Title

Effect of Meat Type, Animal Fat Type and Cooking Temperature on Microstructural and Macroscopic Properties of Cooked Sausages

Authors

Seline Glorieux^{a,b,*}, Liselot Steen^a, Davy Van de Walle^c, Koen Dewettinck^c, Imogen Foubert^b, Ilse Fraeye^a

Affiliations

Seline.Glorieux@kuleuven.be; Liselot.Steen@kuleuven.be; Davy.VandeWalle@UGent.be;
Koen.Dewettinck@UGent.be; Imogen.Foubert@kuleuven.be; Ilse.Fraeye@kuleuven.be

^a KU Leuven Ghent Technology Campus, Leuven Food Science and Nutrition Research Centre (LForCe), Research Group for Technology and Quality of Animal Products, Gebroeders De Smetstraat 1, B-9000 Gent, Belgium

^b KU Leuven Kulak, Leuven Food Science and Nutrition Research Centre (LForCe), Foods & Lipids, Etienne Sabbelaan 53, B-8500 Kortrijk, Belgium

^c Ghent University, Department of Food Technology, Safety and Health, Laboratory of Food Technology and Engineering, Coupure Links 653, B-9000 Gent, Belgium

ORCID of the authors

S. Glorieux: 0000-0002-9744-1771 - L. Steen: 0000-0002-6240-3708

D. Van de Walle: 0000-0003-1018-1696 - K. Dewettinck: 0000-0001-5050-2269

I. Foubert: not available - I. Fraeye: 0000-0002-6238-5810

* Corresponding author.

Tel: +3293316617 - Fax: +3292658638

E-mail address: Seline.Glorieux@kuleuven.be

Abstract

The aim of this study was to simultaneously study the effect of meat type (chicken breast and leg meat), animal fat type (selected pork back fats having a low and high degree of saturation) and cooking temperature (60 and 70 °C) on the microstructural and macroscopic properties (water and fat binding, texture and pH) of cooked sausages. In sausages prepared with the most saturated pork back fat, the emulsion stability significantly decreased and the greater part of the total expressible fluid was fat, while the reverse was seen for the more unsaturated pork back fat. This was probably related to fat coalescence at the microstructural level. The cooking loss was slightly lower for sausages prepared with the most saturated pork back fat. The fat type clearly affected the hardness of the cooked sausages, irrespective of the type of meat or temperature. Thus it can be concluded that small differences in fatty acid composition between the different pork back fats proved to have a great effect on the microstructural and macroscopic properties of the cooked sausages, in contrast with the meat type (muscle fiber type) or temperature which only had a moderate effect.

Keywords

Gel-type emulsified meat products; muscle fiber type; pork back fat; cryo-SEM; fat and water-binding; texture

1. Introduction

Gel-type emulsified meat products such as cooked sausages consist of a finely comminuted lean meat fraction, adipose tissue, ice and additives, which are thoroughly mixed in order to obtain a raw meat batter. In regular emulsion-type meat products, the fat fraction (mostly pork back fat) is macroscopically solid due to the low temperature of the batter (± 12 °C). Therefore, although cooked sausages are often referred to as ‘emulsified’ meat products, they do not form a true emulsion (Feiner 2006). However, the existence of an interfacial protein film (IPF) around the fat globules formed by myofibrillar proteins has been described. This IPF stabilizes the fat phase that becomes liquid as temperature rises during the heating process (pasteurization step), thereby preventing fat coalescence and outward flow of liquefied fat (Barbut and Youssef 2016). Also, during heating, myofibrillar proteins form a three-dimensional gelled protein network wherein fat and water are physically entrapped (Gordon and Barbut 1992). Upon cooling, the protein network is further stabilized, and the fat fraction partially crystallizes. Because the fat particles are coated with an IPF which interacts with the continuous protein network, they act as ‘active filler particles’, contributing to the overall gel rigidity (Wu et al. 2009). As such, it is clear that both the properties of the myofibrillar protein matrix (including IPF) and fat crystallization contribute to the final product structure and texture.

Macroscopic properties such as the water- and fat-holding capacity and the texture of gel-type emulsified meat products depend on various factors such as the nature and amount of the lean meat fraction, fat characteristics, water amount, additives and processing conditions (Santhi et al. 2017). Since these macroscopic properties of semi-solid foods are inseparably related to the mechanical features of the internal structure (Marangoni et al. 2012), evaluation of the microstructure is a prerequisite to fully understanding and predicting the macroscopic properties of meat products processed under various conditions. Analyzing the open literature, only a few studies were found in which the effect of meat type, animal fat type or temperature on both the microstructural and macroscopic properties of meat batters or cooked sausages were studied. With regard to the effect of meat type, Barbut (1997) analyzed the effect of pH on the microstructure, water-holding capacity (WHC) and gel rigidity of meat batters containing white and dark turkey meat. Others (Barbut and Youssef 2016; Youssef and Barbut 2010)

focused on the effect of the substitution of beef fat with alternative solid fats such as hydrogenated palm oil on the microstructural and quality characteristics of meat batters. However, no other studies were found in which both microstructure and macroscopic properties of gel-type emulsified meat products containing different animal fats were investigated. Finally, the effect of cooking temperature on the microstructure, cooking loss and texture of low- and high fat bologna sausages was studied by Carballo et al (1996), while Barbut et al (1996) analyzed the effect of cooking temperature on the microstructure and gel rigidity of meat batters prepared with turkey meat, salt and phosphate.

