of limit values for salt in the label "nøkkelhullet"
- The project has educated 2 PhD-students and 4 MSc students within the field of sodium reduction
- Results from the project have been published in mass media contribution to increased consumer awareness.
- The project has led to spin of projects such as "Salto" and "ProHealth"



#### 6 The project research areas and results

The research was run through three research areas as shown in Figure 1.





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#### 6.1 RA1 Food matrix and water interactions

#### Background

The main factors for quality of salted meat are determined by interactions between water, salt and proteins. In this WP, novel techniques were further developed and applied to study such interactions in low salt products.

It is well known that salt reduction in processed meat and fish affects texture and juiciness negatively. Water is a key component in most food matrices in terms of its content, structure and functionality. It is assumed that the other matrix components like proteins, carbohydrates and salts affect the structuring of the water molecules in the same matrix, which consequently determines structural, functional and sensory properties of the food in question. The role of water in foods and food processes has been widely studied, as has also the structuring of water molecules through H-bonding in different aqueous systems. However, the nature of water is still far from being fully understood, and knowledge has not been well communicated between the different fields of science and research. Investigations on the relationship between water binding and protein denaturation<sup>(36,42)</sup>, showed that increasing the salt from 3% to 6% increases the part of random structures and decreases the  $\alpha$ -helical structures in myofibrillar proteins of meat and fish<sup>(35)</sup>. A further increase of salt increases the  $\beta$ -pleated sheets and related to this we found a loss of myofibrillar water.

In order to process low salt products with adequate sensory properties, new processes and additives was applied. An understanding of how water, salt and proteins interact on a biophysical level is necessary to develop low salt processes. The water binding and biophysical rationale for structuring of water around myofibrillar proteins with respect to additives and low salt levels was studied. FTIR microscopy was used to study protein structural changes as a function of low salt processes and additives. Other techniques, like NIR, microwave and DSC was also applied to study water structure and H-bond characteristics of meat and fish samples.

The overall aim of WP1 was to identify innovations that can reduce the NaCl level to 50 % of the current commercial content of products like minced fish and meat, cured ham or smoked salmon. The topics in this WP formed the basis for the PhD thesis written by Nebojsa Perisic.

#### Tasks

#### Task 1.1: Studying salt-water interactions in protein model systems using new methodology

Techniques like the FTIR, NIR, microwave and DSC will be used to study water structure and waterprotein interactions with respect to the addition of salt substitutes. Models for the structuring of water around proteins under different conditions will be established. Processes and additives will be proposed for low salt products to be tested in the subsequent tasks as well as in WP2 and WP3.

#### Task 1.2: Transferring methodology developed in Task 1.1 to produce low salt minced products

Methodology and models developed in Task 1.1 will be applied to model system products proposed by the reference group (e.g. minced fish and meat products). In addition texture, water-binding, water mobility (LF-NMR) and sensory properties will be measured by standard techniques.

#### Task 1.3: Developing a new low salt process for ripening and curing of ham

Salt substitutes and low salt technologies defined in Task 1.1 and 1.2 will be applied (aim: 50% reduction). Salt diffusion and distribution will be monitored by  $CT^{(22)}$ . The hams will be dried according to standard Norwegian procedures (4-6 months). The final quality of the dry-cured hams will be evaluated by sensory analysis. A reduction of salt content of dry cured meat products is likely to increase the activity of proteases<sup>(43)</sup>. Increased proteolysis may soften the texture of dry-cured hams<sup>(44)</sup>.

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#### 6.2 Results of RA1

#### Salting of beef meat

A salting experiment was carried out using 3 different end concentrations of brines: 1.5%, 6%, and 9% and 3 different salt types. For each brine and concentration a mixture design using three different salts (NaCl / KCl / MgSO<sub>4</sub>) was applied. Different spectroscopic methods have been used to monitor the hydration and protein structural changes. In the first manuscript we have been focusing on the results from FTIR spectroscopy. A second manuscript has been written where the ability of different spectroscopic methods (working on microscopic and macroscopic level) to monitor water and hydration properties have been evaluated.

Conclusions: We observed clear differences between the different salt types when we studied protein structure: Samples, that were treated with mixtures containing MgSO<sub>4</sub> hydrated earlier with increasing salt concentration. An increased hydration of the proteins in meat tissue is related to a partial unfolding of the proteins and thereby to a destabilization of the protein in the long run. This unfolding of the protein may at moderate salt concentrations lead to an increase of hydration, since large parts of the proteins are accessible and able to bind water molecules. When further increasing the salt concentration. According to our findings, MgSO<sub>4</sub> salt brines increase the hydration properties of myofibrillar proteins more efficiently with increasing salt concentration than NaCl and KCl. Therefore the myofibrillar proteins also denature faster with increasing salt concentration in the samples with MgSO<sub>4</sub> than in samples treated with NaCl or KCl. This may be utilized technologically by replacing NaCl partially by even lower parts of MgSO<sub>4</sub>. The results found by FTIR spectroscopy on hydration and protein structure have been further confirmed by an experiment where the water holding capacity has been measured for different salt concentrations.

Further we have investigated how NIR spectroscopy, which was performed on intact meat muscle, relates to FTIR spectroscopy, which was performed on microscopic level. It has been shown that both methods reveal similar information related to water. This is an interesting finding since the FTIR measurements of single myofibers give detailed information on hydration, which can be partially also measured by NIR spectroscopy on macroscopic level. This information may be utilized for on-line process control.

#### Combined marinating and heat-treatment experiment using three salt substitutes

Beef samples were marinated *by injection* and subsequently heat-treated. The marinade was prepared using different types of salts and combinations of salts (NaCl / KCl / MgSO<sub>4</sub>) at the same end concentration of 5.5%.

Conclusions: Evaluation of sensory results showed that marinating brines containing MgSO4 are acceptable from a sensory point of few, at least when the share is 33%. Water loss after storage was slightly lower for the brines of pure NaCl and pure MgSO<sub>4</sub>. This decreased water loss is even more pronounced after heat treatment for the same brines. We may also conclude that MgSO<sub>4</sub> has a positive effect on texture, which has to be further investigated. Spectroscopic analyses by FTIR and Raman microscopy have been performed. There is no difference in protein structure for the different salt mixtures. We may conclude that the marinating with salts containing MgSO4 and KCl is feasible from a sensory point of view and with respect to protein structure. The fact that there were no differences in protein secondary structure for the different salt types may be mainly due to the subsequent heat treatment. We believe that the heat-treatment introduced a variation that masks the variation due to the different salt types. Since we observed that the salting experiment had led to clear differences with respect to salt type, a follow up of the marinating experiment, where we wanted to do the spectroscopic measurements before the heat treatment, but after the marinating step, was planed and

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performed. By this we could measure the effect of the marinating process on the protein structure and obtain a picture of the protein functionality before the subsequent heat-treatment.

