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FTIR Imaging for Structural Analysis of Frankfurter Sausages Subjected to Salt Reduction and Salt Substitution

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ABSTRACT: In this study, the effects of NaCl, KCl, and $MgSO_4$ 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 $MgSO_4$ 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 $MgSO_4$ 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 $MgSO_4$ due to undesired sensory attributes, which underlines the necessity for using taste masking agents.

KEYWORDS: *FTIR imaging, NaCl, KCl, $MgSO_4$, protein hydration, protein secondary structure, WHC, sensory analysis, frankfurter sausages*

■ INTRODUCTION

Excessive sodium intake, which has proven detrimental for human health, is tightly correlated with the consumption of processed meat products.[1,2] Within this food category, products that require NaCl in order to attain the desired textural properties such as hams or sausages contain very high amounts of sodium.[3] For example, frankfurter cooked sausages, which in Western countries are an especially highly consumed meat product, contain a significant amount of sodium, mainly originating from NaCl (~2% and higher).[1,2,4] The current strategies in food industry for addressing the issue of excessive sodium intake include lowering the content of NaCl in food products, as well as partially replacing it with appropriate substitutes.[4] This modification will inevitably affect the characteristics and structure of the final products, as well as the production procedures and shelf life.[2] Partial replacement of sodium with other cations such as potassium, calcium, and magnesium has been proposed for Spanish dry-cured pork loins.[5−7] Nevertheless, the way in which the structural properties are affected by replacing NaCl by some of the commercially available replacers such as $MgSO_4$ is still an open question and needs to be addressed.

The structure or texture in comminuted meat products such as frankfurter sausages is a consequence of processes that differ considerably from those occurring in whole meat products. This is related to broken muscle structures, which cause myofibrillar proteins to be much more exposed and susceptible to structural changes.[3] For instance, when NaCl is added to comminuted meat, the myofibrillar proteins are unfolded, which results in establishment of a dense protein network upon heating. Ionic strength, pH, and heat-treatment regime affect the heat-induced aggregation and thus the microstructure of the product during heating.[8] Besides strongly depending on proteins, the textural and sensory properties as well as water-holding capacity (WHC) of frankfurter sausages also depend on several other ingredients, namely, fats, starch, salt, and water.[3,9] Therefore, it is important to understand salt-induced structural changes in all of the major constituents and ideally follow them simultaneously.[10] However, microstructural examination is tedious and involves staining of individual components on separate sections.[10,11]

As a powerful and versatile analytical technique, 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.[12−15] FTIR microspectroscopy has been extensively used to study protein denaturation in intact meat and fish tissues.[16−20] These studies have shown that salting in combination with heat treatment induces strong changes in the protein secondary structure, mainly characterized by transformation of α-helical structures to aggregated β-sheets.[16,17] It has also been reported that $MgSO_4$ increases the hydration of NH groups in the protein backbone, which has been linked to an increase in WHC of brined intact bovine meat.[21,22]

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. Chemical images represent one chemical characteristic such as total

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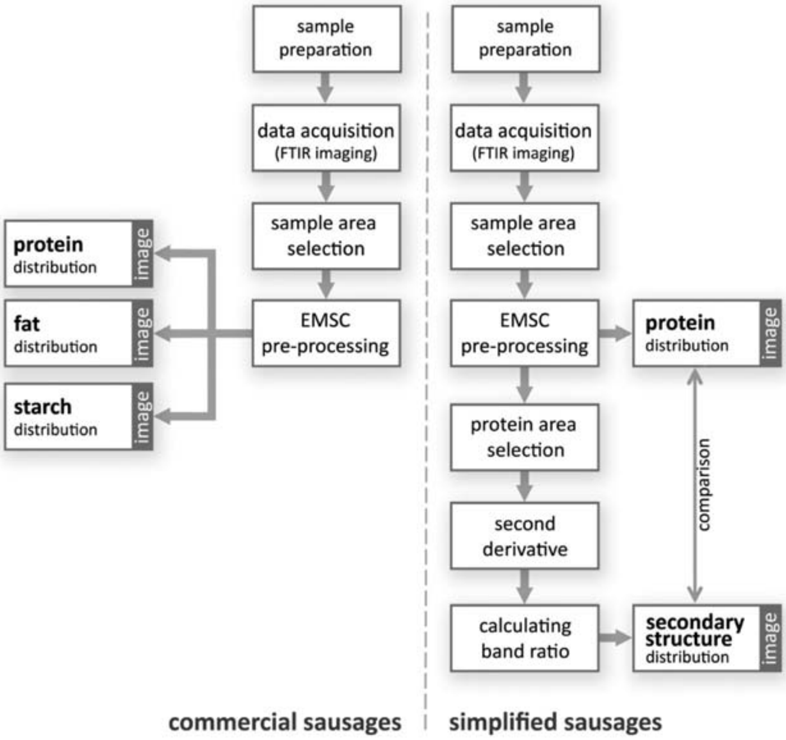

Figure 1. Flow chart illustrating the general strategy for the analysis of the FTIR images.

amount of proteins, fat, and carbohydrates.[23] The majority of FTIR imaging applications are found in biochemical and medical domains, and only a few food-related studies have been reported.[24] Density changes throughout the connective tissue of bovine meat and changes in proteoglycans due to different aging times were investigated by hyperspectral images.[25] The potential of FTIR imaging in studies of matrices of processed foods is thus yet to be outlined.

In this investigation, the main aim was to assess the potential of FTIR microspectroscopic imaging in the study of structural properties of comminuted meat products subjected to different types of salts. In particular, we wanted to investigate the effect of substituting NaCl with KCl and $MgSO_4$ in pure form and in mixtures as well as the effect of different concentrations on structural properties of sausage components. The effects on the structure and distribution of proteins, fat, and starch in the matrix of the frankfurter sausages were followed by FTIR imaging. In order to see if the salt-induced structural changes affected WHC of the sausages, the FTIR results were related to cooking loss measurements.