A previous study proved that meat type, and especially the type of animal fat and temperature have a great influence on the rheological properties of meat batters (Glorieux et al. 2018). Since rheological properties determine the food's perceived quality and have proved to be sensitive to the microstructure (Marangoni et al. 2012; Tornberg 2005), it would be expected that the different types of meat and animal fat, and cooking temperature will affect the cooked sausage's microstructure and therefore, its macroscopic characteristics as a result of protein gelation and/or fat crystallization. The strength of the present study lies in the fact that the influence of meat type (chicken breast and leg meat, containing predominantly white and red muscle fiber types, respectively (Xiong 1994)), fat type (two pork back fats having a distinct fatty acid profile) and cooking temperature (60 and 70 °C) will be studied simultaneously. Cooked sausages will be prepared with the different types of meat and fat then cooked at different temperatures until all samples have reached an F_0 -value of 40 min in order to achieve a commercially safe and stable product under pasteurization conditions. The effect of both composition variables and cooking temperature on the microstructure (using scanning electron microscopy), as well as macroscopic characteristics (fat and water-binding and textural properties) will be analyzed.

2. Material and Methods

2.1. Raw materials (characterization) and ingredients

Fresh chicken breast and leg meat were bought at a local slaughterhouse (Nollens, Kruishoutem, Belgium). The visible fat, connective tissue, skin and bones were removed from the meat. The cleaned

breast and leg meat was chopped, ground and homogenized to generate one large homogeneous batch of each raw material, which was then vacuum-packed then stored at -18°C until further analysis. Fresh Belgian and Italian pork back fat was provided by De Lausnay (Zelee, Belgium) and Spekindustrie Van Maele (Diksmuide, Belgium), respectively, trimmed of visible meat, ground then stored at -18°C until further analysis (within a four-month period). The water, protein and fat content of the raw materials were analyzed according to ISO procedures 1442-1973, 937-1978 and 1443-1973, respectively (Table 1).

The fatty acid composition of Belgian (generally having a lower degree of saturation) and Italian (generally having a higher degree of saturation) pork back fat was determined by gas chromatography (Steen et al., 2015). Only details on palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2) will be presented, as these were the four main fatty acids accounting for about 90% of the total fatty acids present. In addition, the overall degree of saturation was also calculated (based on all fatty acids measured). Due to the different degrees of saturation, Belgian pork back fat and Italian pork back fat will be further referred to as the ‘unsaturated’ (U) and ‘saturated’ (S) pork back fat, respectively. Lard was obtained from the fat fractions by a rendering process (Steen et al. 2015), equilibrated at 7°C for 1 week, and subsequently the melting profiles were analyzed using differential scanning calorimetry (DSC) and hardness via texture analysis. The DSC experiments were performed on a Q2000 DSC with a refrigerated cooling system and autosampler (TA Instruments, New Castle, DE, USA). The instrument was calibrated prior to analysis with indium (TA Instruments). Nitrogen was used to purge the system. A hermetically sealed aluminum pan with about 10 mg of lard was transferred to the cell that was preset at 7°C , and subsequently heated from 7°C to 80°C at $20^{\circ}\text{C}/\text{min}$. An empty pan was used as the reference. The peak maxima (peak temperature) of the melting peaks, together with the total enthalpy were calculated from the DSC melting curves. Bulk fat hardness was evaluated using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments, Bognor Regis, UK). The lard samples (approximately 3 cm in height) were axially penetrated (33% penetration) using a load cell of 100 N and a conical probe (diameter 20 mm) at a speed of 10 mm/min. Hardness was directly obtained from the recorded force-distance curves and described as the maximum force (N) needed to penetrate the sample.

All food ingredients/additives and spices (nitrite curing salt, sugar, white pepper, foil, ascorbic acid, glutamate, coriander and cardamom) were bought from Solina Group (Eke-Nazareth, Belgium).

2.2. Cooked sausage manufacturing

The cooked sausages were produced with chicken breast or leg meat (30% w/w), U or S pork back fat (35% w/w) and ice (35% w/w), together with nitrite curing salt (1.8% w/w), sugar (0.5% w/w), white pepper (0.2% w/w), foil (0.05% w/w), ascorbic acid (0.05% w/w), glutamate (0.05% w/w), coriander (0.025% w/w) and cardamom (0.025% w/w). The latter 8 ingredients were calculated in relation to the total mass of raw materials (meat and fat) and ice. The cooked sausages were prepared in the pilot plant of the research group 'Technology and Quality of Animal Products' (KU Leuven Ghent Technology Campus, Belgium). The raw materials were thawed overnight before manufacturing. First, the raw meat (chicken breast or leg meat) was pre-chopped together with the ice and nitrite curing salt in a bowl cutter for ~ 8 min (Stephan Cutter UM12, Hameln, Germany) until the temperature reached 5 °C. Next, the U or S pork back fat was added together with the remaining food additives, and the total mass was cut under vacuum for 3 min to obtain a homogenous raw meat batter. The temperature did not exceed 12 °C during processing to avoid excessive protein denaturation and fat coalescence. Part of the meat batter was immediately analyzed for emulsion stability. The remainder of the batter was filled into cans of standardized dimensions (diameter 7 cm, height 5 cm, mass ~ 250 g, Crown Verpakking Belgie NV, Hoboken, Belgium). The cans were cooked at 60 ± 1 °C or 70 ± 1 °C (core temperature) in a cooking chamber (Rational Climaplust Combi CPC 61, Claes Machines, Paal, Belgium) until all samples had received an F_0 -value treatment of 40 min (reference temperature 70 °C and respective z-value 10 °C) in order to achieve a safe and stable product under pasteurization conditions. After cooking, the cans were cooled to 4 °C and stored until further analysis. One week after the manufacturing process, the cooked sausages were observed using cryo-SEM and analyzed for cooking loss, textural properties, and pH.