#### Marinating of beef meat II

For the follow-up marinating experiment the experimental design was as for the first experiment, with the difference that only two animals were used and two different aging times were used before the marinating. For the second experiment no sensory analysis was performed. The muscles were aged 2 day and 14 days. After the ageing samples were cut into 4x4x1 cm slices. The slices were cut into thinner slices compared to the first experiment, since samples were brine-marinated and not needle injected this time. Using a thickness of approximately 1 cm ensured that the salt brines could reach all parts of the samples within short time. As in the first experiment, marinating brines have been prepared using the different types of salts NaCl / KCl / MgSO4 in different combinations. A full mixture design with same end concentration of 5.5% for all samples has been used.

The hypothesis generated after experiment 1, that for moderate salt concentrations the hydration properties of proteins are increased when NaCl is replaced by MgSO4, was confirmed. Under heat-treatment, samples that were marinated by brines containing shares of MgSO4 loose less water. Since it was shown in experiment 1 that brines with shares of MgSO4 have no detrimental effect on sensory properties of the marinated meat, we may propose to replace parts of NaCl by MgSO4 for marinating beef muscles. Spectroscopic data has not yet been evaluated. A manuscript is in preparation.

#### Production of sausages using salt mixtures

Salts that were used for salting fresh beef meat samples were used in different sausage recipes, as one of commercially available beef meat products. Sausages were produced in 2 ways using the aforementioned salts: (1) experimental sausages that contained basic constituents and did not contain starch and other additives for enhancing the taste and (2) sausages that were produced in a commercial way. Both kinds of sausages produced were analyzed by: FTIR microspectroscopy and FTIR imaging, NIR spectroscopy, texture analysis and pH. Besides this, the sausages produced in a commercial way were additionally analyzed by sensory panel.

Conclusions: FTIR imaging was used to map the distribution of sausage components, such as proteins, carbohydrates and fats. This distribution was found to be different between the recipes used due to different salts applied. Generally, it was observed that the structure of the sausages (both experimental and commercial), was more homogeneous in sausages with higher percentage of salts used. However, there was a trend observed that showed that homogeneity was the highest in sausages containing NaCl, while it was the lowest in KCl recepy. Sausages prepared with MgSO<sub>4</sub> were in between regarding the homogeneity.

#### Publication: Food Chemistry, 2013, 12; 138(1) 679-686

Authors: Nebojsa Perisic, Nils Kristian Afseth, Ragni Ofstad, Sahar Hassani, Achim Kohler

## Title: Characterizing protein, salt and water interactions with combined vibrational spectroscopic techniques.

**Abstract:** In this paper a combination of NIR spectroscopy and FTIR and Raman microspectroscopy was used to elucidate the effects of different salts (NaCl, KCl and MgSO(4)) on structural proteins and their hydration in muscle tissue. Multivariate multi-block technique Consensus Principal Component Analysis enabled integration of different vibrational spectroscopic techniques: macroscopic information obtained by NIR spectroscopy is directly related to microscopic information obtained by FTIR and Raman microspectroscopy. Changes in protein secondary structure observed at different concentrations of salts were linked to changes in protein hydration affinity. The evidence for this was given by connecting the underlying FTIR bands of the amide I region (1700-1600 cm(-1)) and the water region (3500-3000 cm(-1)) with water vibrations obtained by NIR spectroscopy. In addition,

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Raman microspectroscopy demonstrated that different cations affected structures of aromatic amino acid residues differently, which indicates that cation- $\pi$  interactions play an important role in determination of the final structure of protein molecules.

#### Publication: Meat Science, 2013, 5;95(3) 576-85

# Authors:Nebojsa Perisic, Nils Kristian Afseth, Ragni Ofstad, Bjørg Narum, Achim KohlerTitle:Characterizing salt substitution in beef meat processing by vibrational<br/>spectroscopy and sensory analysis

Abstract: In this investigation, the effect of NaCl, KCl and MgSO4 on bovine meat was studied, where the salts were used in standard marinades in 5.5% concentration. The effect of salts on secondary structure of the myofibrillar proteins, protein-water interactions, WHC, and sensory properties of the meat was followed by carrying out FTIR and NIR measurements, cooking loss and sensory analysis. The information obtained by spectroscopic analysis was integrated by using CPCA. This revealed that MgSO4 increased ratio of  $\alpha$ -helices and CO and NH groups (followed by FTIR) that are involved in H-bonding with surrounding water molecules (followed by NIR). This was also supported by increased WHC. Conversely, KCl reduced WHC of meat and was correlated to non-hydrogenated CO and NH groups. Furthermore, the sensory analysis confirmed that MgSO4 was acceptable only when the share of this salt in the mixture was one third.

#### Publication: J Agric Food Chem, 2011, 23;59(18) 10052-10061

## Authors:Nebojsa Perisic, Nils Kristian Afseth, Ragni Ofstad, Achim KohlerTitle:Monitoring protein structural changes and hydration in bovine meat tissue due<br/>to salt substitutes by Fourier transform infrared (FTIR) microspectroscopy

**Abstract:** The objective of this study was to investigate the influence of NaCl and two salt substitutes, MgSO4 and KCl, in different concentrations (1.5, 6.0, and 9.0%) on meat proteins by using Fourier transform infrared (FTIR) microspectroscopy. Hydration properties and secondary structural properties of proteins were investigated by studying the amide I, amide II, and water regions (3500-3000 cm(-1)) in FTIR spectra. By applying multivariate analysis (PCA and PLSR), differences between samples according to salt concentration and salt type were found and correlated to spectral bands. The most distinctive differences related to salt type were obtained by using the water region. It was found that samples salted with MgSO4 exhibited hydration and subsequent denaturation of proteins at lower concentrations than those salted with NaCl. Samples salted with KCl brines showed less denaturation even at the 9.0% concentration. The FTIR results were further supported by water-binding capacity (WBC) measurements.

