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Characterizing salt substitution in beef meat processing by vibrational spectroscopy and sensory analysis

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ABSTRACT

In this investigation, the effect of NaCl, KCl and $MgSO_4$ 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 $MgSO_4$ increased ratio of α-helices and C═O 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 C═O and NH groups. Furthermore, the sensory analysis confirmed that $MgSO_4$ was acceptable only when the share of this salt in the mixture was one third.

1. Introduction

Due to the negative health effects associated with excessive intake of sodium, the food industry aims at lowering its content in processed foods (Asaria, Chisholm, Mathers, Ezzati, & Beaglehole, 2007; Chrysant, 2000; Desmond, 2006; Ripoll, Albertí, Panea, Olleta, & Sañudo, 2008; Ruusunen & Puolanne, 2005). This reduction is, however, not straightforward, since NaCl has important positive effects on taste, textural properties, water holding capacity (WHC) and shelf life (Barrett, Peticolas, & Robson, 1978; Böcker, Ofstad, Bertram, Egelandsdal, & Kohler, 2006; Crehan, Troy, & Buckley, 2000; Gimeno, Astiasarán, & Bello, 2001). Therefore, NaCl is still the main source of excessive sodium intake (He & MacGregor, 2008). Substituting NaCl with other types of salts is thus a leading strategy in the food industry (Ruusunen & Puolanne, 2005), and the assessment of the effects of potential substitutes on taste, textural properties, and WHC is a crucial requirement for a successful substitution.

A large range of potential NaCl replacers is readily available and several are already in use in industry (He & MacGregor, 2008). NaCl replacers are frequently added as complex mixtures in order to attain the desired sensory effects and to overcome undesired taste effects of individual replacers (Desmond, 2006; Kilcast & Angus, 2007). Among currently used inorganic salts are KCl and $MgSO_4$ (He & MacGregor, 2008). These salts are usually used in combination with taste enhancers and masking agents due to their off taste (Gou, Guerrero, Gelabert, & Arnau, 1996; Ruusunen & Puolanne, 2005). Although KCl is chemically and physically comparable to NaCl, it is not able to fully substitute NaCl because of its different sensory properties (Gimeno et al., 2001). On the contrary, while $MgSO_4$ is chemically and physically notably different from NaCl, it has similar effects related to some of the macroscopic characteristics of meat, such as increased WHC (Perisic, Afseth, Ofstad, & Kohler, 2011).

One of the most common methods of introducing salt into meat products is marinating, and its use in the meat industry is steadily increasing (Björkroth, 2005). This processing method is used for a wide range of meat products, from fresh meat products to hams and sausages (Lawrie, 1998). Another common meat processing method that improves meat quality is conditioning or ageing (Boakye & Mittal, 1993). Ageing is defined as a post-mortem process of keeping unprocessed meat for varying lengths of time under controlled conditions (Boakye & Mittal, 1993). It is shown that increasing the ionic strength of the meat during ageing by addition of salts affects the pH, tenderness and WHC considerably (Offer & Knight, 1988; Offer et al., 1989). Wu et al. (2006) found that ageing does not induce the notable formation of aggregated β-structures at the expense of the α-helical structures, which was pronounced upon salting of meat with NaCl. However, a complete and unifying theoretical explanation of WHC and its connection to changes in processing methods are still lacking.

Vibrational spectroscopy has shown to be a powerful tool for chemical and structural analysis of biological samples on the macroscopic and microscopic level (Siebert & Hildebrandt, 2008). NIR

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spectroscopy is today widely used for on-line quality control (Elmasry, Barbin, Sun, & Allen, 2011; Siesler, 2002) and for the prediction of general qualities of main food constituents, such as water, fat and proteins (Ripoll et al., 2008). Structural NIR analysis of food proteins and other food constituents are also reported (Pieters et al., 2011), but since the NIR region is comprised of strongly overlapping combinational bands and overtones, the interpretation of the spectra is difficult (Prieto, Roehe, Lavín, Batten, & Andrés, 2009). FTIR microspectroscopy has proven to be an indispensable tool for following changes in the secondary structure of proteins, and recently the technique also has been used for studying effects of salting on meat (Bertram, Kohler, Böcker, Ofstad, & Andersen, 2006; Carton, Böcker, Ofstad, Sørheim, & Kohler, 2009; Perisic et al., 2011; Wu et al., 2006). It has been demonstrated by the use of FTIR microspectroscopy that brining has a strong effect on the secondary structure of proteins in salmon (Carton et al., 2009). We have recently linked changes in secondary structure of beef meat proteins with their hydration affinities caused by different inorganic salts (Perisic et al., 2011). Thus, these techniques could be used rapidly and non-destructively to determine protein structure and hydration properties in animal tissue. Yet, the potential of FTIR microspectroscopy for the monitoring of the influence of NaCl replacers on meat proteins is to be fully utilised.

