TeRiFiQ

Project no. 289397

Combining Technologies to achieve significant binary Reductions in Sodium, Fat and Sugar content in everyday foods whilst optimizing their nutritional Quality

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Deliverable D2.2
Optimisation of functional properties of salts and protein in sausage production

Abstract: *Report on the effects of formulation and processing conditions during sausage production on functional properties of proteins (water- and protein-binding)*

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Lead contractor/partner for this deliverable: NOFIMA
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Dissemination level	
PU Public (must be available on the website)	[X]
PP Restricted to other programme participants (including the Commission Services)	[ ]
RE Restricted to a group specified by the consortium (including the Commission Services)	[ ]
CO Confidential, only for members of the consortium (including the Commission Services)	[ ]

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Glossary

FTIR	Fourier-Transform Infrared Spectroscopy
K	Potassium
Na	Sodium
NaCl	Sodium chloride or Salt
NMR	Nuclear Magnetic Resonance

1. Context and objective

The formulation of sausages comprises lean muscle, salt, fat and binders. The technological function of salt in cooked sausages is mainly to improve protein and water binding of the batter. Salt alters the muscle proteins' secondary and tertiary structures and thereby improves their protein and water binding capacities, which again affects the micro- and macrostructure of sausages and thus influence the processing and eating quality characteristics. These binding capacities are crucial during the production of cooked sausages, and reduction of salt concentration will lead to weaker binding of both protein and water. We have applied several approaches in order to study and improve the functional properties of meat proteins; use of pre-rigor meat, use of different meat raw materials, additives and optimisation of processing conditions.

Three activities are associated to this deliverable:

- Evaluation of the effect of several Na-reduction activities on commercial sausages
- Effect of meat raw materials and pre-salting
- Effect of pre-rigor salting and degree of emulsification

2. Evaluating the effect of sodium reduction activities

As a result of their participation in TeRiFiQ, Leiv Vidar has during the last two years performed several adjustments to their standard hot dog recipe in order to reduce the Na content of their products: replacement of Na-lactate with K-lactate and a gradual reduction of added NaCl from 1.9 to 1.7%. The actual effect of these changes on the salt content in consumer-ready hot dogs has now been evaluated. In addition, a consumer acceptance study in collaboration with Nofima has been performed on hot dogs with a combined reduction of Na and fat.

2.1 Sodium-reducing activities

In the middle of 2012, Leiv Vidar replaced the original Na-lactate in their hot dog recipe with K-lactate. During the last two years, they have also performed a gradual reduction of NaCl-addition during batter production from 1.9 to 1.7%. In order to evaluate the actual effect these changes have had on salt content in the finished product, the salt content (measured as Cl^- content and converted to NaCl content) has been analysed in hot dogs over the last two years. The salt content has also been evaluated in three other products (OnionDog, CheeseDog and Frankfurter) as a comparison over the same period.

Despite their efforts to reduce the salt content of their hot dogs, they observed that the calculated salt content of these sausages was about 2.1%, which was around 0.5% points higher than for the other sausage products. In addition, there was a considerable variation in salt content (1.9-2.4%) over the last two years, with an increasing trend from the middle of 2013 until April 2014.

The basic recipe for the hot dogs is identical to the OnionDogs (with the exception of additional onion in the latter), and the only obvious difference between these two sausage types is related to the cooling method after cooking. The hot dogs are the only sausages that are cooled by submersion in saturated undercooled salt brine. Although the distributors claim that the submersion time (20 min) is so low that this procedure should not contribute towards an elevated salt content in the final product, this is the most likely reason for the 0.5% points higher salt content in this product compared to the other.

Moreover, the increasing trend for salt content towards the end of this period can be assigned to a flaw in this cooling system that has caused the submersion time to increase to one hour in this period. By adjusting, and possibly replacing, the cooling technique for the hot dogs, it should be feasible to reduce the salt content in the hot dogs from the current 2.1% to 1.6% (as for the other sausages) without major texture problems, although consumers may notice a reduction in saltiness if the change is too abrupt.

Another challenge that this activity reveals is the lack of easily available sodium/Na measurements. Since the measurements of NaCl most commonly used are based on actually measuring the content of chloride ions, this method would not be able to reveal changes in Na content as a result of replacing Na-lactate with K-lactate.

2.2 Combined reduction of sodium and fat – consumer acceptance

In collaboration with Nofima, Leiv Vidar performed a consumer acceptance test on hot dogs with a combined reduction of sodium and fat. Two types of hot dogs were tested; standard hot dog with about 2% salt and 17% fat, and a healthier hot dog with about 20% reduction of salt and 24% reduction of fat. The test was performed as a preference test at a seminar for the private Norwegian meat industry, and each participant was given one sample of each sausage types and asked to indicate which sausage they preferred. The first half of the responders (72 participants) received no information about the two sausages, while the second half (70 participants) were given information regarding which sausage was standard and which was with less fat and sodium. Among the group of responders who received no information, the preference of the sausages was random (50:50 splitt). For the responders who received information, 70% preferred the standard hot dog while 30% preferred the healthy hot dog.