#### Publication: J Agric Food Chem, 2013, 21;61(13) 3219-3228

# Authors:Nebojsa Perisic, Nils Kristian Afseth, Ragni Ofstad, Jan Scheel, Achim KohlerTitle:FTIR imaging of structural analysis of frankfurter sausages subjected to salt<br/>reduction and salt substitution

**Abstract:** In this study, the effects of NaCl, KCl, and MgSO4 in various concentrations on structural and sensory properties of frankfurter sausages were investigated. FTIR was used to analyze the overall homogeneousness of the sausages by simultaneously following the distribution of main sausage ingredients, i.e., proteins, fats, and starch. A more homogeneous distribution of the main ingredients was observed with higher concentration of added salts, while it was most pronounced for the MgSO4 recipe. Furthermore, FTIR imaging was used in order to follow the distribution of protein secondary structure motifs throughout the sausage matrix. It was confirmed that KCl inhibited the partial denaturation of proteins, unlike that observed for MgSO4 recipes, where an additional increase in protein hydration was detected. These findings were unequivocally supported by WHC measurements. However, the sensory analysis clearly



distinguished the sausages prepared with MgSO4 due to undesired sensory attributes, which underlines the necessity for using taste masking agents.

# Thesis:PhD, NMBU / NOFIMACandidate:Nebojsa PerisicTitle:Vibrational spectroscopy analysis of interactions between proteins, salts and<br/>water

**Abstract:** The increased amounts of sodium in the modern diet are connected to health problems, mostly through increased blood pressure that can eventually lead to heart attack (1). Because of this, the food industry is developing strategies for reducing and substituting NaCl, which is the main source of excessive sodium intake. Processed meat products often contain high amounts of NaCl and therefore represent an important food category for applying this strategy. However, there are various obstacles related to NaCl reduction/substitution which are mainly reflected by the number of beneficial effects this mineral has, both with respect to properties of food products and with respect to industrial production.

In this thesis, we investigated the influence of NaCl, KCl and MgSO4 in various concentrations and combinations on the structure and properties of meat and meat product. The main focus was placed on changes in the protein secondary structure and the protein hydration. For this purpose a platform of several vibrational spectroscopy methods was used, namely FTIR microspectroscopy, FTIR imaging, Raman microspectroscopy and NIR spectroscopy.

FTIR spectroscopy revealed that the main effects of salts were visible in changes in the hydration of proteins when salts were used without the heat treatment. This was determined by analysing the vibrational bands in the FTIR water region (3500-3000 cm-1), which showed that MgSO4 caused the highest increase in protein hydration through increased amount of hydrogenated NH groups on the protein backbone. A novel approach of using FTIR imaging to study structural changes in food matrices caused by different salts included: (I) further development of EMSC pre-processing of FTIR images, (II) following the changes in the secondary structure of proteins (mainly denatured  $\beta$ -sheet structures and native  $\alpha$ -helices) and (III) mapping the intensity of these changes throughout the matrix of meat products, such as in Frankfurter sausage. The use of FTIR imaging also showed that combination of different salts and heat treatment caused different patterns of denaturation.

PLSR and CPCA were performed in order to integrate the information obtained by different vibrational spectroscopy methods. By doing so, the information obtained on molecular structure was linked to the changes in macroscopic properties of meat, resulting in a more complete insight into the processes that occurred under the studied treatments. By correlating FTIR and Raman spectroscopy, it was revealed that different cations (Na+, K+ and Mg2+) caused distinct changes in the structure of aromatic amino acid

side chains. Supplementing our studies with NIR spectroscopy revealed clear differences in the effect of different salts on the interaction of water molecules and myofibrillar proteins as well as on the overall colour of the meat. Furthermore, the importance of validating the CPCA results was also underlined.

The findings of spectroscopic investigation were additionally supported and validated by the analyses of changes in water holding capacity and sensory attributes.

#### Rapid determination of sodium content in foods – sodium electrode

A rapid method for measurement of sodium content in different foodstuffs was established. The chosen method was sodium electrode. A master student was educated as part of the development of the method.

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# Publication:Master thesis, UiT, the Norwegian College of Fishery ScienceAuthor:Anette AlmåsTitle:Determination of salt content in food using sodium selective electrode and<br/>potentiometric chloride titration

Abstract: Addition of salt (NaCl) has been applied in the food industry for many years, both to improve the sensorial properties and to preserve food. However, the salt intake in the Western countries including Norway is generally too high. It is well known that high salt intake contributes to increased blood pressure. Since high blood pressure increases the risk of many life threatening diseases, it is important to reduce the salt intake. Approximately <sup>3</sup>/<sub>4</sub> of the salt intake is caused by processed food, and the focus has therefore been to reduce salt addition to these types of food products. It is the sodium ion in sodium chloride-molecule that may be injurious to health. Still chloride titration is the most commonly applied measurement technique to decide salt content. In many processed food products, sodium is added through other sources than sodium chloride (NaCl). Measurement of chloride will therefore give a wrong estimate of the actual sodium content in the product. It is a need to apply methods that measure sodium content directly. In the future, the industry may also be obliged to label their products with sodium content. The purpose of this thesis was to compare salt content in foods by use of sodium selective electrode and potentiometric chloride titration. Sodium selective electrode measures the sodium content, while chloride titration measures the chloride content. The total sodium content may be estimated from the measured values. Three products produced at the lab (thawed fish mince, cooked ham and fish pudding), and three industrial produced products (cooked ham, potato chips and smoked salmon) were investigated. The results showed that sodium selective electrode may easily be applied to determine sodium content in foods. The sodium selective electrode gave the best results when applying the direct measurement compared to the so called single known addition method. In products where only sodium chloride is the only sodium source and the concentration is approx. 2-3%, the chloride titration gave relatively good results similar to what was achieved by the sodium selective electrode. In many processed foods such as ham, other ingredients containing sodium are also added, in such cases sodium selective electrode is the only alternative method to determine sodium content. The experiments on smoked salmon showed that the product contained a relatively high salt content, and that the sampling is of high importance for the result of the analysis. It is necessary to develop guidelines for how to sample if producers are obliged to label sodium content in these products. The overall conclusion of this master study is that sodium selective electrode is a method that may be applied by the food industry to estimate sodium content in food products.

#### Nondestructive measurements of the content of salts in low-salt fish mince

Collaboration between The Universitat Politècnica de València and SINTEF Fisheries and Aquaculture was established to strengthen the research on measuring-techniques of salt reduction in muscle based foods at both institutes. This came in place by extra funding given by the Norwegian Research Council to PhD student Kirsti Greiff for a 3 months overseas fellowship. The collaboration was a part of the PhD-study for Kirsti Greiff at SINTEF Fisheries and Aquaculture and NTNU with the working title of the thesis "Salt reduction in meat and fish products"

The goal was to establish closer collaboration between The Universitat Politècnica de València and SINTEF Fisheries and Aquaculture to find innovative non-destructive measuring- techniques to determine sodium content in meat and fish products.