2.3. Water and fat binding

2.3.1. Emulsion stability

Emulsion stability (water and fat loss caused by forced destabilisation of the meat batter) was measured as described by Hughes et al (1997). A sample of raw (unheated) meat batter (30 g) was placed in a pre-weighed centrifuge tube (VWR International, Leuven, Belgium) and weighed. The sample was heated in a cooking chamber (30 min at 60 or 70 °C) and centrifuged (4230 × g, 3 min, 25 °C, Hettich Universal 320 R, Newport Pagnell, UK). The drip (supernatant containing a mixture of water and fat) was poured into an aluminum dish (VWR International) and weighed. The percentage of total expressible fluid (% TEF) was calculated by the formula:

$$TEF (\%) = \frac{\text{drip loss}}{\text{initial mass sample}} * 100$$

Aluminum dishes containing the drip were subsequently left overnight in an oven at 103 °C (Memmert, Schwabach, Germany) to evaporate the water then reweighed to calculate the contribution of water and fat to TEF, determined as the percentage weight loss of TEF after heating overnight, and 100% – *water part of TEF*, respectively.

2.3.2. Cooking loss

One week after processing, the cooking loss (CL) was calculated by determining the initial sausage mass as well as the drip loss (jelly and/or fat separation). CL was expressed as follows:

$$CL (\%) = \frac{\text{drip loss}}{\text{initial mass sample}} * 100$$

2.4. Textural properties

The hardness of the cooked sausages was evaluated using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments). The cooked sausages were axially penetrated (2 cm) using a cylindrical probe (diameter 12 mm, Lloyd Instruments) connected to a load cell of 100 N at a speed of 100 mm/min. Hardness was directly obtained from the recorded force-distance curves as the maximum force (N) required to penetrate the sample (Steen et al., 2014).

2.5. pH

One week after processing, the pH of the cooked sausages was measured with a Portable Meat pH Meter (HI 99163, HANNA Instruments Nieuwegein, Netherlands).

2.6. Microstructural properties

After one week in a refrigerator (4 °C), the microstructure of the cooked sausages was visualized using scanning electron microscopy. The cooked sausage samples were quickly frozen in slushed nitrogen (−210 °C), fractured at -140 °C, sublimated (45 min at -70 °C), sputter-coated with platinum then observed using a Jeol JSM-7100 F scanning electron microscope (Jeol Europe B.V., Zaventem, Belgium). The accelerating voltage was 3 kV.

2.7. Statistical analysis

Three independent preparations of cooked sausage were prepared for each variation. All analyses were performed in triplicate on each sausage sample. The results are expressed as mean ± standard error (n = 3). Statistical analysis was performed using IBM SPSS Statistics 22 (Armonk, NY, USA).

A balanced fixed effects design was chosen for this study. The effects of meat type (chicken breast *vs.* leg meat), animal fat type (U *vs.* S pork back fat) and temperature (60 *vs.* 70 °C) on emulsion stability (TEF, and water/fat part of TEF), cooking loss, hardness and pH were evaluated by three-way ANOVA. No three-way interactions were observed. However, two-way interactions that were significant were further interpreted, as well as the significant main effects.

Tukey's post hoc test was performed in the case of significant two-way interactions. A significance level of $p < 0.05$ was used to identify significant differences for all statistical analyses.

3. Results and Discussion

3.1. Pork back fats characteristics

The chemical composition and physicochemical characteristics of the pork back fats are shown in Table 1. In general, the chemical composition of the pork back fats was well in line with other studies (Glorieux et al. 2018; Marrikar and Yanty 2014; Steen et al. 2015). The S pork back fat in our study contained more saturated fatty acids (higher degree of saturation) compared with the U pork back fat (Table 1). The melting peak area, an indication of the solid fat content (SFC), and the bulk fat hardness of lard from the S pork back fat (Table 1) were significantly higher than those of the U pork back fat. A higher degree of saturation has been correlated with a higher SFC and hardness of lard in other studies (Campos et al. 2002; Gläser et al. 2004). The peak temperature (Table 1) was also obtained from the DSC melting curves and denotes the temperature at which the greatest proportion of lipids melt with the maximum thermal effect (da Silva et al. 2013). The melting peak temperature of lard from the S pork back fat was higher than that of the U pork back fat. This could be explained by the higher melting temperature of saturated fatty acids which were more abundant in the S pork back fat (Casutt et al. 1999; Svenstrup et al. 2005).

3.2. pH

Statistical analysis of the pH values revealed no three-way interaction ($p = 0.904$), no significant two-way interactions for temperature $\times$ meat type ($p = 0.804$), temperature $\times$ fat type ($p = 0.802$) or fat type $\times$ meat type ($p = 0.804$), but an overall effect of meat type ($p < 0.001$) and fat type ($p < 0.001$) but not for temperature ($p = 0.373$).

The pH values of cooked sausages prepared with chicken leg were significantly higher than of those prepared with chicken breast meat irrespective of the type of fat or the temperature (Table 2). The pH value of chicken leg meat (6.18) was higher than that of chicken breast meat (6.07), which probably explains the higher pH of the corresponding cooked sausage samples. Different pH values between chicken breast and leg meat have also been reported by Wattanachant et al (2004) who found that different animal fat types also resulted in significant differences in pH of the final sausages. However, the authors did not offer a clear explanation for this observation.

229

230 3.3. Emulsion stability

231 3.3.1. Total expressible fluid (TEF)

232 Statistical analysis of TEF revealed no three-way interaction ($p = 0.197$), a two-way interaction for
233 temperature $\times$ meat type ($p = 0.011$) and temperature $\times$ fat type ($p < 0.001$) but not for fat type $\times$ meat
234 type ($p = 0.069$), and an overall effect of meat type ($p = 0.001$), fat type ($p < 0.001$) and temperature (p
235 < 0.001).