■ MATERIALS AND METHODS

Sample Preparation. For the purpose of this investigation two types of sausages were prepared: simplified sausages and commercial sausages. The simplified sausages did not contain starch or caseinate, ingredients regularly added to commercial sausages. The reason for this was that simplified sausages were used to study the correlation between the structure of main meat constituents (such as protein and fat) in a model system and changes in quality traits of sausages (such as texture and WHC). NaCl (Akzo salt and basic, Netherlands) and two potential salt replacers, namely, KCl (Kali group, Germany) and $MgSO_4$ (Sigma-Aldrich, Switzerland), were added to simplified and commercial sausages in pure states as well as in mixtures, making seven different salt compositions: 100% NaCl, 100% KCl, 100% $MgSO_4$, 50%−50% NaCl−KCl, 50%−50% NaCl−$MgSO_4$, 50%−50% KCl−$MgSO_4$, and 33%−33%−33% NaCl−KCl−$MgSO_4$. These salt compositions were added in three total concentrations of 0.9%, 1.8%, and 2.7%, which makes 21 different recipes in total.

Raw Ingredients and Chemicals. Pork meat grading, cattle meat grading, and pork fat rind used for making the meat blending were obtained from a commercial slaughter (Furuseth, Norway). The caseinate (Tine, Norway), potato starch (Hoff, Norway), and spice blend (Geovuremûller GMBH, Germany) were also used.

Simplified Sausages. The meat batter, which constituted 68% of the final mixture, was made of approximately 48% pork meat grading, 37% cattle meat grading, and 15% pork fat rind, ground in a small-sized Stephan mixer (Stephan Machinery GMBH, Germany) and mixed with water (totaling 31% of the final mixture) and salts. The meat batter had an initial temperature of 4 °C, and mixing was performed until the final temperature reached 14−16 °C. In the final mixture used for producing simplified sausages there was 18.0% fat and 10.6% proteins. Around 40 g of the final mixture was stored in 60 mL plastic tubes using individual plastic bags per recipe for filling; three replicates were made per recipe and then stored at 4 °C until further analysis.

Commercial Sausages. The meat batter, which constituted 59% of the final mixture, was made of approximately 43% pork meat grading, 34% cattle meat grading, and 23% pork fat rind. The pork and cattle meat, at an initial temperature of 4 °C, was mixed together with salt in an industrial bowl cutter (Type30-1-Vacu-20009, Kilia Fleischereimaschinenfabrik, Kiel, Germany) for 5−10 s, after which the water was added portionwise (totaling 31% of the final mixture), before the addition of potato starch (∼7%), caseinate (1.3%), pork fat rind, and spices. Mixing was performed until the final temperature reached 14−16 °C. In the mixture used for producing the commercial sausages there was 18.2% fat and 9.8% proteins. This final mixture was filled into artificial casing (Viscofan cellulose casing, Viscofan SA, Pamplona, Spain) by using an industrial filler (Tipo E-25-1, Fatosa S.A., Sabadell, Spain). Sausages prepared in this way were further processed in the processing chamber (Unitronic SC2000, Doleschal Austria, Steyr, Austria), which included heating and drying of the sausages for 20 min at 60 °C, preparing for smoking for 10 min at 60 °C, effective smoking for 20 min at 60 °C and 60% humidity in the chamber, removing the smoke from the chamber for 10 min, and cooking for 25 min at 78 °C and 99% humidity in the chamber, after which the steam was removed from the chamber. As a final step, the sausages were sprayed with 5 °C water for cooling, vacuum packed, and stored at 4 °C for 20 h.

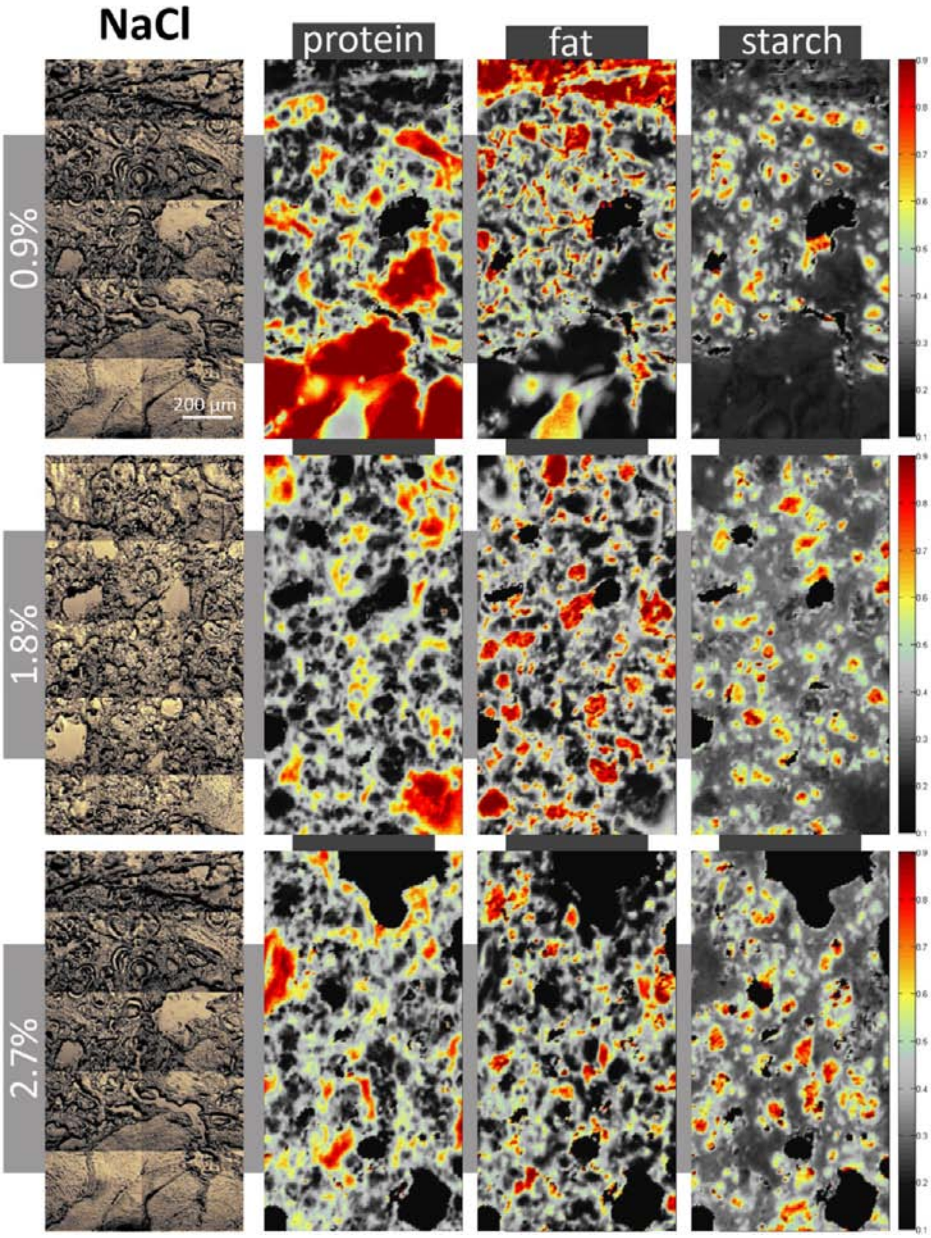

Figure 2. FTIR images of commercial sausages made with only NaCl: the first column presents micrographs of sausage cross sections; second column, distribution of proteins; third column, distribution of fats; and fourth column, distribution of starch throughout the sausage, while the first row presents 0.9%, second 1.8%, and third 2.7% of added salt.