The sub-goals were:

- i) To create a joint publication in the area: *Measuring sodium in low-salt products*,
- ii) to exchange knowledge on the field the protein/water matrix in food with low salt/sodium content, and

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iii) to measure/predict the amount of other cations (eg. K+, Mg2+, Ca2+) for substitution of sodium in foods.

The experiments focused on fish products. Brines and model products of minced fish with salt was used to compare the different analytical methods and to transfer the measure techniques from brines to food matrix. The following methods were applied:

- i) Traditional measuring techniques
- ii) Low Field NMR
- iii) Electronic sensors (impedance spectroscopy)

The collaboration resulted in a common publication.

#### Publication: J Agric Food Chem, 2014, 62(12), 2496-2505

Authors: Kirsti Greiff, Ana Fuentes, Ida G. Aursand, Ulf Erikson, Rafael Masot , Miguel Alcañiz, and Jose M. Barat

Title:Innovative Nondestructive Measurements of Water Activity and the Content of<br/>Salts in Low-Salt Hake Minces

Abstract: Impedance spectroscopy (IS), low-field proton nuclear magnetic resonance (LF 1H NMR), chloride titration, ion chromatography, and an ion selective electrode were used to investigate the physicochemical parameters and measure sodium and potassium contents in low-salt brines and fish. Salt solutions (0–3 w/w, %) and model products of minced hake with added NaCl (0.5–3.0 w/w, %), or a mixture of NaCl and KCl (50/50 w/w, %), were analyzed. Good correlation was observed between the sodium content determined by using the ion selective electrode method and ion chromatography (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 1H NMR seems to be a suitable technique for studies of low-salt products.

#### Case study: Soft Light – half fat margarine

A case with focus on reducing salt in the product Soft Light which is a half-fat margarine was performed together with Mills. Experiments have been performed together with Mills to measure FTIR ATR spectra of Soft Light that was produced with lower salt concentrations and salt replacers.

Conclusions: Replacement of all added NaCl with KCl resulted in increased pH. NaCl and particularly KCl reduced the viscosity in the water phase. The pilot scale test gave unstable emulsions. Sensorial tests showed that NaCl correlated strongest with the parameters salty taste, body, sourish smell/taste, and solidity whereas KCl correlated stronger with the parameters bitterness, rancidity. Furthermore, a bitter taste was reported if 50% of the NaCl is replaced by 50% KCl. Replacement by 25-30 % KCl might be a possibility. The consumer test showed that consumers were positive to both 25 % and 50 % KCl in half-fat margarine. The content of 25% KCl gave good results compared to reduction of NaCl without substitution with KCl. No microbial growth was observed in any of the samples, not even the sample with no added salt.

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#### 6.3 RA2: Shelf-life prediction and design of alternative preservation strategies

#### Background

The shelf-life of foods can be defined as the period for which it will retain an acceptable level of eating quality, from a safety and sensory point of view<sup>(45)</sup>. The shelf-life may be limited by microbiological, enzymatic or physiochemical reactions, but in this project we will focus on microbial spoilage as this is considered most relevant for low salt products.

The reduced preservative effect of salt in low-salt products may be compensated by adding chemical preservatives (e.g. benzoate, sorbate, etc), ingredients containing natural antimicrobial compound (e.g. spices), by improving the hygiene of the production process, by improved killing of micro-organisms during the production process (e.g. increased heat treatment), or by improved storage conditions (e.g. lower storage temperature, modified atmosphere packing, etc.). The question, from an applied point of view, is how one can best and most cost-effective compensate the reduced salt content. The aim of WP2 is to provide the food industry with a practical tool to assess possible strategies.

The microflora that colonizes a particular food or beverage depends on the characteristics of the product (e.g. composition, pH, etc.) and the way it is processed and stored<sup>(45)</sup>. The response of the isolates to different preservatives will be determined as a basis for design of new preservation strategies and modelling. A problem with this strategy is that even though the number of isolates may be limited, and the number of possible preservative strategies is limited, the number of possible combinations to be tested rapidly becomes large. Laboratory robots that are able to handle a large number of samples within a reasonable time span will be applied. Once the responses of the isolates have been determined, available models for microbial food spoilage may be adapted to develop a predictive model for the specific product, and hopefully also for related products.

#### Tasks

#### Task 2.1: Selection of model products

In discussions with the industrial partners model products will be selected and low-salt versions produced. The salt content should be significantly reduced, but still be at a level considered realistic for a low-salt version of the original product. It is important that the shelf-life of the products is limited by microbial growth and not other processes (e.g. chemical deterioration). Furthermore, the reduction in shelf-life between the original product and the low-salt version must be significant.

#### Task 2.2: The effect of salt reduction on the shelf-life and isolation of the main spoilage organisms

The selected products and low salt versions of them will be stored until they spoil, and the main spoilers isolated. A challenge at this stage is to identify the real culprits. Sometimes specific microorganisms that constitute only a fraction of the flora, but produce off-flavour compounds may limit shelf-life. Examples include *Photobacterium phosphoreum* in fresh fish packed in modified atmosphere and *Shewanell putrefaciens*-like H<sub>2</sub>S producing bacteria in aerobically stored fresh fish<sup>(46)</sup>. Thus, the initial isolation of microorganisms from the product must employ a variety of selective media in order to identify possible critical species.

*Task 2.3: Determine the sensitivity of the obtained isolates towards different preservative hurdles* The spoilage isolates will be screened against a number of alternative preservative methods and combinations of them. Laboratory robots will be used to handle the large number of samples.

*Task 2.4: Application of possible alternative preservation strategies in the model products* Based on the results of Task 2.3, possible preservation strategies will be tested in low salt products.

Task 2.5: Modelling

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Based on the results from Task 2.3 and 2.4 it will be attempted to develop models for the main spoilage organisms in the product. The models will be based upon models described in the literature.