236 The first of the two-way interactions, temperature $\times$ meat type revealed that, independent of the type of
237 fat, the effect of temperature on TEF depended on the type of meat. However, *post-hoc* tests revealed
238 no significant differences between individual means. Statistical analysis also revealed that the main
239 effect of meat type was significant. Indeed, independent of the type of fat or temperature, TEF was
240 slightly higher for the cooked sausages prepared with chicken leg than those prepared with chicken
241 breast meat, indicating a poorer emulsion stability of the former sausages. Both emulsification and
242 gelation contribute to the stabilization of water and fat in cooked sausages. From the literature, it is clear
243 that the functional properties of myofibrillar proteins such as emulsification (Zorba and Kurt 2006) and
244 gelation (Sun and Holley 2011) differ between meat sources (*e.g.*, chicken *vs.* pork meat) and within
245 meat types (*e.g.*, white *vs.* red muscle fiber types) (Glorieux et al. 2017). Although cooked sausages
246 containing chicken breast meat contained more protein (1%) compared to chicken leg meat, it is likely
247 that the higher emulsion stability of these cooked sausages compared with leg meat can be attributed to
248 the better emulsifying (Sun and Holley 2011) and/or gelation properties of chicken leg proteins
249 compared to chicken breast proteins, especially at 70 °C. At this temperature, gel strength of myofibrillar
250 proteins from chicken breast meat is significantly higher compared to chicken leg meat (Glorieux et al.
251 2017). In contrast with our results, Barbut (1997) concluded that batters prepared with turkey thigh (leg)
252 raw meat exhibited a higher WHC compared with turkey breast meat batters if the meats were compared
253 at their original pH (6.5 for thigh and 5.8 for breast meat) but showed similar values of WHC if the pH
254 of the breast meat was adjusted to 6.4. However, it is unlikely that the pH of the meat types affected

TEF in our study, because the pH of the chicken breast meat was lower than that of the chicken leg meat but the cooked sausages prepared with chicken breast meat still resulted in the lowest TEF (Table 2). Also, [Barbut \(1997\)](#) evaluated the effect of meat type on the WHC of unheated meat batters after 30 min of incubation at 0 °C, while the emulsion stability of cooked meat batters was tested in our study. These different results could also be caused by the different origins of meat (chicken vs. turkey), or the absence of a fat fraction in their meat batters. Additionally, low-fat sausages containing pork meat showed higher TEF compared with duck meat, followed by chicken meat ([Ali et al. 2011](#)), reaffirming the fact that TEF is influenced by the type of meat.

Regarding the second two-way interaction, temperature $\times$ fat type (Table 2, underlined text) it was observed that, irrespective of the type of meat, the effect of temperature on TEF was dependent on the type of fat. TEF significantly increased with increasing temperature for the cooked sausages with U pork back fat but did not change for sausages with S pork back fat. For the same temperature, TEF was always considerably higher for sausages containing S pork back fat than those containing U pork back fat, the effect being more pronounced at 60 °C than at 70 °C. In addition, the main effect of fat type was found to be significant ($p < 0.001$). [Shao et al. \(2011\)](#) also observed significant differences between the TEF of meat batters containing pork fat (1.66% TEF) or dairy butter (2.88% TEF). They stated that meat batters containing added butter resulted in the lowest emulsion stability because the proteins of the butter displaced the protein film around the fat globules. Also, although not mentioned in their study, one could assume that dairy butter was more saturated than pork fat. In that case, this would confirm the results in our study, namely, the higher TEF of meat batters containing the most saturated fat.

It was also clear from the main effects that TEF was significantly affected by temperature. Irrespective of the type of meat or fat, TEF increased with increasing temperature. The lower ability to hold water and/or fat as temperature increased, was probably due to the known phenomenon of more protein denaturation and aggregation, as well as the build-up of interior pressure as the temperature increased ([van der Sman 2013](#)).

3.3.2. Water and fat part of TEF

Statistical analysis of the contributions of water or fat to TEF (%) revealed in both cases no three-way interactions ($p = 0.938$ or 0.927), no significant two-way interactions for temperature $\times$ meat type ($p = 0.285$ or 0.279), temperature $\times$ fat type ($p = 0.278$ or 0.273) and fat type $\times$ meat type ($p = 0.675$ or 0.665), and an overall effect of fat type ($p < 0.001$ in both cases) but not for meat type ($p = 0.481$ or 0.472) or temperature ($p = 0.264$ or 0.271).

Irrespective of the temperature or type of meat, the water part of TEF was always higher from sausages prepared using U pork back fat than from those using S pork back fat, whereas the fat part of TEF was always higher from sausages prepared using S pork back fat than from those using U pork back fat (Table 2). It has been suggested that the fat needs to be partially melted to coat the fat particles properly with an IPF and to prevent the fat from coalescing and exudation (Shao et al. 2011). This IPF already exists in raw meat batters, prior to heating (Zhang et al. 2013) so it is suggested that a thin layer of liquid fat is likely to surround each fat particle, because of tissue disruption and frictional heat during chopping (Jones 1984), thus facilitating the formation of IPF. Also, the adsorption of myofibrillar protein on the surface of the fat globules through hydrophobic binding decreases if the degree of saturation of the fat particles increases (Smith et al. 1983). The S pork back fat in our study contained more saturated fatty acids, resulting in a higher SFC and bulk fat hardness compared with the U pork back fat (Table 1). Although the SFC of lard in emulsified systems is lower than that in bulk lard (Campbell et al. 2002), it is likely that due to the remarkably higher SFC and hardness of lard from the S pork back fat, the fat particles from the S pork back fat were less ‘liquefied’ and coated by myofibrillar proteins than those from the U pork back fat during the comminution process. This probably decreased the ability of the myofibrillar proteins to coat the fat particles from the S pork back fat properly and retain them in the matrix after heating and subsequent centrifugation. This hypothesis presumably explains the higher amount of TEF of meat batters containing S pork back fat. Shao et al (2011) observed significantly higher water and fat losses from meat batters containing dairy butter compared with pork fat. This agreed with our results on fat losses, again supposing that dairy butter is the most saturated fat. No other studies

have been found where the effect of meat type, fat type or temperature on the emulsion stability or the water/fat part of TEF of meat batters was analyzed.