WHC Measurements: Cooking Loss. *Simplified Sausages.* The plastic tubes with simplified sausages were placed into a centrifuge for 10 min at a relative centrifuge force of 197.2*g*, in order to ensure the settling of the meat batters. After that, the tubes were put into a water bath at 80 °C for 30 min, followed by cooling in ice water for 30 min. The simplified sausages were taken out, and the weights of the tubes, including released water and fat, were recorded, representing the cooking loss. The percentage of cooking loss was calculated as weight of loss multiplied by 100 and divided by the weight of initial meat. Finally the simplified sausages were put back into the plastic tubes and stored at 4 °C for further analysis. Cooking loss of simplified sausages was measured in accordance with the Claus and Sørheim method.[26]

Sensory Analysis. Prior to sensory analysis, the commercial sausages were boiled in a steam heater for 10 min at 80 °C, after which 2 cm × 4 cm cylinders were excised from the middle of the sausages. Intensity in odor (smoke, sweet, spicy, sour, metal, meat), taste/flavor (salty, sour, umami, meat, bitter, spicy, smoke, metal, aftertaste), and texture (doughiness, roughness, juiciness, fatness, hardness, cohesiveness) were evaluated by a panel of nine judges, on a scale of 1 to 9, where 1 stands for the lowest and 9 for the highest intensity of the evaluated attribute. The panel received two pretraining sessions of sausage samples, based on which the samples containing 2.7% pure $MgSO_4$, pure KCl, and a mixture of these two salts were excluded from further considerations due to strong off-tastes. In order to obtain a balanced experimental design, all samples made with 2.7% salts were excluded from final analysis of sensory data.

Data Analysis. *FTIR Imaging.* The steps of the FTIR imaging experiments are summarized in Figure 1. In the first step after spectra acquisition, all of the FTIR images of both types of sausages were checked for average intensity of spectra in order to select only the sample area; spectra with very low average intensity were removed from further consideration, because these were the spectra obtained on air inclusions. These spectra were marked as “bad spectra”, and their positions were used in order to make a “mask” of spectra that will be further treated as zero spectra. The “good” spectra were further preprocessed by performing EMSC individually on each of the FTIR images.[25]

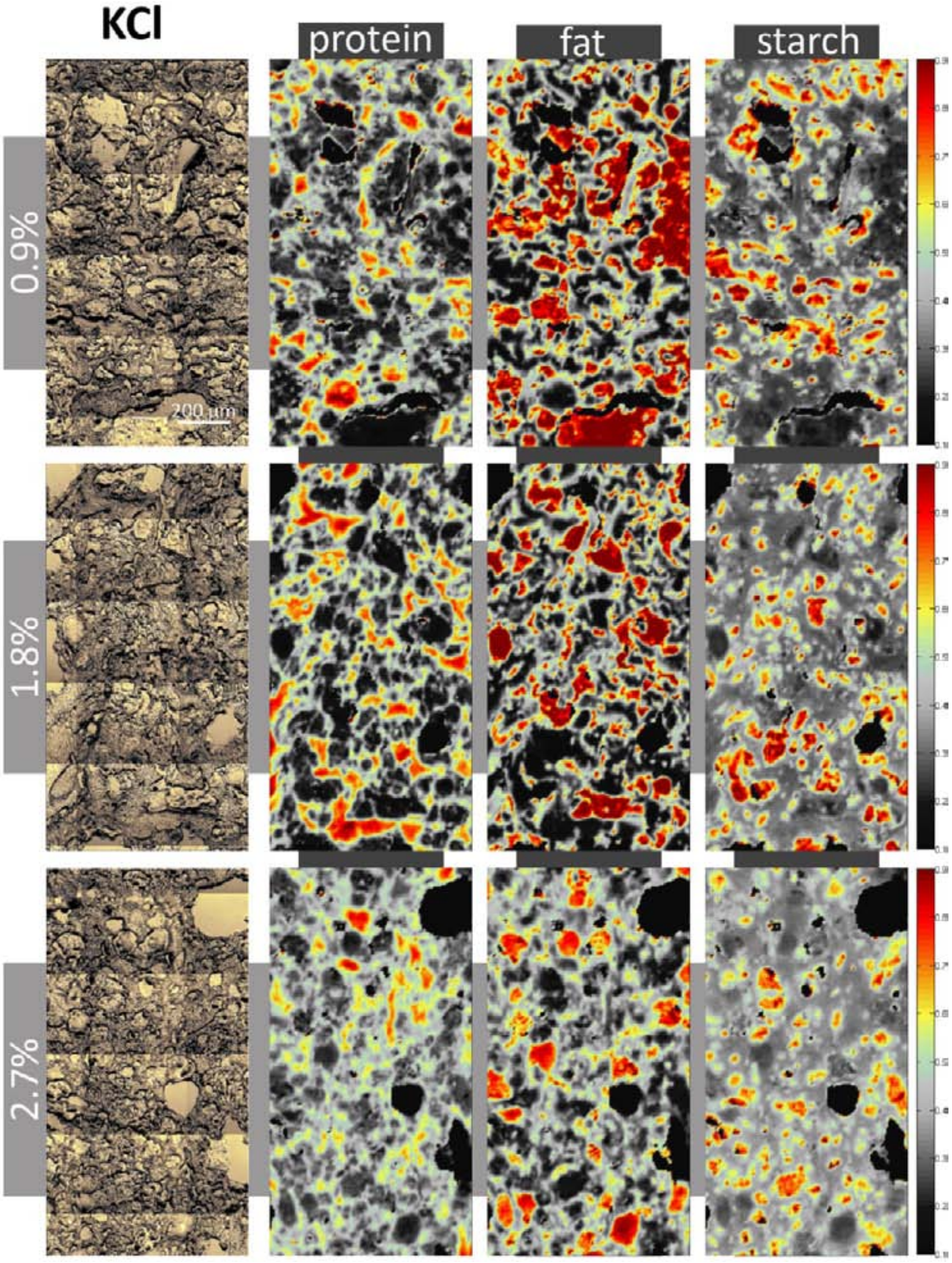

Figure 3. FTIR images of commercial sausages made with only KCl. Columns and rows are organized as in Figure 2.