#### 6.4 Results of RA2

#### **Theoretical studies**

#### Publication: SINTEF-report

#### Author: Kjell D Josefsen

#### Title: Salt, water activity and microbial growth. Some theoretical aspects.

Abstract: Sodium (Na) is required for normal human body functions. An intake of 1.5 g Na per day, corresponding to 3.8 g salt (NaCl), is considered adequate, but the estimated daily salt intake in Norway is approx. 10 g per day for adults. Epidemiological studies have demonstrated that high salt intake is associated with an increased risk of cardiovascular disease and strokes. Norwegian public health authorities therefore aim to reduce the salt intake to 5 g per day ( $\sim 2$  g Na/day). In the affluent Western societies the natural Na content of food provide 10-12% of the Na intake, while 11-20% is added as salt during cooking and the at the table. The remaining 70-80% is derived from Nacompounds, mainly salt, added by manufacturers during processing. Thus, if the daily Na intake is to be halved, the average salt content in processed food should be reduced by 55-60%. This is a considerable challenge for the food industry. Salt is not only a taste additive and a flavor enhancer, but alto affects texture, processability and ripening processes, and help to preserve the product against pathogenic and spoilage microorganisms. The importance of the preservative effect varies from essential to not very important depending on the food product. The preservative effect of salt is due to its ability to reduce the water activity (a<sub>w</sub>) of food products. This report gives a short introduction to the water activity concept and how it affects microbial growth and survival, as well as empirically and theoretically based wquations that can be used to estimate a<sub>w</sub> in food products.

#### Case study: Shrimps in brine

A case study on reducing the salt content in shrimps was performed together with Brødrene Remø. Different salt blends were tested, and it was found that salt can be reduced by 20% maintaining shelf-life. However, sensory properties need to be improved for the low salt shrimps. A Master thesis was performed on the same topic.

#### Publication: Master thesis, NTNU, dept. Biotechnology

#### Author: Marita Helen Kvammen

## Title: Microbial shelf life of low-salt products. Effect of salt (NaCl) reduction in shrimps in brine

Abstract: Salt (NaCl) is the most used additive in the world, because of its preservative ability, sensorial quality, and because of the effect it has on texture and processing. It is also a cheap ingredient. Several studies have shown that a high sodium (Na) intake results in increased risk for cardiovascular diseases, a reduction of the NaCl content of food is therefore recommended. Processed food is responsible for 75 % of the Na intake, and reduction must therefore first and foremost be implemented within this genera. Replacement of NaCl with potassium chloride (KCl) has been tested to reduce Na concentration in several products. The main objective of this study was to determine the effect of reduced NaCl content on the microbial shelf life of brined shrimps. Four commercial shrimps in brine products were analysed with respect to pH, water activity (a<sub>w</sub>), and Total Viable Count (TVC). A HPLC (High Performance Liquid Chromatography) analysis of the brines was also performed. Three experiments were conducted to try to determine if the Na content could be reduced without compromising the microbial stability was studied. The effect of equimolar amounts of KCl/NaCl and the effect of acetic acid was studied in the next one. The last experiment examined the pH, salt and preservative tolerance of strains isolated from the commercial products.

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Analyses of four commercial products showed that high TVC was correlated with high pH. This finding was confirmed in the first experiment, where pH was found to be the most important parameter with respect to microbial growth in brined shrimps. By reducing pH, a 50 % reduction of the Na content is feasible. This reduction can be obtained by just removing NaCl or by replacing parts of it by KCl. In fact, the reduction in water activity (a<sub>w</sub>) resulting from adding 20 or 40 g/l NaCl compared to not adding salt, did not have a significant effect on shelf life. KCl had a significant effect on log TVC in the first experiment, and unexpectedly TVC was increasing with increasing KCl concentration. This was also confirmed in the experiment with equimolar amounts of the two salts, as replacing NaCl with equimolar amounts of KCl seemed to increase TVC. The difference between log TVC obtained with KCl and NaCl at similar a<sub>w</sub> was, however, not significant. If a low-salt brined shrimps product is to be produced, a sensorial evaluation of the effect of lowering the pH and also the effects of removing NaCl or replacing NaCl with KCl is needed to optimize the flavour and texture of the product.

Brined shrimps with low concentrations of acetic acid (0.8-1.6 g/l) had significantly higher log TVC than corresponding brined shrimps without acetic acid. This indicates that acetic acid actually promotes growth rather than inhibiting it in the concentrations applied in the commercial products. Further studies are required to determine if low concentrations of acetic acid actually stimulates microbial growth in brined shrimps.

Strains isolated from the commercial products grew best at high pH (6.0-6.5) in medium without preservatives, and as expected, as the concentration of preservatives increased, growth was limited to the media with the highest pH. The four tested bacterial strains were in general more sensitive to the combination of preservatives and pH in the cultivation medium in the wells, than in the brined shrimps they were isolated from.

The microflora of brined shrimps is expected to be dominated by lactic acid bacteria. The observed lack of preservative tolerance, when testing isolated pure cultures, however, indicated that the bacteria are not lactic acid bacteria. A comparison of TVC on PCA and a selective agar medium (MRS), however, indicated that lactic acid bacteria could be dominating also in this study.

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#### 6.5 RA3: Process and technological improvements

#### Background

When a product is salted in salt solution or dry salt, two main simultaneous flows are usually generated: Salt uptake and water loss or gain depending on the salting procedure<sup>(47,48)</sup>. Salt uptake depends on many factors including species, muscle type, size, thickness, weight, composition, physiological state, salting method, brine concentration, duration of salting step and temperature<sup>(20)</sup>. The transport phenomena of the salting operation are believed to significantly affect the quality and safety of the salted product. Diffusion is said to be the most important mass transfer mechanism responsible for sodium and chloride transport<sup>(49)</sup>. Unfortunately, not much attention has been given to these phenomena. As, a consequence most processes in the food industry today are based on trial and error and dominated by empirism<sup>(50)</sup>. To obtain a better understanding of the industrial process applications it is important to apply the basic concept of food engineering related to modelling, kinetics quantification, process yield and economic implication.

This work package will be continuously developed in close cooperation with industry. As the need of the participating industry will change during the project period, the research institutes will be open for changes in the current proposed plans. In these work packages we will look at:

#### Tasks

#### Task 3.1: Salt replacers, taste enhancers and masking ingredients

The RTD will collect commercially available salt replacers, taste enhancers and masking ingredients. Together with the participating industry these ingredients will be tested in already existing products. The result from this work will be an overview over which ingredients are optimal for different types of product in terms of yield, taste and shelf-life. This WP3.1 will be coupled to WP1.1 to extract theoretical (molecular) explanations for the observed results. Results will be exchanged with WP2 to optimize shelf-life.