It also needs to be mentioned that the U pork back fat contained more protein (collagen) than the S pork back fat (3.60% vs. 1.91%, respectively). Regarding the meat batter with 35% added pork back fat, this would imply a difference of approximately 0.60%. The presence of some collagenous material may help to improve the structure the product and retain the fat, although meat products are finely chopped (Barbut and Youssef 2016). Collagen may also play a role as a secondary emulsifier (with myosin being the first), due to its partially hydrophobic nature (Bailey and Light 1989). In this respect, earlier studies compared the effect of beef fat with that of rendered beef fat (and thus, the presence or absence of collagen) on the cooking loss and texture of meat batters. Their findings seem to depend on the temperature and amount of protein in the meat batter (Barbut and Youssef 2016; Youssef and Barbut 2010). Taking into account the amount of protein and temperature in our study (7 to 8% protein and 60 or 70 °C heating temperature), Youssef and Barbut (2010) reported no differences in fluid loss and significantly lower fat losses from the meat batters (8% protein and cooked to 72 °C) containing beef fat and rendered beef fat, respectively. In our study, TEF and the fat part of TEF was lower from sausages containing U pork back fat (containing more collagen) than from those containing S pork back fat. So, apart from the physical characteristics of the fat particles, it is possible that the higher amount of collagen in sausages prepared using U pork back fat also contributed slightly to the fat holding capacity.

3.4. Cooking loss

The statistical analysis of cooking loss data revealed no three-way interaction ($p = 0.290$), no significant two-way interactions for temperature $\times$ meat type ($p = 0.637$), temperature $\times$ fat type ($p = 0.271$) or fat type $\times$ meat type ($p = 0.637$), but an overall effect of meat type ($p = 0.009$), fat type ($p < 0.001$) and temperature ($p = 0.023$).

Table 2 shows that irrespective of the type of fat or temperature, the cooking loss (CL) was always higher for cooked sausages prepared with chicken leg than those prepared with chicken breast meat,

which could be due to the lower gelling ability of myofibrillar proteins from chicken leg meat (Glorieux et al. 2017), or the slightly lower protein content (1% lower) compared with sausages containing chicken breast meat. However, the differences were rather small. In contrast, Barbut (1997) evaluated CL after heating meat batters without a fat fraction from 20 °C to 75 °C at 0.5 °C/min and subsequent cooling, finding that the cooking loss of turkey breast meat was significantly higher than that from turkey thigh meat if they were compared at their original pH (6.5 for thigh and 5.8 for breast meat). Again, when the pH of turkey breast meat was adjusted to 6.4, the CL of meat batters containing turkey breast meat was similar to that of turkey thigh meat. However, in our study, it is unlikely that differences in CL were attributable to the different pH values of the meat fractions, because the pH of the chicken breast meat was lower than that of the chicken leg meat but the former resulted in the lowest cooking losses. In a study of Ali et al (2011), low-fat sausages containing pork meat showed higher CL than duck meat, followed by chicken meat, which confirms that CL can be influenced by the type of meat.

Cooked sausages prepared with S pork back fat had a significantly lower CL than those prepared with U pork back fat, irrespective of the type of meat or temperature. This seems to conflict with our findings for TEF (see section 3.1.1), where batters containing S pork back fat resulted in the lowest emulsion stability. However, whereas TEF was measured after 30 min of heating and the subsequent centrifugation of the meat batter, the CL was measured one week after processing. This implies that during cooling and storage, the fat crystallizes and further protein-protein and protein-fat interactions occur, which enhanced the fat stabilization. It is clear from the literature that the effect of fat type (and thus indirectly, the degree of saturation) on CL strongly depends on the amount of protein in the meat batter. Barbut and Youssef (2016) found no significant differences in CL between meat batters (15% protein and 25% fat) containing beef fat, rendered beef fat and hydrogenated palm oil, while Youssef and Barbut (2010) reported significantly lower CL values for meat batters (8% protein and 25% fat) containing hydrogenated palm oil compared with those prepared with beef fat and rendered beef fat. The meat batters in our study contained (depending on the type of meat and/or fat) 7 to 8% protein and 28 to 31% fat. The results of this study thus agreed with those of Youssef and Barbut (2010), *i.e.* a lower CL occurred when using the most saturated fat.

Irrespective of the type of meat or fat, the CL significantly increased with increasing temperature, probably because of greater protein denaturation, protein aggregation and internal pressure build-up. Higher values for the CL of cooked sausages as temperature increased has also been reported by [Barbut and Youssef \(2016\)](#), while [Carballo et al \(1996\)](#) reported no effects of final internal product temperature differences on CL. This could however have been due to the different experimental conditions in their study (internal temperature of 63, 70 or 78 °C and a cooking chamber temperature of 90 °C).

3.5. Textural properties

Statistical analysis of hardness revealed no three-way interaction ($p = 0.535$), no significant two-way interactions for temperature $\times$ meat type ($p = 0.669$), temperature $\times$ fat type ($p = 0.079$) or fat type $\times$ meat type ($p = 0.409$), but an overall effect of meat type ($p < 0.001$) and fat type ($p < 0.001$) but not for temperature ($p = 0.915$).