This was done in order to find the best suitable extended multiplicative scatter correction (EMSC) model with a reference spectrum containing intensive signals of all of the investigated ingredients. The EMSC model was afterward used for preprocessing all of the FTIR images obtained on one sausage type. This was done in order to make the information contained in all of the FTIR images of one type of sausages directly comparable. After this step the two types of sausages were treated differently.

Commercial Sausages. For presenting the distribution of particular chemical compounds, a characteristic wavenumber of that particular chemical compound was selected in EMSC preprocessed FTIR spectra and used for calculating the FTIR image. The distribution of proteins is presented by differences in the intensity of the amide I band at 1650 cm^{-1}, that of fats is presented by the C=O stretching band at 1750 cm^{-1}, while starch was depicted by the C−O stretching band at 1030 cm^{-1}.[19]

Simplified Sausages. Chemical images were calculated in the same way as for commercial sausages. After this, the protein-rich areas on the FTIR images were selected for further analysis. As criterion for selecting the protein pixels we required that the intensity of the amide I band was higher than 30% of the maximum value observed on the FTIR images. Thereafter, the second derivative of the FTIR spectra was calculated by using the Savitzky−Golay algorithm[27] with a window size of nine smoothing points (~9 cm^{-1}). This was further used to calculate 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 C=O groups involved in intramolecular hydrogen bonds and aggregated β-sheets occurring between, while bands that occur in the area between 1658 and 1670 cm^{-1} are the bands assigned to α-helical structures and non-hydrogenated C=O groups.[12−14] In this way the difference between the denatured and native protein structures is emphasized: the band ratios taking values above 1 indicate that aggregated β-structures are more abundant in that particular area, while the band ratios taking values below 1 indicate that α-structures are dominant. Since the intensity values of the bands in second derivative spectra take both positive and negative values, the spectra were shifted to positive values in order to obtain the appropriate values of area ratios. This was done by adding a value of 0.1 to all of the spectra. In order to visualize the distribution of more denatured structures from

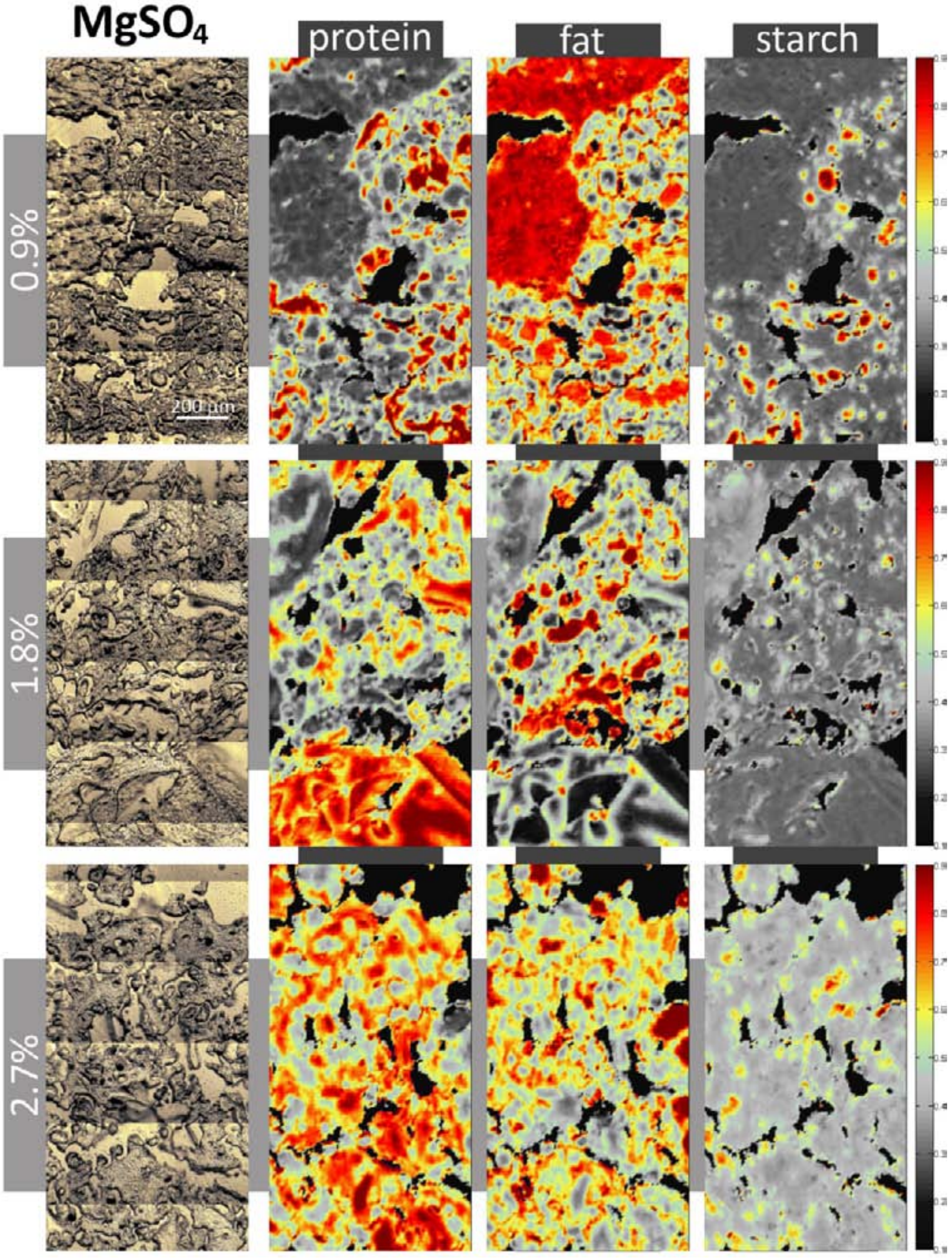

Figure 4. FTIR images of commercial sausages made with only $MgSO_4$. Columns and rows are organized as in Figure 2.

the 1628 and 1640 cm^{-1} area, the values of the calculated ratio that are higher than 1 were used for calculating FTIR images. This was applied on simplified sausages prepared with 1.8% salts in pure form. The FTIR images obtained in this way were compared to the FTIR images of the overall protein distribution.