3. Effect of meat raw materials and pre-salting

The aim of this lab-scale experiment was to study the effect of meat raw materials, pre-salting and salt concentration on yield and texture. The effect of these factors on protein structure and water organisation were further assessed by light microscopy, FTIR microspectroscopy and low-field NMR measurements.

3.1 Approach

Main factors included in the 3x2x2 factorial design:
Meat raw materials: pork with 23% fat (pork 23), pork with 6% fat (pork 6), and beef with 14% fat (beef)
NaCl addition: 1.5% and 0.9%
Pre-salting: no pre-salting and pre-salting with 1% NaCl 2 days prior to batter production

The composition of the batters was adjusted so that the content of protein and fat was identical for all batters, at 12.8% and 18.0% respectively. The different meat raw materials had varying content of connective tissue: 4.2% (pork 6), 14.0% (pork 23), and 18.7% (beef).

The experiment was performed at lab-scale, where 1-kg batches of batter were produced. Each batter was then filled into plastic tubes (8x40g) and cooked in a water bath at 80°C for 30 min, followed by cooling in iced water for 30 min. Yield was expressed as cooking loss, measured by weighing the batter prior to cooking and comparing this to the weight of the cooked batter after cooling. Texture was measured using a standard TPA-analysis, and the peak force was used in the statistical analysis. Samples for microstructure and FTIR microspectroscopy were snap-frozen in liquid nitrogen immediately after batter production, and low-field NMR measurements were performed on fresh batter at the same time. These analyses were only performed on the samples subjected to pre-salting.

3.2 Results

3.2.1 Yield and texture

With respect to yield, significant effects were observed for both meat raw material and NaCl addition, while no effect was seen of pre-salting. Batters produced with beef raw material had a higher cooking loss compared to the pork raw materials, while increased NaCl addition lead to reduced cooking loss. Similarly for texture, the highest peak force was observed for beef, followed by pork 23, and the lowest for pork 6. Pre-salting had no effect on texture for pork 6 and pork 23, while pre-salting of beef resulted in a reduced peak force. The difference observed in texture between the different meat raw materials could be a result of the varying connective tissue content in these batters. There was also an interaction effect between pre-salting and NaCl addition level, with a reduced peak force for the pre-salted batters at high NaCl addition, and an increased peak force for the pre-salted batters at the low NaCl addition.

3.2.2 Microstructure

Samples for light microscopy was performed by freezing blocks of batter in liquid nitrogen and 5µm sections were made in a cryostat at -22°C (Wild Leitz GmbH, Wetzlar, Germany). The sections were mounted on glass slides and immediately stored at -20°. The sections were stained with Nile Red overnight at 4° and with Movat pentachrome stain. Nile red is highly soluble in oils and fat will appear with a bright yellow fluorescence in the microscope. The Movat pentachrome stain was used in order to visualise and differentiate between muscle proteins and collagen/connective tissue. Image acquisition was made using a Leica DMLB microscope and a digital camera.

No effect of NaCl addition was observed for the distribution of fat in the batters, however the pork 6 batters seemed to be better emulsified than pork 23 and beef that both still contained large particles of intact fat tissue (Figure 1). It was difficult to get a good differentiation between the muscle and connective tissue components, since the optimal chemical for good connective tissue differentiation (i.e. picric acid) could not be used due to health and safety regulations in the lab.

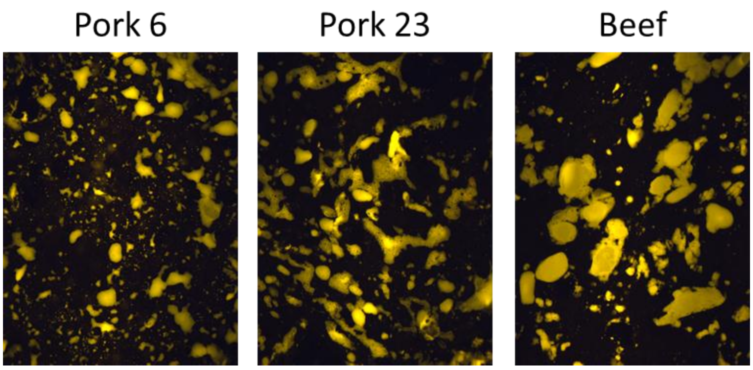

Figure 1. Light microscopoy images showing the fat distribution in batters produced with three different meat raw materials; pork 6, pork 23 and beef. Fat is stained yellow.