As shown in Table 2, the hardness of cooked sausages prepared with chicken breast meat was significantly higher than those prepared with chicken leg meat, irrespective of the type of fat or temperature. It is known that gelation of myofibrillar proteins contributes to the textural properties of processed meat products and is affected by the muscle fiber type ([Xiong 1994](#)), and that myofibrillar proteins from chicken leg meat exhibit poorer gelation properties than those from chicken breast meat ([Glorieux et al. 2017](#)). This may explain the significant effect of meat type on the hardness of cooked sausages in our study. Also, the higher amount of protein (1%) of sausages containing breast meat could have enhanced the hardness, compared with sausages containing chicken leg meat. Two other studies have also reported significant differences in the textural properties of meat batters prepared with different types of meat ([Ali et al. 2011](#); [Barbut et al. 1996](#)).

Irrespective of the type of meat or temperature, hardness was significantly higher for cooked sausages prepared with S pork back fat than for those prepared with U pork back fat. Fat particles in sausages act as active fillers because they are covered by an IPF which is connected to the gelled protein matrix. It is known that the rigidity of active filler particles can affect overall gel strength ([Dickinson 2012](#)). Due to

differences in their fatty acid composition, the U and S pork back fats (Table 1) have a different SFC, and hence a different hardness (Table 1). It is plausible that the large difference in hardness between the lard of the U and S pork back fat also influences the rigidity of the filler particles and consequently, the hardness of the cooked sausage. These differences in chemical composition and physicochemical properties presumably explain why cooked sausages containing S pork back fat yielded higher hardness values. In a previous study, the viscoelastic properties of meat batters containing chicken meat and two types of pork fat with a distinct fatty acid profile were studied during heating and cooling. The results indicated a strong increase in gel strength during cooling, probably attributed to fat crystallization, with this effect being more pronounced for the most saturated pork back fat (Glorieux et al. 2018). Measuring the viscoelastic properties is a good indication of the final textural properties such as the hardness of semi-solid foods (Maruyama et al. 2007). Significant differences in hardness have also been reported in a study of Barbut and Youssef (2016), in which the hardest meat batters were obtained with hydrogenated palm oil (melting point 54 °C) compared with beef fat, rendered beef fat, canola oil and palm oil. In another study, Tan et al (2006) reported that the hardness of chicken frankfurters increased when the chicken fat (slip melting point 28.1 °C) was partially or totally replaced with palm stearin (slip melting point 34.7 °C). The fact that the hardest meat batters in our study were obtained with the type of fat having the highest peak temperature (Table 1), agreed with the results of Tan et al (2006).

Although progressive cooking causes more denaturation of meat proteins and the formation of a more aggregated protein network (Barbut and Youssef 2016), the hardness was not affected by temperature, irrespective of the type of meat or fat. However, in the present study, the sausages were cooked to two different core temperatures until an F_0 -value of 40 min had been attained. Practically, this implies a much longer heating time for the sausages cooked at 60 °C ($\pm$ 385 min), compared with 70 °C ($\pm$ 85 min). Since isothermal heating for a prolonged period of time also enhances protein denaturation and aggregation (Glorieux et al. 2017), it is postulated that both phenomena (longer heating time vs. higher temperature) cancelled each other out, resulting in equal hardness values.

3.6. Microstructural properties

The microstructure of the different cooked sausages was observed using electron microscopy. Rapid freezing fixed the microstructure of the cooked sausages and subsequent sublimation removed the water, thereby exposing the protein network. No substantial changes in microstructure between the different types of meat (chicken breast and leg) were observed. Therefore, only the micrographs of cooked sausages containing chicken breast meat are shown (Fig. 1).

In general, the micrographs of cooked sausages (Fig. 1A-D) and meat protein matrices at high magnification (Fig. 1E-F) corresponded well with those in the literature (Barbut 1997; Barbut and Youssef 1996; Youssef and Barbut 2010). Overall, an orderly homogenous structure was observed, with fat particles being evenly dispersed in a compact gelled protein matrix (Fig1A-F), regardless of the fat type. The fat particles embedded in the protein matrix were surrounded by a protein film (IPF), which is clearly seen in a micrograph (Fig. 1G). In meat batters, not all fat particles are of uniform size and shape (Zhang et al. 2013), which could also be clearly observed in this study (Fig. 1A-D). Also, there was clear evidence of fat coalescence in sausages prepared with the S pork back fat (Fig. 1C-D) compared with sausages prepared with the U pork back fat (Fig. 1A-B). This could again be explained by the fact that the fat particles from the U pork back fat were probably more 'liquefied' and coated with myofibrillar proteins than fat particles of the S pork back fat. Youssef and Barbut (2010) observed fat particles with sharp edges when hydrogenated palm oil was used instead of beef fat and ascribed this phenomenon to the higher melting point (54 °C) of the hydrogenated palm oil, which kept it rigid during chopping. This more or less agreed with our results. Also, in oil-in-water emulsions, protruding fat crystals at the interface (which are more present when the degree of saturation increases), could lead to emulsion destabilization and partial coalescence (Douaire et al. 2014). Both phenomena could possibly explain the fat coalescence in sausages containing the S pork back fat compared with the U pork back fat and the higher amount of the fat part of TEF in sausages prepared with the most saturated pork back fat. No other studies have focused on the visualization of the microstructure of sausages prepared with different animal fats, so any comparison with other research is not possible.