WHC/Sensory Analysis. Data of WHC obtained by cooking loss measurements were averaged over recipe used, with calculation of standard deviation. Data obtained by sensory analysis were analyzed using principal component analysis (PCA) in order to find the underlying causes of distinction between sample treatments and measured variables.

All steps of data analysis, calculations, preprocessing, and plotting were performed by using in-house-developed routines written with MATLAB software (version 7.12.0.635 (R2011a), The MathWorks, Natick, MA, USA).

■ RESULTS

FTIR Imaging of Commercial Sausages. The FTIR images of commercial sausages are presented in Figures 2, 3, and 4, where the overall distribution of proteins, fat, and starch is depicted together with optical micrographs of the corresponding sausage sections. For the sake of clarity, only FTIR images of samples treated with salts in pure form are presented. As it can be seen in Figure 2, the concentration of added salt plays an important role in the distribution of proteins, fats, and starch when pure NaCl is used in the recipe. When NaCl is added at 0.9%, the protein and fat fractions occupy voluminous/large discontinuous areas of the sausage matrix, with starch being evenly distributed. When NaCl is added in concentrations of 1.8%, it can be seen that the overall distribution of proteins, fats, and starch is significantly more homogeneous, and the fat droplets have a spherical form. In the case of 2.7% of NaCl, it is observed that the fat droplets are not as round as in sausages with 1.8% of added NaCl, while the overall distribution of proteins, fats, and starch is highly homogeneous.

In sausages made with KCl recipes (Figure 3), a similar trend to the case of NaCl recipes can be observed regarding the increased homogeneity of sausage components. A notable difference in KCl recipes is observed, however, where the fat droplets have circular/spherical shapes only at the highest concentration of 2.7%.

Finally, in FTIR images of sausages prepared with $MgSO_4$ (Figure 4), it can be observed that in the case of 0.9% and 1.8% there are large discontinuous areas of fats and proteins. Nonetheless, the remaining part of the sausage samples shows a notable homogeneity in the protein, fat, and starch distribution. It is also observed that the highest concentration of $MgSO_4$ causes a highly homogeneous distribution of all of the analyzed ingredients.

A general trend was observed on the FTIR images for all of the salt types: the higher the concentration of salt, the higher the homogeneity of protein distribution in the sausage matrix. This trend is observable via the shapes of air pores in the sausage matrix, where it is obvious that more circular shapes corresponds to the more homogeneous sausage structures (the observations of air pore shapes are performed on wide areas on samples as well as on a larger number of sausage sections than presented in Figure 2). In the case of the NaCl recipe, the pores at 0.9% have notable irregular shapes, while at 1.8% and 2.7% the shapes are more regularly and circularly shaped. This trend is not as apparent for the KCl recipe, where the regularity of air occlusions does not increase notably with concentration. Finally, the $MgSO_4$ recipe caused a notable regularity of the air occlusions already at 0.9%, which was further increased with increased concentrations.

FTIR Imaging of Simplified Sausages. As simplified sausages were prepared without the addition of starch and spices, the effect of added salt on the protein structure is not disturbed by these ingredients. Therefore, the effect of different salt types and concentrations on protein secondary structure is easier to study for the simplified sausages. These effects were also studied in the commercial sausages, but the trends in the differences between the salts were not as visible as in simplified sausages, most probably due to the presence of starch and caseinate. Therefore, only results obtained on the simplified sausages are presented here.

As a further step in analysis, the chemical images of simplified sausages showing the distribution of secondary structure motifs and thereby depicting denaturation were additionally calculated. The distribution of band ratio values was shown to be the most distinct for the 1.8% salt concentrations. Accordingly, in Figure 5 the distribution of band ratio values over the whole range of protein pixels in three FTIR images of sausages prepared with 1.8% pure salts is plotted. As shown in the figure, for sausages with NaCl, two populations with maximum values around 0.7 and around 1.2, respectively, are found. The sausages prepared with KCl and $MgSO_4$ have only one maximum value around 1.6 and 1.5, respectively.

Representative FTIR images of simplified sausages prepared with 1.8% salts in pure states are presented in Figure 6, where the band ratio values are presented in the range from 1 to 2; in the first column the distribution of proteins throughout the samples is presented, in the second column the distribution of secondary structures is plotted, while the third column presents the FTIR spectra of proteins with the lowest and highest values of band ratio.

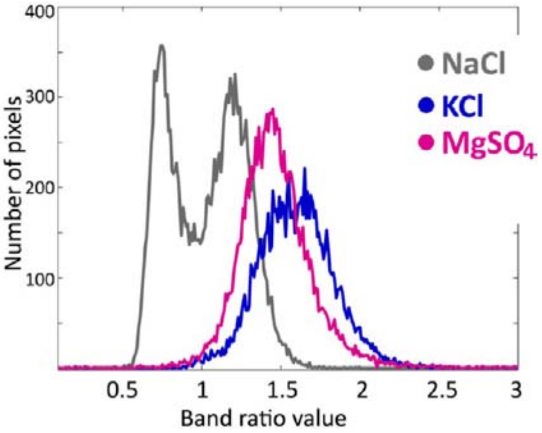

Figure 5. Distribution of band ratio values in FTIR images of simplified sausages treated with 1.8% salts in pure form. Ratio is calculated between aggregated β-sheet band and α-helical bands.

NaCl sausages display two distinct protein regions (Figure 6a): (I) a large continuous area in the upper section of the FTIR image with high protein signal, most probably originating from remnants of intact meat, and (II) the remaining section of the image with evenly distributed protein signals. As shown in Figure 6b, the areas of intact proteins have a low ratio between β- and α-structures (corresponding to the left maximum of the histogram for NaCl in Figure 5), while the areas with evenly distributed proteins exhibit a high ratio of these bands (corresponding to the right maximum of the histogram for NaCl in Figure 5). The area of the intact protein corresponds to ratio values below 1. From the amide I region of the FTIR spectra (Figure 6c), it is observed that the α-helical band in the spectra of the large continuous area (low ratio value) is shifted and more intensive (black spectra) compared to this band found for the evenly distributed proteins (red spectra). Correspondingly, the aggregated β-sheet band was found to be more intensive in areas with evenly distributed proteins. The band assigned to non-hydrogenated C=O groups is found to be significantly more intensive in the large continuous area showing low band ratios. At the same time, the H-bonded C=O groups were also found to be higher in low-ratio regions.