#### *Task 3.2: Modelling salting processes*

In collaboration with chemical engineers mathematical models describing mass transfer of salts and water will be constructed based on knowledge from chemical process industry. These models will be experimentally validated. Concentration gradients of sodium and water in actual dry salting and brine salting experiments will be quantified by already developed analytical techniques based on NMR imaging. The outcome of WP3.2 will be a simulation program giving the process developer a tool to decide the best salting process for the product. This decision tools will be used together with industry to introduce new or improve existing salting processes.

#### Task 3.3: Economic implications

Changes in technology will influence the production cost. A simple tool for economic analysis of process changes that combines yield, production rates and investment and running cost will be developed for selected salting processes. This simulation and decision tool will help industry in choosing the economic optimal production process for their salted product.

#### *Task 3.4: Combination of generated knowledge*

Based on the generated knowledge in WP1, WP2 and WP3, general recommendation to the industry in salt reduction and designing of new low salt products will be given. This will ensure that industry will have salting processes based on the best possible salting technology, which will satisfy both consumers, health authorities and shareholders in the industry.

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#### 6.6 Results of RA3

#### Case study: Low salt sausages

A case study on low salt cooked sausages was performed. The aim of this study was to investigate the effect on low-salt formulation of the production process and end-product such as water holding capacity, drip loss, water activity, shelf –life and texture. Industry partners involved are the following: NORTURA; Finsbråten, Berggren, Stabburet.

#### **Publication: SINTEF-report**

#### Authors: Kirsti Greiff, Ida G. Aursand, Kjell D. Josefsen and Revilija Mozuarityte Title: Pølser med redusert innhold av natrium. The effect of sodium- reduction and replacing with potassium on physiocochemical properties and taste in cooked sausages.

**Abstract:** No-smoked sausages containing beef- and pork meat and a reduced concentration of sodium (Na) down to a salt content of 1.1% was produced by (1) substituting 11-42% of the added salt (NaCl) with KCl and (2) reducing the addition of NaCl with 11-42%. The reference sausage was added 1.9% NaCl. The sausages were produced at Nortura's pilot plant at Løren. The results indicate that reduction of NaCl leads to increased water activity and slightly reduced WHC. The texture in cold sausages were not affected by the salt content. The hardness increased by increasing salt content in heated sausages. Reduced of salt content caused a slightly increase in pH, and at the last used-by-date the pH was slightly reduced. Drip loss in the package increased slightly for the lowest salt concentrations and increased slightly with storage time. Replacement of NaCl with KCl had no apparent effect on water activity, WHC, texture, pH or drip loss. An untrained sensory panel was not able to distinguish between the salty taste in sausages containing 1.5% and 1.9% NaCl. The sensory panel was able to taste the bitter taste when 42% NaCl was replaced by KCl. The results of this experiment indicate that the effect of salt (1.1-1.9%) on sausage self-life was very limited.

#### Case study: Low salt ham

A case study on cooked ham of pork was performed in close collaboration with the meat industry, Espeland AS. The aim of this work was to investigate the sensorial changes of cooked ham with reduced sodium content and to develop a cooked ham containing 25-50% less sodium than the reference ham.

The experiments in this case were co-financed by SALTO- Saltreduksjon gjennom prosessog produktoptimalisering i næringsmiddelindustrien, prosjektnr. 210431/O10

Publication: Food Chemistry, to be submitted September 2014 .

Author: Kirsti Greiff, Ekrem Misimi, John Reidar Mathiassen, Margrethe Hersleth og Ida G. Aursand.

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

Abstract: The European diet today generally contains too much sodium (Na<sup>+</sup>). 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 1.4%, and <sup>1</sup>/<sub>4</sub> of the Na<sup>+</sup> was replaced by K<sup>+</sup>. 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<sup>+</sup>-ions by K<sup>+</sup>-ions of <sup>1</sup>/<sub>4</sub> gave no significant

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changes in WHC, moisture, pH, expressed moisture, the sensory profile attributes or the surface lightness and shininess.

The experiments in this case were co-financed by SALTO- Saltreduksjon gjennom prosessog produktoptimalisering i næringsmiddelindustrien, prosjektnr. 210431/O10

#### Salt replacers

# Publication:SINTEF-report, 2012Author:Kjell JosefsenTitle:Salt (NaCl) replacers

Abstract: Sodium (Na) is required for normal human body functions. An intake of 1.5 g Na per day, corresponding to 3.8 g salt (NaCl), is considered adequate, but the estimated daily salt intake in Norway is approx. 10 g per day for adults. Epidemiological studies have demonstrated that high salt intake is associated with an increased risk of cardiovascular disease and strokes. Norwegian public health authorities therefore aim to reduce the salt intake to 5 g per day (~2 g Na/day). In the affluent Western societies the natural Na content of food provide 10-12% of the Na intake, while 11-20% is added as salt during cooking and the at the table. The remaining 70-80% is derived from Nacompounds, mainly salt, added by manufacturers during processing. Thus, if the daily Na intake is to be halved, the average salt content in processed food should be reduced by 55-60%. This is a considerable challenge for the food industry. Salt is not only a taste additive and a flavour enhancer, but also affects texture, process-ability and ripening processes, and help to preserve products against pathogenic and spoilage micro-organisms. The aim of the "Low salt products" KMB project is to build up competence on how the food industry can reduce the Na content of products without sacrificing the desirable properties obtained by adding NaCl. One strategy is to replace NaCl with other mineral salts such as KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub>. However, these salts do not have a clean salt taste and often have a metallic or bitter taste or aftertaste, limiting the amount that can be added unless masking agents are included. Another strategy is to replace NaCl with organic flavour compounds such as spices, yeast extracts, amino acids, and 5'-nucleotides. However, the other functional properties of NaCl (effects on texture and process-ability, preservative effect, etc.) are then lost. In order to maintain shelf-life in low salt products, low-taste preservatives such as K-lactate may be added. In practice, a combination of several strategies may be the best option. Collectively these alternatives to NaCl are often called salt replacers, and during the last decade a number of such replacers have become commercially available. The aim of this report is to give an overview over commercially available salt replacers with particular emphasis on their effect on food safety.

#### Modelling

One publication has been written based on the experiments with the bowl cutter for meat. Chemical engineering models for salt diffusion are under development. Equations for salt diffusion in homogeneous matrix of different geometric configurations are found and mathematical description of salt transport in the liquid surrounding the matrix has been developed. Experimental setup to study the simultaneous salt transport and diffusion has been constructed and experiments are underway.