3.2.3 Low-field NMR

Low-field NMR is a fast and non-invasive method for the determination of water and fat in foods; amongst others is it a useful tool to estimate water mobility/binding in muscle foods. Therefore it was used to investigate the water binding in these batters. The raw meat batters were measured by low-field NMR and the collected spectra were exposed to different data-treatment methods and subsequently analysed by Principal Component Analysis. The results revealed that the differences were dominated by the type of meat used in the batter (beef vs pork). The mixture containing beef shows a water population (shorter relaxation times) with more restricted mobility than samples based on pork, i.e. water appears to be tighter bound in these samples. At the same time, differences due to salting methods were less consistent and much less distinct, and therefore inconclusive.

3.2.4 FTIR microspectroscopy

As a powerful and versatile analytical technique, Fourier-transform Infrared (FTIR) microspectroscopy enables monitoring structural changes in different components simultaneously, such as bulk proteins, fats, and carbohydrates. This is done by acquiring single FTIR spectra of tissue components such as myofibers in microscopic mode. In addition, more detailed structural information on protein secondary structure can also be obtained. In FTIR microspectroscopic imaging, infrared spectra are obtained in each image pixel in a given region of interest. From an FTIR microspectroscopic image, representing a three-dimensional image cube with two spatial and one spectral dimension, chemical images of thin tissue sections can be obtained. In this investigation, the main aim was to assess the potential of FTIR microspectroscopic imaging in the study of the structural properties of meat batter subjected to different salt concentrations and varying raw material qualities.

Meat batter was sectioned on a cryostat (Leica CM 3050, Leica Microsystem Wetzlar, GmBH, Heidelberg, Germany). Adjacent sections of 10 µm were transferred to a ZnSe window for FTIR microscopic imaging. FTIR spectral images were acquired in transmission mode using a Spectrum Spotlight FT-IR Imaging system (Perkin-Elmer, Bucks, UK). All data analysis were performed using in-house developed software based on Matlab (TheMathWorks,Natick, MA, USA). After spectral acquisition, all FTIR images were subjected to a quality check in order to remove all spectra associated with significantly low S/N ratios and air inclusions etc. Subsequently, all spectra with a significant content of protein were selected for further processing, whereas spectra of other components (e.g. lipids) were discarded. Then all spectra were subjected to mathematical pre-processing using Savitzky–Golay derivatisation and SNV correction. Chemical images were then calculated by using the ratio between (I) the area in the range 1628–1640 cm−1 and (II) the area in the range 1658–1670 cm−1. Bands that occur in the area between 1628 and 1640 cm−1 are assigned to aggregated β-sheets occurring, while bands that occur in the area between 1658 and 1670 cm−1 are the bands assigned to α-helical structures. In this way the difference between the denatured and native protein structures is emphasized.

For all samples, chemical images of the ratio between the aggregated β-sheets and the α-helical structures present were obtained. An example of two chemical images from meat batter made from the same raw material quality, but having different salt concentrations, is provided in Figure 2.

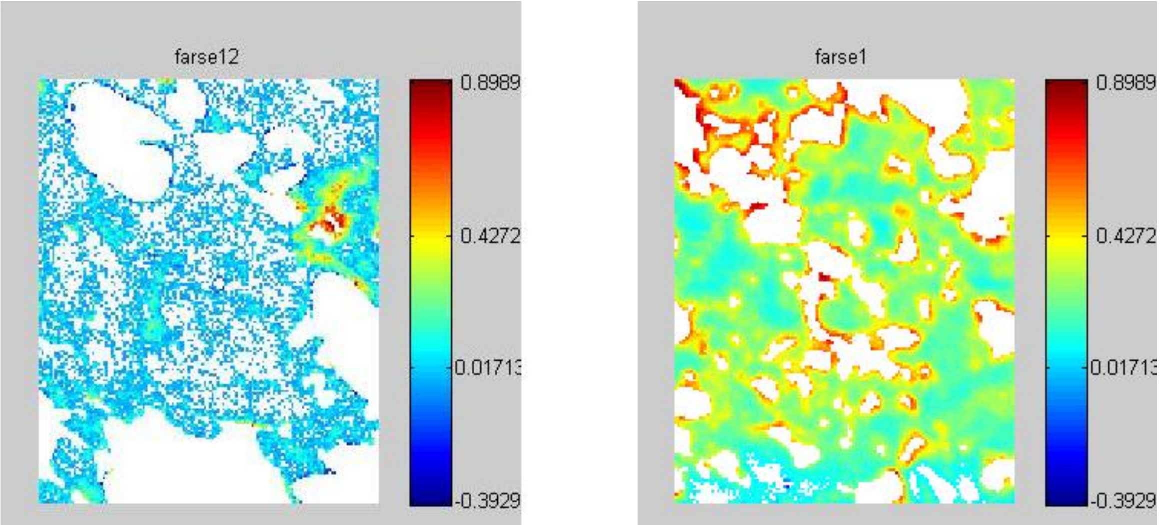

Figure 2. Chemical images showing the ratio between aggregated β-sheets and the α-helical structures in meat batter made from the same raw material quality, but having different salt concentrations (left: 0.9 %, right: 1.5 %).