Samples of KCl sausages display an evenly distributed protein signal throughout the sample surface (Figure 6d). From a closer inspection of the secondary structure distribution (Figure 6e), it can be seen that the aggregated β-sheets are predominant in almost all of the homogeneous protein areas. Similarly to the NaCl recipe, the larger protein areas were higher in α-helical bands. For the spectra presented in Figure 6f, it is shown that areas with low band ratios have more intensive bands related to non-hydrogenated C=O groups ($\sim$1667 cm^{-1}) and H-bonded C=O groups at around 1635 cm^{-1}, and no shift in the bands was observed.

In samples of $MgSO_4$ sausages the distribution of proteins is highly homogeneous (Figure 6g), while most of the protein areas have a high band ratio (Figure 6h). From the spectra of this sample (Figure 6i), it can be seen that the variation is quite different from the variation observed for NaCl and KCl. Besides the intensity of the aggregated β-structures being visibly higher in all of the spectra, there are no visible shifts in positions of the bands, nor do the bands of non-hydrogenated or H-bonded C=O groups (at 1667 and 1635 cm^{-1}, respectively) exhibit increased intensities.

When the distribution of proteins in FTIR images of simplified sausages prepared with 0.9% and 2.7% salts was analyzed, similar trends to those in commercial sausages were observed, but were less pronounced (results not shown). In the case of 0.9% salt, the protein matrix is comparably similar to all of the used salts, but to

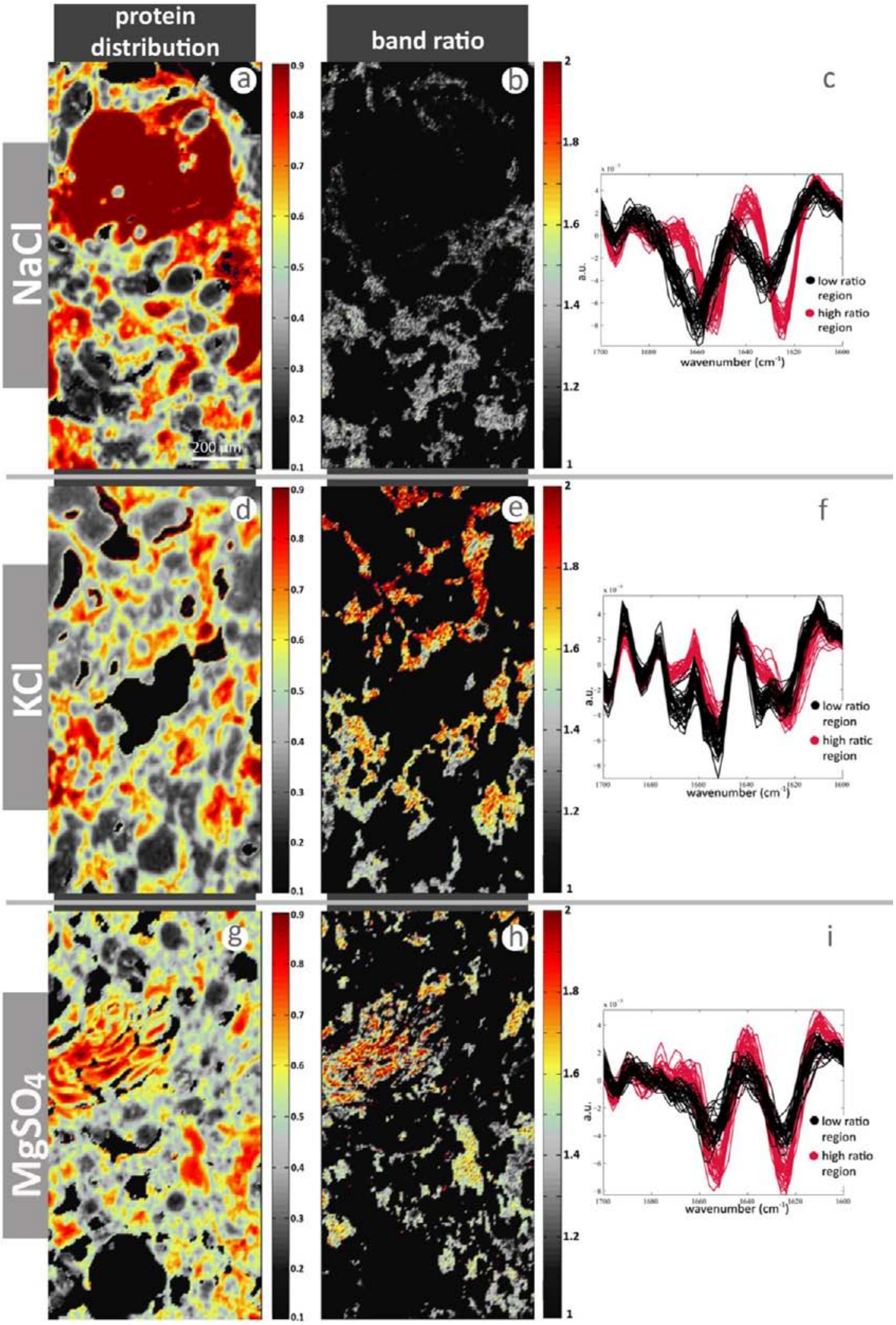

Figure 6. FTIR images of simplified sausages made with pure salts: Sausages made with NaCl are depicted in the first row (a, b, c), with KCl in the second row (d, e, f), and sausages made with $MgSO_4$ are presented in the third row (g, h, i). The FTIR images of the distribution of proteins are plotted in the first column (a, d, g), the distribution of secondary structures is shown in the second column (b, e, h), while spectra in the amide I region are presented in the third column (d, f, i).

some extent less homogeneous in the KCl sausage. In sausages made with KCl it is also observed that the proteins were more aggregated and do not make a generally developed protein matrix. When the salts are added at 1.8%, the homogeneity of the protein matrix is increased for all of the salts, while the $MgSO_4$ recipe causes the production of the most homogeneous protein network.

In the case of the highest concentration, all of the salts show similar distributions of the protein fraction.