The main aim of this study was to determine microscopic and macroscopic changes of meat with respect to ageing and salting with different types of salt in order to relate molecular changes directly to macroscopic properties such as WHC and sensory attributes. For this purpose beef meat (*longissimus dorsi*) was marinated with NaCl and two of the commercially available replacers KCl and $MgSO_4$, and aged for different durations of time. Samples were analysed by FTIR microspectroscopy and NIR spectroscopy, sensory analysis and WHC analysis. The spectroscopic results were linked by multi-block method Consensus Principal Component Analysis (CPCA) to find the connections between changes in secondary structure of proteins and water interactions. Chemical and sensory results were further compared to spectroscopic results in order to relate the changes in molecular structure of proteins to sensory characteristic and WHC, as one of the most important properties of meat products for the meat industry.

2. Materials and methods

In order to investigate the effects of different salt types and different ageing times on various properties of beef meat (*longissimus dorsi* muscles from Norwegian Red Cattle), three separate studies were performed:

Study 1: The effect of different ageing times and the effect of different animals were studied using FTIR microspectroscopy.
Study 2: The effects of ageing and salting with different types of salt on the meat protein secondary structure, protein–water interactions and WHC were investigated. For this purpose FTIR microspectroscopy, NIR spectroscopy and cooking loss measurements were performed.
Study 3: The effect of different salt types on sensory properties of beef meat was investigated by measuring cooking loss in combination with sensory analysis.

2.1. Study 1

2.1.1. Sample preparation

Beef muscle blocks were excised from 5 different carcasses 45 min post mortem. Consecutively, muscle blocks in approximately 5 mm × 5 mm × 2 mm size were excised and prepared for FTIR analysis for samples that were aged for 0 days. The rest of the muscle was packed in polyethylene bags under slight vacuum and aged at 4 °C for 2, 7 and 21 days. Samples obtained from all 5 carcasses were aged for all ageing times. After each of the ageing time points, muscle blocks were excised and further prepared for FTIR analysis.

2.1.2. FTIR analysis

Excised muscle blocks (5 mm × 5 mm × 2 mm) were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Electron Microscopy Sciences, Hatfiles, USA), frozen in liquid N_2 and stored at −80 °C until sectioning. The samples were sectioned at −22 °C transversally to the fibre direction. A cryostat (Leica CM 3050S, Nussloch, Germany) was used, and 10-μm-thick sections were prepared and thaw-mounted on ZnSe slides for FTIR microspectroscopic measurements. The sections were finally freeze-dried and stored under dry conditions.

The acquisition of FTIR spectra was performed on an IRScopeII FTIR microscope coupled to an Equinox 55 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany), equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Spectra were collected from single myofibres in transmission mode in the range from 4000 to 1000 cm^{-1}, with spectral resolution of 4 cm^{-1} using a 15× objective lens.

For each spectrum 64 interferograms were collected and averaged. Both spectrometer and microscope were sealed by a specially designed box and were continuously purged with dry air in order to reduce the spectral contribution of water vapour and CO_2. Additional compensation for water vapour/CO_2 variation was accomplished by taking background spectra of the ZnSe substrate.

2.2. Study 2

2.2.1. Sample preparation

Samples of *longissimus dorsi* muscle were obtained from two different animals (Norwegian red): samples from one animal were aged for 2 days, while samples from the other animal were aged for 14 days. The conditions during ageing of these samples were set as in Study 1: samples were packed in polyethylene bags under slight vacuum and stored at 4 °C. After this, samples were marinated by immersing in marinade solutions for 48 h. Marinades used in this experiment are composed of 87% of water, 5.5% of salt in different mixtures, 4% sodium lactate/diacetate, 3% dextrose and 0.5% sodium ascorbate. The salts used in the marinades are NaCl, KCl and $MgSO_4$ in their pure states as well as in mixtures of two and all three salts in equal proportions (with the constant final concentration of 5.5%), resulting in a total of 7 different marinades. From each animal 14 samples were taken: 2 replicates per each of the 7 marinades resulting in 28 meat samples in total.

2.2.2. FTIR analysis

After brining, samples were prepared for FTIR analysis and consecutively analysed by FTIR microspectroscopy according to the protocol described for Study 1. The resulting FTIR data set consisted of 20 single-myofibre spectra per one experimental treatment (one animal treated with one marinade). The final data set consisted of 140 spectra.

2.2.3. NIR analysis

Immediately after brining, samples were analysed by NIR spectroscopy without further sample preparation. VIS/NIR spectra were obtained by a NIRSystems XDS Rapid Content Analyzer (Foss NIRSystems, Silver Spring, MD, USA) equipped with a quartz halogen lamp and a PbS/Si detector. The spectra were collected in the reflectance mode employing an internal ceramic reference standard. The spot size of the incoming light was set at 17.25 mm (diameter), and all spectra were acquired in standard sample cups with quartz windows. Each spectrum was the average of 32 scans, and all spectra covered the spectral region of 400–2500 nm with a digital resolution of 0.5 nm.

2.2.4. Cooking loss

Meat pieces of 3.5 cm × 3.5 cm × 3.5 cm were weighed and consecutively vacuum packed in polyethylene bags. The samples were first submerged in water bath with temperature of 70.5 °C and kept