It can also be seen from two micrographs (Fig. 1E and F) that the cooking temperature (60 or 70 °C) clearly affected the density of the protein matrix. The number of connections between protein strands increased when the sausages were heated at 60 °C, compared with those heated at 70 °C. The higher protein matrix density at 60 °C was accompanied by a reduction in pore sizes (Fig. 1E-F). A denser protein matrix due to protein aggregation when the temperature increased, has been reported by [Barbut et al. \(1996\)](#). Therefore, a denser protein matrix was expected at 70 °C, compared with 60 °C. However, as already mentioned, the heating time of sausages heated at 60 °C was considerably longer than at 70 °C. Although the temperature was lower, more protein-protein interactions possibly occurred during the long heating process at 60 °C, resulting in more aggregated proteins. Also, [Tornberg \(2005\)](#) has stated that a gel with a good water-holding capacity suggests a fine-stranded gel with higher numbers of junction points per unit volume. This fine-stranded gel at 60 °C in our study probably explains the lower CL compared with that at 70 °C, irrespective of the type of meat or fat used.

4. Conclusions

Significant differences in fatty acid composition between the different pork back fats proved to have a great effect on the microstructural and macroscopic properties of the cooked sausages, in contrast to meat type (and thus, muscle fiber type) or temperature which only had a moderate effect. For sausages prepared with the most saturated pork back fat, the emulsion stability significantly decreased and the greater part of TEF was fat, while the reverse effect was seen for the more unsaturated pork back fat. This was probably related to fat coalescence at the microstructural level. The cooking loss was slightly lower for sausages prepared with the most saturated pork back fat, but the fat type clearly affected the hardness of cooked sausages, irrespective of the type of meat or temperature. Because fat particles act as active filler particles and thereby contribute to the overall gel strength, the presumably higher rigidity of the filler particles of the most saturated pork back fat could probably be attributed to the higher hardness values of the cooked sausages containing this type of fat. These results are extremely relevant for all manufacturers of cooked sausages, since the fatty acid composition of different animal fats, even

originating from the same anatomical location, differs widely. Also, fundamental insights into the exact mechanism of the stabilization of meat batters, and especially the factors of influence, such as the physical properties of the fat, need to be further elucidated, as they can greatly affect the final product structure and texture.

Acknowledgements

This research was funded by a Ph.D. grant from the Agency for Innovation & Entrepreneurship.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

References

- Ali, M.S., Kim, G.D., Seo, H.W., Jung, E.Y., Kim, B.W., Yang, H.S., & Joo, S.T. (2011). Possibility of making low-fat sausages from duck meat with addition of rice flour. *Asian-Australasian Journal of Animal Sciences*, 24, 421-428.
- Bailey, A.J., & Light, N.D. (1989). *Connective tissue in meat and meat products*. New York, U.S.A.: Elsevier Applied Science.
- Barbut, S. (1997). Microstructure of white and dark turkey meat batters as affected by pH. *British Poultry Science*, 38, 175-182.

486 Barbut, S., & Youssef, M.K. (2016). Effect of gradual heating and fat/oil type on fat stability,
 487 texture, color, and microstructure of meat batters. *Journal of Food Science*, 81, 2199-
 488 2205.

489 Barbut, S., Gordon, A., & Smith, A. (1996). Effect of cooking temperature on the
 490 microstructure of meat batters prepared with salt and phosphate. *LWT – Food Science and*
 491 *Technology*, 29, 475–480.

492 Campbell, S.D., Goff, H.D., & Rousseau, D. (2002). Comparison of crystallization properties
 493 of a palm stearin/canola oil blend and lard in bulk and emulsified form. *Food Research*
 494 *International*, 35, 935–944.

495 Campos, R., Narine, S.S., & Marangoni, A.G. (2002). Effect of cooling rate on the structure
 496 and mechanical properties of milk fat and lard. *Food Research International*, 35, 971-
 497 981.

498 Carballo, J., Fernández, P., Barreto, G., Solas, M.T., & Jiménez Colmenero, F. (1996).
 499 Characteristics of High- and Low-Fat Bologna Sausages as Affected by Final Internal
 500 Cooking Temperature and Chilling Storage. *Journal of the Science of Food and*
 501 *Agriculture*, 72, 40-48.

502 Casutt, M.M., Scheeder, M.R.L, Escher, F., Dufey, P-A., & Kreuzer, M. (1999). Relating
 503 texture properties and composition of bovine fat tissue. *Fett/Lipid*, 101, 283-290.

504 Da Silva, R.C., Ribeiro, A.P.B., Soares, F.A.S.M., Capacla, I.R., Hazzan, M., dos Santos, A.O.,
 505 Cardoso, L.P., & Gioielli, L.A. (2013). Microstructure and thermal profile of structured
 506 lipids produced by continuous enzymatic interesterification. *Journal of the American Oil*
 507 *Chemists' Society*, 90, 631–639.

508 Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil
 509 droplets. *Food Hydrocolloids*, 28, 224-241.

510 Douaire, M., di Bari, V., Norton, J.E., Sullo, A., Lillford, P., & Norton, I.T. (2014). Fat
 511 crystallisation at oil–water interfaces. *Advances in Colloid and Interface Science*, 203, 1–
 512 10.

513 Feiner, G. (2006). *Meat products handbook - Practical science and technology*. U.K.:
 514 Woodhead Publishing Limited.

515 Gläser, K.R., Wenk, C., & Scheeder, M.R.L. (2004). Evaluation of pork backfat firmness and
 516 lard consistency using several different physicochemical methods. *Journal of the Science*
 517 *of Food and Agriculture*, 84, 853-862.