Water Holding Capacity. Results of cooking loss measurements on simplified sausages (no starch added) are presented in Figure 7, where the influences of salt concentration and salt type are clear and systematic. A general trend that is observable is that cooking loss decreases significantly with addition of higher quantities of salt, in this case from 0.9%, to 1.8%, to 2.7%. When pure salts are added, it can be seen that KCl causes a decrease in WHC at both 0.9% and 1.8%. On the contrary, the presence of $MgSO_4$ causes an increase in WHC at 1.8% compared to NaCl, while it is comparable to sausages made with NaCl in case of 0.9%. The salt mixtures do not cause a significant change in WHC compared with NaCl, except for the salt mixture that does not contain NaCl at all, the mixture of KCl and $MgSO_4$.

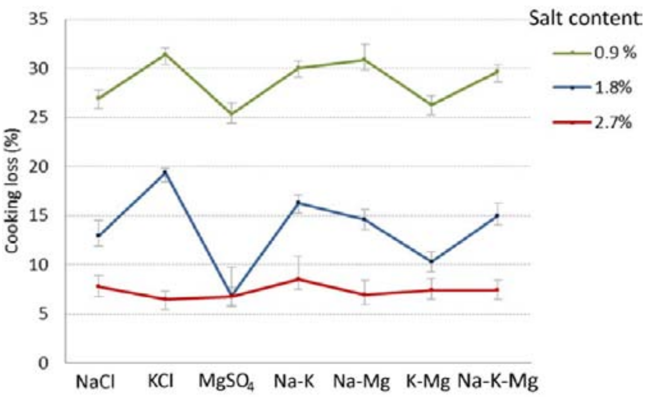

Figure 7. Cooking loss measurements depicting the changes in WHC of simplified sausages.

The WHC of the commercial sausages was also measured, but the changes in WHC due to different salts alone were no longer as systematic as in simplified sausages (results not shown). This might be due to the presence of starch, but since these sausages were not made in replicates for economical reasons and were measured on bulk scale with a low precision, these results were not taken into consideration.

Sensory Analysis. PCA results of sensory analysis of the commercial sausages are presented in Figure 8. Sausages prepared with 2.7% pure $MgSO_4$, pure KCl, and a mixture of these two salt were rejected by the sensory panel due to strong off-tastes. Therefore, in order to have a balanced experimental design, only the sausages prepared with 0.9% and 1.8% salt were analyzed.

Score plots of PC1 and PC2 are plotted in Figure 8a, where samples are labeled in accordance with salt concentration, and Figure 8b, where samples are in accordance with salt type. As shown in Figure 8a, the samples are clearly separated due to different salt concentrations, mostly along PC2. In Figure 8b, it is apparent that along PC1 samples are clustered according to the presence and absence of $MgSO_4$.

In the correlation loading plot presented in Figure 8c, it is apparent that the differences between the effects of salt types on sensory attributes were mostly caused by the differences between NaCl and $MgSO_4$. More precisely, the sausages that contain $MgSO_4$, either in pure form or in the mixture with NaCl, are found high in undesired sensory attributes such as bitter taste, metal flavor, aftertaste, and metal odor. Quite oppositely, the sausages made with only NaCl and the mixture of NaCl and KCl

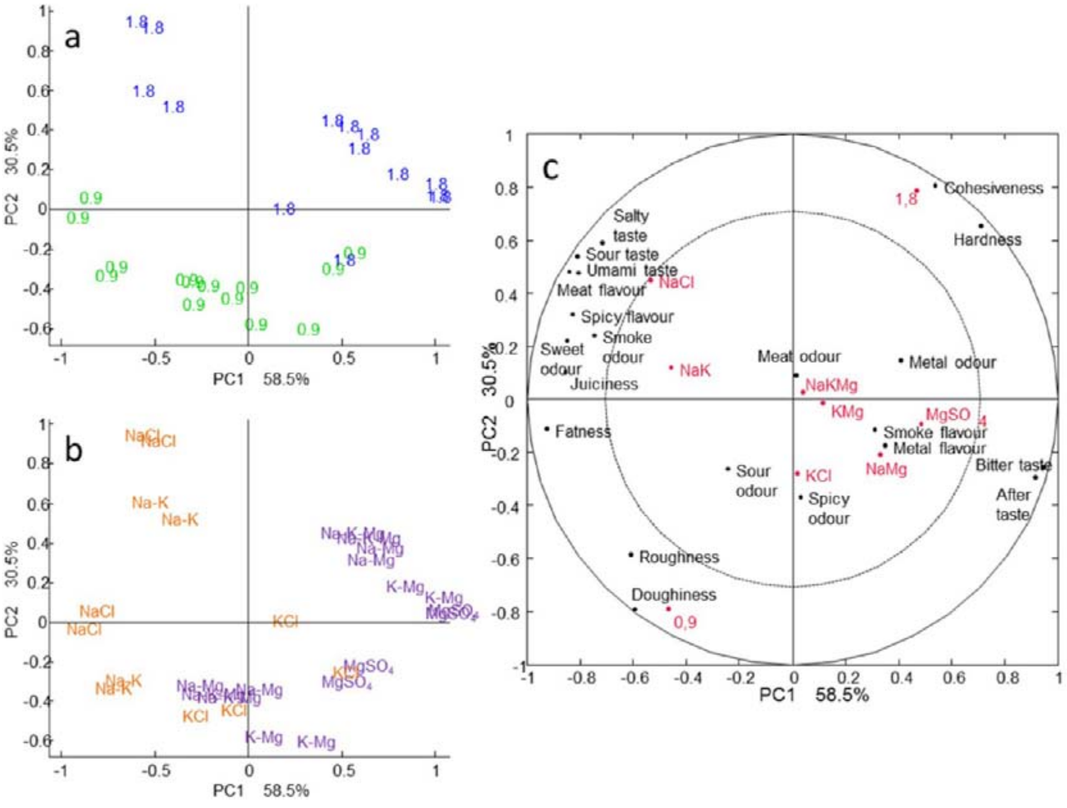

Figure 8. PCA of sensory analysis in PC1 and PC2: (a) score plots with samples labeled according to salt concentration, (b) score plots with samples labeled according to salt type, and (c) correlation loading plot.

were found high in salty and umami taste, meat flavor, sour taste, spicy flavor, smoke odor, and sweet odor, as well as in juiciness and fatness. The mixture of all three salts did not contribute significantly to this PCA model.