Figure 2 illustrates some general features of the FTIR imaging technique. A turn towards more red colours in the images indicates that the β-sheets signal has increased and the signal of the α-helical bands is reduced. In the present example this indicates that the level of β-sheets is increasing with increasing salt concentrations within this small concentration range, which could also be expected from previous experiments. It has previously been shown that increasing β-sheet structure could indicate increased water binding, which also fits well with the present experiment. This could indicate that 0.9 % salt concentration is at the lower border of concentrations needed for appropriate water binding properties. Chemical images like the ones shown in Figure 2 could also be obtained for the other raw material qualities.

Figure 2 also shows that both images reveal small deviations from the general texture of the images, such as the small red spot seen at the right side of the 0.9 % image. These deviations could indicate heterogeneity issues of the protein matrices and could thus be used to evaluate quality aspects of the processing of the batters.

4. Effect of pre-rigor salting and emulsification

Pre-rigor meat has better binding properties than post-rigor meat due to the increased pH level, but this raw material is not much utilized in the meat industry. Pre-rigor meat can be used directly for batter production, or the meat can be salted before the meat enters the state of rigor in order to maintain the high pH and thus the improved binding properties. A challenge with using pork trimmings is the short time for pigs to enter rigor, approximately 1 hour compared to several hours for cattle. Trimmings from sows are commonly used in sausages recipes. The purpose of this trail was to conduct an integrated slaughtering, deboning, salting and sausage production experiment to evaluate the effects of using pre-rigor sow meat trimmings on sausage quality.

4.1 Approach

The experiment was performed in collaboration with Leiv Vidar, and the sows were slaughtered at Fatland Jæren. At the day of slaughter, the meat from the forequarters of two sows with 142 and 176 kgs carcass weights were ground, immediately blended with a 20 % NaCl brine and set to chill, all within 60 minutes *postmortem*. The trimmings were transported to Nofima for sausage production 2 days *postmortem*. Six series of sausages were tested: salting of pre-rigor meat, salting of post-rigor meat and salting directly in batter (control). All three meat types were used for the production of both emulsion hot dog sausages and coarse non-emulsion sausages. The sausages were cooked to 74 °C core temperature. Analyses included pH, cooking yield, instrumental texture and taste and texture evaluation with two in-house panels at Leiv Vidar and Nofima.

4.2 Results

The pH data at different stages during the production, ranging from fresh meat at slaughter to final sausage, showed that pH was consistently approximately 0.2 units higher for the pre-rigor compared to the post-rigor series. This result demonstrated that the selected preparation of the pre-rigor trimmings was efficient in regulating the rigor development to higher binding ability, despite the short time available for adding salt. The instrumental texture analysis showed that the texture was much firmer for the pre-rigor than post-rigor coarse sausages. Panel testing verified that pre-rigor sausages, both emulsion and coarse, were more firm than sausages produced with post-rigor meat. Cooking losses were low for all series and not different between sausage types.

The current trial was promising in showing the potential for using pre-rigor sow trimmings with high binding. Therefore, this raw material will be used for in a larger upcoming experiment to investigate if this raw material can compensate for the reduced binding in low salt sausages.

5. Conclusions

Based on the activities and experiments performed in this task, we can conclude that:

- There are challenges related to easily available sodium/Na measurements, since the most common measurements of NaCl are based on measuring chloride ion content and then converting these to NaCl content
- Cooling hot dogs by submersion in saturated undercooled salt brine seems to substantially increase the NaCl content of the final products
- A reduction of NaCl content from 2.1% to 1.6% (25% reduction) in hot dogs should be feasible without major changes to batter recipe
- The liking of hot dogs with a combined 20% salt reduction and 24% reduction of fat was similar to a standard hot dogs, provided that no information was given to the responders
- Informing consumers about the content in the sausages significantly reduced the liking of the healthier hot dog
- Reducing the salt content in meat batters leads to increased cooking loss and softer texture
- Meat raw material has a significant effect on both cooking loss and texture (beef vs. pork)
- Meat raw material with a low fat content gave rise to better emulsified batters compared to more fatty meats
- Low-field NMR revealed differences in water mobility for beef and pork batters
- FTIR micropscopy has a potential for imaging of protein structural changes in meat batters of different salt concentrations, and revealed that the level of ß-sheets (favourable for water and protein binding properties) is increasing with increasing salt concentrations

- Pre-rigor salting of meat improves the binding properties of the sausage batter, and thus may be used to compensate for reduced binding properties observed at low salt concentrations