518 Glorieux, S., Steen, L., De Brabanter, J., Foubert, I., & Fraeye, I. (2018). Effect of meat type,
 519 animal fat composition and isothermal temperature on the viscoelastic properties of meat
 520 batters. *Journal of Food Science* (accepted). <https://doi.org/10.1111/1750-3841.14182>

521 Glorieux, S., Steen, L., Paelinck, H., Foubert, I., & Fraeye, I. (2017). Isothermal gelation
 522 behavior of myofibrillar proteins from white and red chicken meat at different
 523 temperatures. *Poultry Science*, 96, 3785-3795.

524 Gordon, A., & Barbut, S. (1992). Mechanism of meat batter stabilization: a review. *Critical*
 525 *Reviews in Food Science and Nutrition*, 32, 299-332.

526 Hughes, E., Cofrades, S., & Troy, D.J. (1996). Effects of fat level, oat fibre and carrageenan on
 527 frankfurters formulated with 5, 12 and 30% fat. *Meat Science*, 45, 273-281.

528 Jones, K.W. (1984). Protein Lipid Interactions in Processed Meats. *Reciprocal Meat*
 529 *Conference Proceedings*, 37, 52-57.

530 Marangoni, A.G., Acevedo, N., Maleky, F., Co, E., Peyronel, F., Mazzanti, G., Quinn, B., &
 531 Pink, D. (2012). Structure and functionality of edible fats. *Soft Matter*, 8, 1275-1300.

532 Marrikar, J.M.N., & Yanty, N.A.M. (2014). Effect of chemical and enzymatic modifications on
 533 the identity characteristics of lard: a review. *International Journal of Food Properties*,
 534 17, 321-330.

535 Maruyama, K., Sakashita, T., Hagura, Y., & Suzuki, K. (2007). Relationship between rheology,
 536 particle size and texture of mayonnaise. *Food Science and Technology Research*, 13, 1-
 537 6.

538 Santhi, D., Kalaikannan, A., & Sureshkumar, S. (2017). Factors influencing meat emulsion
 539 properties and product texture: A review. *Critical Reviews in Food Science and Nutrition*,
 540 57, 2021-2027.

541 Shao, J.H., Zou, Y.F., Xu, X.L., Wu, J.Q., & Zhou, G.H. (2011). Evaluation of structural
 542 changes in raw and heated meat batters prepared with different lipids using Raman
 543 spectroscopy. *Food Research International*, 44, 2955-2961.

544 Smith, L.M., Fantozzi, P., & Creveling, R.K. (1983). Study of triglyceride-protein interaction
 545 using a microemulsion-filtration method. *Journal of the American Oil Chemists' Society*,
 546 60, 960- 967.

547 Steen, L., Fraeye, I., Goemaere, O., Sifre, L., Goderis, B., Paelinck, H., & Foubert, I. (2014).
 548 Effect of Salt and Liver/Fat Ratio on Microstructure, Emulsion Stability, Texture and
 549 Sensory Mouth Feel of Liver Paste. *Food and Bioprocess Technology*, 7, 2855–2864.

550 Steen, L., Rigolle, A., Glorieux, S., Paelinck, H., Fraeye, I., Goderis, B., & Foubert, I. (2015).
 551 Isothermal crystallization behavior of lard at different temperatures studied by DSC and
 552 real-time XRD. *Food Research International*, 69, 49-56.

553 Sun, X.D., & Holley, R.A. (2011). Factors Influencing Gel Formation by Myofibrillar Proteins
 554 in Muscle Foods. *Comprehensive Reviews in Food Science and Food Safety*, 10, 33-51.

555 Svenstrup, G., Brüggemann, D., Kristensen, L., Risbo, J., & Skibsted, L.H. (2005). The
 556 influence of pretreatment on pork fat crystallization. *European Journal of Lipid Science*
 557 *and Technology*, 107, 607-615.

558 Tan, S.S., Aminah, A., Zhang, X.G., & Abdul, S.B. (2006). Optimizing palm oil and palm
 559 stearin utilization for sensory and textural properties of chicken frankfurters. *Meat*
 560 *Science*, 72, 387-397.

561 Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality
 562 of meat products. *Meat Science*, 70, 493–508.

563 Van der Sman, R.G.M. (2013). Modeling cooking of chicken meat in industrial tunnel ovens
 564 with the Flory–Rehner theory. *Meat Science*, 95, 940–57.

565 Wattanachant, S., Benjakul, S., & Ledward, D.A. (2004). Composition, color, and texture of
 566 Thai indigenous and broiler chicken muscles. *Poultry Science*, 83, 123-128.

567 Wu, M., Xiong, Y.L., Chen, J., Tang, X., & Zhou, G. (2009). Rheological and microstructural
 568 properties of porcine myofibrillar protein–lipid emulsion composite gels. *Journal of Food*
 569 *Science*, 74, 207-217.

570 Xiong, Y.L. (1994). Myofibrillar protein from different muscle fiber types: implications of
 571 biochemical and functional properties in meat processing. *Critical Reviews in Food*
 572 *Science and Nutrition*, 34, 293-320.

- 573 Youssef, M.K., & Barbut, S. (2010). Physicochemical effects of the lipid phase and protein level
574 on meat emulsion stability, texture and microstructure. *Journal of Food Science*, 75, 108-
575 114.
- 576 Zhang, Y., Wang, Z., Peng, Z., Jin, H., Wang, R.R., & Yao, Y. (2013). Distribution of fat
577 droplets/particles and protein film components in batters of lean and back fat produced
578 under controlled shear conditions, *CyTA - Journal of Food*, 11, 352-358.
- 579 Zorba, O., & Kurt, Ş. (2006). Optimization of emulsion characteristics of beef, chicken and
580 turkey meat mixtures in model system using mixture design. *Meat Science*, 73, 611–618.