#### Publication: J Food Eng, 2012, 144-151

#### Authors: IV Vodyanova, I Storrø, A Olsen, T Rustad

#### Title: Mathematical modelling of mixing of salt in minced meat by bowl-cutter

**Abstract:** Salting is one of the basic procedures, used in food processing for prolongation of shelf life and to give desired taste. The NaCl concentration influences the properties of food systems, such as water holding properties, viscosity, texture, emulsification etc. Thus it is important to study and model mixing of NaCl by mathematical methods not only to understand the mixing process, but also to predict changes in concentrations during the process and their influence on the properties of the matrix

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and the food quality. The objective of this study was to develop a mathematical model of the mixing of salt and meat in a bowl cutter and verify this with experimental data. A mathematical model of mixing as mass exchange was developed based on knowledge from chemical processing. The bowl cutter was described as a continuous stirred tank reactor model combined with a plug flow reactor in a repetitive series-model. The mixing was modelled as a first order system. The theoretical model is presented as graphs. The salt concentration was determined experimentally and compared to the theoretical model. The experimental curves for the spread of salt fit well with the theoretical model. The theoretical model shows that 30 rounds are sufficient to get an equal salt concentration in the whole bulk of meat. The comparison of the theoretically predicted graphs and the results showed that the current mathematical equation is appropriate for description of the process and can be used in further study. The developed model can, with some adjustments and taking the variable conditions of real systems into account, be used to model mixing of salt in a bowl cutter.

#### Model system: Reduction of sodium in fish mince

Fish mince as a model product was used in different experiments. The aim of the studies were to investigate the effects of cations  $(Na^+, K^+, Mg^{2+})$  on gelation and physicochemical properties of raw and cooked haddock mince.

Publication: Manuscript in preparation, to be submitted December 2014.

Authors: Kirsti Greiff, Ida G. Aursand, Ulf Erikson, Kurt I. Draget and Turid Rustad

#### Title: Sodium reduction in minced fish products: Effect on physicochemical properties

Abstract: There is an increasing interest for salt reduction in food, due to the known negative health effect of consumption of foods with high amounts of sodium. Food manufacturers are faced with the dilemma of how to reduce the salt content of foods without losing their palatability, texture, processing yield and long shelf-life. This requires a full understanding of the technological problems associated with salt reduction. The aim of this study was to investigate the effect of different cations (Na<sup>+</sup>, K<sup>+</sup>, Mg<sub>2</sub><sup>+</sup>) on gelation and physicochemical properties of minced fillets and cooked haddock (Melanogrammus aeglefinus) mince. Fresh or frozen/thawed filets were minced together with water and different salts (NaCl, KCl, MgCl<sub>2</sub>) at different concentrations between 0.4% and 3.2% w/w. The minces were analyzed before and after heating to a core temperature of 80°C. Low field NMR T<sub>2</sub> relaxation, texture, rheology, water content, pH, water-holding capasity (WHC), and cooking loss were measured. The rheological analyses showed differences in viscoelastic properties between the different minces, and the low field NMR T<sub>2</sub> relaxation data showed differences in water distribution within the mince protein network. Generally, increased salt content seemed to result in a higher watercontent in the cocked minces, and to a decline in pH. The water content of the cooked minces was dependent on type of cation added. In addition, the pH of the cooked minces varied due to different salts. The minces containing MgCl<sub>2</sub> had the lowest pH independent of salt content. There were small differences in the water-holding capacity of minces with different salts. A summary of the results will be presented.

**Publication**: J Aquatic Food Product Technology, accept (30-Nov-2013) Archiving completed on 24-Mar-2014

Authors: Andreetta-Gorelkina, I.V., Greiff, K., Rustad, T. and Aursand, I.G.

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

Abstract: The daily intake of salt in the western world is around 8-11 g/day which is twice the amount needed. Due to negative health effects of the high sodium intake, it is recommended to reduce the salt intake to around 6 g/day. To achieve this there is a need to develop products with reduced sodium content. One way to reduce dietary sodium intake is to exchange sodium salts with potassium or magnesium salts. The effect of sodium, potassium and magnesium chlorides on extractability of muscle proteins from fresh and frozen haddock muscle as well as minces made from the same raw material was studied. Extractability of salt soluble proteins (SSP) in

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solutions with different concentrations is an indicator of how the different ions will affect the functional properties (water holding, gelling) of proteins. Salting with KCl and MgCl<sub>2</sub> instead of the NaCl at various concentrations changed extractability of the proteins. The highest solubility of the proteins was achieved using Na<sup>+</sup> but at low concentrations, extractability in K<sup>+</sup> and Mg<sup>2+</sup> is on the same level as for Na<sup>+</sup>, showing that it is possible to substitute a certain amount of NaCl with KCl or MgCl<sub>2</sub>. Freezing affected the structure of tissue and protein properties molecules, resulting in decrease of the amount of SSP. Further studies of influence of salt mixtures on the quality of the fish raw materials are needed.

**Publication:** Manuscript in progress, to be submitted December 2014 in LWT – Food Science and Technology

Authors: Kirsti Greiff, Charlotte Jatteau Staurem, Berit Nordvi, Turid Rustad

#### Title: Salt Reduction in Fish Pudding using Milk-Based Ingredients

Abstract: Two different powders were tested; TINE whey permeate (TW), which is based on whey obtained in cheese production, and TINE A experimental variant (TA), which is based on milk. The former has relatively high lactose content, while the latter contains less lactose but higher amounts of salts. Use of the powders has previously been experimented by TINE in meat-based products like liver patty and sausages. In order to investigate the outcome of their addition in fish-based products, fish pudding was used as a model product. Favourable health effects from intake of fish combined with the need to lower salt intake in the population lead to the desire of producing fish-based products with high fish and low salt content. Not all commercially available products based on fish mince are labelled with salt content, but among those that are labelled, the salt content ranges from 1 % to 2 %. In addition to potentially allowing a salt reduction, the use of whey permeate in food for human consumption is favourable due to the fact that whey is a by-product and large amounts of whey get discarded without exploitation of its potential as a food ingredient. The food industry employs various milk-based ingredients in form of powders such as whey, whey permeate, lactose, skimmed milk and whole milk, which act as fillers and contribute with desirable milk, sweet and umami flavour. In a product with a high percentage of milk and/or cream such as fish pudding, the flavour of milk and umami are desired. Challenges connected to the use of whey permeate and other dairy-based powders are mainly connected to their contribution of flavours that might be unexpected in some foods. Therefore, it was investigated how addition of TW and TA affect the flavour of fish puddings, and also their effect on other physical properties such as texture, colour, water-holding properties and protein solubility.