In the same plot it can be seen along PC1 that the concentration of salts affects the textural properties of sausages the most; when sausages were prepared with 0.9% salt, the doughiness and roughness were high, while sausages prepared with 1.8% salt were perceived harder and more cohesive. This finding is in agreement with a separate study of texture profile analysis of sausages (unpublished results), which showed that the effect of salt concentration on the sausage texture is highly significant, while salt type and interaction of salt type and salt concentration were not statistically significant.

■ DISCUSSION

FTIR imaging provides the opportunity to simultaneously follow several different constituents in the same sample, without the need for staining. It is hereby shown by FTIR imaging that the distribution of compounds in sausages, both simplified and commercial ones, is more homogeneous for higher salt concentrations. It is further shown that $MgSO_4$ causes the most homogeneous structure of the sausages, particularly the sausages made with starch (Figures 2, 3, and 4). This strong effect of $MgSO_4$ is observed already at 0.9% salt concentration, while for NaCl a similar effect was observed at 1.8%, and KCl seems not to follow the same trend. Furthermore, when the hydration of the proteins increases by $MgSO_4$, the structure of the proteins is more unfolded, making the protein matrix more homogeneous and thus the water binding capacity is higher. This is most probably due to the fact that $MgSO_4$ increases the hydration of proteins[21,22] and therefore repels fat droplets more due to the presence of polar water molecules.[8]

In the case of simplified sausages (Figure 6) there is neither added starch nor caseinate and the main ingredients are proteins and fat. The structure and distribution of these two components are therefore highly important for macroscopic attributes of the simplified sausages, such as its texture and WHC. This is hereby used as a model system for better understanding the connection between the changes in the structure of proteins caused by different salts and the overall quality traits of commercial sausages, such as WHC and sensory attributes.

More precisely, it was shown that the WHC of simplified sausages is significantly affected by both salt type and salt concentrations. It was also found that NaCl strongly increases hydration of C═O groups on the protein backbone and stabilization of β-turns (Figure 6c). The establishment of denatured aggregated β-sheets at the expense of native α-helical structures is observed to be strong. This is in agreement with the literature, where it is stated that the addition of NaCl in combination with heat treatment strongly affects the secondary structure of proteins and causes denaturation of native structures.[16,28]

For simplified sausages made with KCl, it was found that the protein backbones were not as heavily hydrated as in the case of NaCl, visible through a notable intensity of non-hydrogenated C═O groups in all of the spectra (Figure 6f). It was also found that β-turns were highly stabilized by the establishment of a H-bond on the C═O groups. This is also visible through WHC measurements, where sausages made with pure KCl have the highest cooking loss (Figure 7a). In addition to this, a significant degree of denaturation was observed. This finding indicates that the mechanism of interaction between KCl and meat proteins is comparable to that of NaCl, most probably due to the presence of Cl^- ions in both salts, which are shown to have a stronger effect on protein structure than the cations.[8] The difference between Na^+ and K^+ is most probably causing the decreased ability of KCl to induce higher WHC.

Simplified sausages made with $MgSO_4$ showed an increased capacity to retain water compared to NaCl (Figure 7a). This is also connected to the distribution of protein secondary structure, where it was found that the proteins were highly hydrated, visible through a low intensity of non-hydrogenated and H-bonded C═O groups (Figure 6i). Additionally, the denaturation of proteins is also found to be high throughout the sausage sample, indicating a different type of mechanism, which is observable through different variation patterns in the spectra. This is due to the presence of divalent ions (Mg^{2+} and SO_4^{2-}) that express boldly different physicochemical properties than monovalent sodium, potassium, and chloride ions.[8] The fact that commercial sausages did not show any clear patterns in WHC according to the types of salts used might be due to the presence of starch, which is known to form highly hygroscopic gels at temperatures above 70 °C.[29] Moreover, since the WHC of sausages made without starch does not change with different salts, but only with different concentrations, it can be stated that NaCl substitution can be performed without affecting the ability of sausages to maintain water. However, according to our findings, the lowering of salt amounts will affect the WHC of sausages.

Similar conclusions can be drawn from the texture profile analysis of sausages, where it was found that the salt concentration is the only significant factor. These finding were in correlation with the literature, where it was also found that various combinations of NaCl, KCl, and $MgCl_2$ did not cause any statistically significant change in the texture of the dry cured hams.[30] In another study it was also shown that the concentration of NaCl is a very important factor for the texture of frankfurter sausages.[9]

However, the results of sensory analysis showed that both salt concentration and salt type are important for various sensory attributes. Moreover, it was found that the presence of $MgSO_4$ in the pure form or in a mixture with NaCl or KCl causes unwanted sensory attributes of sausages, such as bitter taste and metallic odor and flavor. Since it was demonstrated that $MgSO_4$ has a beneficial influence on texture and WHC, it can be used as a NaCl replacer only in smaller proportions. The finding that the mixture of all three salts in equal proportions did not contribute significantly to the variation in the data set supports using $MgSO_4$ in smaller ratios in salt mixtures. According to Hsieh et al.[31] and Weinberg et al.,[32] both the ionic strength and the specific ion affect the extent of swelling and hence the water holding ability of minced fish muscle. The ionic strength and pH will affect the degree of aggregation of myofibrils unfolded with the aid of salt and thus the microstructure of myosin gels, thereby influencing the water-holding properties.[33] Since $MgSO_4$ is divalent, it is expected that the concentration of $MgSO_4$ could be reduced even more and still affect WHC in a positive way. Furthermore, FTIR imaging is presented as a well-suited versatile analytical tool for analyzing the distribution of chemical components throughout the surface of a complex sample. Along with this, it enables following of changes in the structure of these components. It is hereby shown that the effects that NaCl, KCl, and $MgSO_4$ have on the distribution of proteins, fats, and starch in frankfurter sausages are clearly distinguishable by FTIR imaging. In-depth analysis further showed that these salts induce distinct changes in secondary structure of the meat proteins, as well as different distribution patterns of these secondary structures throughout the matrix of the sausages. Measurements of WHC and texture of the sausages supported the FTIR imaging findings: $MgSO_4$ has the strongest influence on the increment of WHC, while the texture was influenced by salt concentration rather than the salt type. Sensory analysis showed that the use of $MgSO_4$ has a negative effect on the taste of the sausages, underlining the necessity for adding taste-masking agents.

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