The experiments in this case were co-financed by SALTO- Saltreduksjon gjennom prosessog produktoptimalisering i næringsmiddelindustrien, prosjektnr. 210431/O10

#### Publication: Master thesis, NTNU, Dept. Biotechnology

#### Author: Ida Karoline Sivertsen

**Title:** Low salt products of fish. Different salts effect on muscle proteins

Abstract: Processed foods are one of the main sources of sodium in the western diet. An increased intake of sodium is connected to high blood pressure, which increases the risk of cardiovascular disease. This knowledge has led to increased interest in meat and fish products with a lower content of sodium – so-called low salt products. Reduced sodium content can be achieved by reducing the amount of added NaCl and/or by the use of salt replacers. The aim of this study was to investigate the effects of different concentrations of NaCl, KCl and MgCl2 on protein solubility in fresh and frozen pollock (*Pollachius Virens*), haddock (*Melanogrammus Aeglefinus*), great silver smelt (*Argentina Silus*) and cod (*Gadus Morhua*)

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in an attempt to find out if KCl and MgCl2 could be used as salt replacers in these fish species. Measurements of protein solubility were linked to measurements of pH and water holding capacity (WHC) in these species. The influence of Na-alginate, Na-alginate+NaCl and NaCl on protein solubility in mince of fresh and of frozen haddock was investigated, and linked to the functional properties of a heat-treated model product. The effect of heating on WHC in mince with and without added NaCl was investigated. Protein solubility was highest in fillets of fresh fish and was reduced due to freezing and mincing.

For fresh fish, no differences in amount of exctracted salt soluble proteins (SSP) were found between the different species studied. A larger amount of SSP was extracted from frozen fillet of haddock than from frozen fillet of pollock and cod. For mince of fresh fish, the largest amount of SSP was extracted from cod. For mince of frozen fish, the largest amount of SSP was extracted from great silver smelt. 0.6 M NaCl was judged as the best protein extractant when taking in to consideration the amount of extracted SSP and SDS-PAGE profile, and was followed by 0.3 M MgCl<sub>2</sub> and 0.3 M NaCl. 0.3 M KCl, 0.6 M MgCl<sub>2</sub> and 0.6 M KCl were judged as the poorest protein extractants. The reason for this was the general lack of the myosin heavy chain (MHC) in these extracts, which could influence the gelling capacity and WHC of the product. Extraction of MHC with 0.3 M KCl seemed to depend on the raw material. On the basis of this 0.3 M MgCl<sub>2</sub> was judged as the best salt replacer. The additives Na-alginate, Na-alginate+NaCl and NaCl all had a positive effect on protein solubility in mince of haddock and could compensate for use of a poor protein extractant or a protein extractant of low concentration. Heat-treatment of minces/puddings with and without additives showed that the WHC was lowest at 50°C and increased with increasing temperatures, probably due to the gelation of myosin. The WHC was lowest in the raw material without additives and increased with addition of NaCl, Na alginate+NaCl and Na-alginate. The best effect on the WHC, drip loss and texture was obtained when Na-alginate (with or without NaCl) was added.

The pH varied in pollock, haddock, great silver smelt and cod, but increased during freezing. There was a weak positive correlation between WHC and pH in fresh fish (10% significance level), but not in frozen fish. The WHC of cod and haddock increased during freezing (5% significance level), while it was reduced in pollock.

#### Publication: Master thesis, NTNU, Dept. Biotechnology

#### Author: Lise Merete Sitter Bersmo

#### Title: Fish mince with reduced salt content (NaCl) and added sodium alginate

Abstract: Research has revealed that the salt intake among human beings is higher than the recommended daily intake. A high intake of salt increases the risk of elevated blood pressure and cardiovascular diseases. The major part of the salt intake results from processed food. A reduction in the salt content of processed food would not only influence the taste, salt is also important for physico-chemical properties like water-binding capacity (WHC), cooking loss, gelation, solubility of proteins and texture. To maintain the functional properties it is necessary to acquire knowledge about additives that can be used in addition to low salt concentrations. The purpose of this assignment was to study the functional properties in a model of fish pudding (heat treated fish mince) where NaCl (0.4 %) or sodium alginate (SA) (1.1 %) and these two in combination were added. For minced fish with SA added in a gel system together with CaCO<sub>3</sub> and glucono- $\delta$  lactone (GDL) a rheological analysis was carried out. Addition of SA (1.1 %), CaCO<sub>3</sub> (0.2 %) and GDL (0.6 %) to minced fish showed a significant increase in WHC and reduced cooking loss in minced fish where SA was added compared to minced fish where only NaCl was added. The tendency was also that minced fish where SA was added formed a stronger gel than minced fish where only NaCl was added. According to the protein solubility there was an indication that SA had a

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stronger contribution to the gel formation than the myofibrillar proteins. By adding SA (1 %), CaCO<sub>3</sub> and GDL to minced fish (fish and water) a gel that increased in strength over the 20 hours of measuring time, was formed. This suggests that addition of SA CaCO<sub>3</sub> and GDL to minced fish can enhance the functional properties of heat-treated fish mince both for fresh and frozen raw materials.

## Publication:PhD thesis, NTNU, dept. Biotechnology / SINTEF Fisheries and AquacultureAuthor:Kirsti Greiff

Preliminary title: Sodium reduction in muscle foods- Measuring techniques and effect on quality parameters

**Abstract:** The thesis will be submitted spring 2015

#### 7 Media

Some of the popular scientific papers written about the project are:

- Salt verstingene i hverdagen HELSE, Dagbladet 30.05.13
- Mindre salt, samme smak, Handelsbladet FK, 29.05.13
- Saltinnholdet skal reduseres, Adressavisa 28.05.13
- Mindre salt, samme smak, Aftenposten morgen, 09.06.13
- Less salt in our food, Alpha Galileo, 03.06.13
- Mindre salt i maten, Gemini.no, 27.05.13
- Mindre salt, samme smak, Forskning.no, 29.05.13
- Salt verre enn tobakk, Forskning.no, 14.11.13
- Det farlige natriumet, Aftenposten Innsikt, April 2014.(Sunnhetsbladet, August, 2014 i og Pensjonisten, August/September 2014).
- Sans salt, International Innovation, March 2013
- Sodium surplus, International Innovation, March 2013



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