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4 Effect of Meat Type, Animal Fatty Acid Composition and Isothermal Temperature on the Viscoelastic
5 Properties of Meat Batters

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ABSTRACT

The aim of this research was to simultaneously study the effect of meat type (chicken breast and leg meat), animal fatty acid composition (selected pork backfats having a low and high degree of saturation, respectively) and isothermal temperature (50, 60, 70 and 80 °C) on the viscoelastic properties of meat batters during and after application of different time-temperature profiles. Gelation of meat proteins contributed most to the viscoelastic properties of meat batters during heating, while crystallization of the lipids especially contributed to the viscoelastic properties during the cooling phase. Although the meat type had little effect on the final viscoelastic properties of the meat product, the fatty acid composition had a clear impact on the melting peak area (and therefore solid fat content) of lard, and subsequently on the final viscoelastic properties of meat batters prepared with different types of fats, with higher G' (elastic modulus) values for the most saturated animal fat. The crystallization of the fat clearly transcended the effect of the meat type with regard to G' at the end of the process. With increasing (isothermal) temperature, G' of meat batters increased. Therefore, it could be concluded that the structural properties of heated meat batters mainly depend on the heating temperature and the fatty acid composition, rather than the meat type.

Keywords

Rheology; Differential scanning calorimetry; chicken breast and leg meat; degree of saturation; isothermal heating

Practical Application

Quality characteristics of cooked sausages depend on multiple factors such as the meat and fat type, non-meat ingredients and processing conditions. From this study it could be concluded that the structural properties of cooked sausage batters mainly depend on the heating temperature and the fatty acid composition, rather than the meat type. Since the fatty acid composition of different animal fats differs widely, these results may be a concern for all manufactures of cooked sausages products with regard to the product structure and final texture, keeping in mind that rendered fat was used in this study, which is not common in sausage making.

1. Introduction

Meat batters can be defined as complex multiphase systems consisting of finely comminuted meat, fat, water, salt (NaCl) and other additives, in which myofibrillar protein-coated fat globules are dispersed in a myofibrillar protein gel matrix (Dickinson, 2012). Meat batters are heated and subsequently cooled, to obtain a gel-type emulsified meat product such as a cooked sausage. In meat batters, the existence of an interfacial protein film around the fat globules has been described, stabilizing the fat phase that becomes liquid during the heating process (Barbut and Youssef, 2016). In addition, during heating, a three dimensional gelled protein network is formed by the myofibrillar proteins from the meat. In this way, fat and water are physically entrapped, thereby hindering the water and fat from separating out from the product (Gordon and Barbut, 1992; Tornberg, 2013). Upon cooling, the fat fraction partially crystallizes. The meat batter is thus transformed from a meat emulsion into a gel-like product exhibiting viscoelastic behavior, whose characteristics depend on its composition and processing conditions. However, the composition of the raw materials (meat and fat) can vary greatly, which is expected to have an impact on the structure and quality of the final product. Meat differs in chemical composition such as the amount of water, proteins, connective tissue and intramuscular fat, as well as physical properties such as color, water-activity, firmness, pH, *etc.* Muscle fibers (75-92% of the total muscle volume) have different biochemical properties, dependent of the muscle fiber type, of which type I and type IIb are most different from each other (Tornberg, 2005; Listrat et al. 2016; Xiong, 1994). The effect of muscle fiber type (among and within species) on the gelation properties of myofibrillar proteins has been described in detail in a review of Sun & Holley (2011). Barbut (1997) studied the microstructure of meat batters containing white and dark turkey meat, while the effect of pork, chicken or duck meat on the quality characteristics (e.g., cooking loss, color and texture) of low-fat sausages has been studied in other study (Ali et al. 2011). Also, the chemical composition of pork fats can vary widely and is dependent on the anatomic location within the animal and influenced by rearing factors such as age, sex, breed, feeding and physiological state. Fats with a different chemical composition and therefore different physicochemical properties, affect the distribution of fat and protein-fat interactions in the product, the crystallization behavior during cooling which is important with regard to structure formation (Svenstrup, Brüggemann, Kristensen, Risbo, & Skibsted, 2005), the textural properties of meat batters (Shao, Zou,

77 Xu, Wu, & Zhou, 2011; Barbut & Youssef, 2016) and final product characteristics (textural, sensorial
78 and visual properties) of cooked sausages (Baer & Dilger, 2014). Furthermore, product characteristics
79 are also affected by the applied thermal processing conditions. Different temperatures can influence the
80 meat protein interactions and their gelation properties (Glorieux, Steen, Paelinck, Foubert, & Fraeye,
81 2017; Liu, Stevenson, & Lanier, 2013) but also the microstructure, color and gel strength of meat
82 batters/cooked sausages (Barbut, Gordon, & Smith, 1996; Barbut & Youssef, 2016; Carballo,
83 Fernández, Barreto, & Solas, 1996). An interesting tool to study structure formation in meat batters is
84 by recording their viscoelastic properties during heating and cooling, as it provides in depth
85 understanding of how the product structure is formed during the different processing steps.
86 Comprehension of the matrix characteristics of the intermediate product (meat batter) aids to control its
87 functionality during cooked sausage manufacturing, and steer the quality characteristics of the final
88 product (Steen et al., 2014). Analysing the scientific literature with regard to viscoelastic properties of
89 meat batters, some studies were found in which the effect of meat (Mittal & Barbut, 1989; Zhang &
90 Barbut, 2007), temperature and/or animal vs. vegetable fat on the viscoelastic properties of meat batters
91 were investigated (Alvarez, Xiong, Castillo, Payne, & Garrido, 2012; Ferris, Sandoval, Barreiro,
92 Sánchez, & Müller, 2009; Liu & Lanier, 2016). Although the effect on the viscoelastic properties of
93 meat batters of each of these factors (meat type, animal fatty acid composition and temperature)
94 independently has been established, these factors have not been studied simultaneous. Therefore, the
95 aim of this study was to evaluate the viscoelastic properties of meat batters prepared with chicken breast
96 or leg meat (containing predominantly white and red muscle fiber types, respectively) and lard from two
97 pork backfats with a distinctly different fatty acid composition during isothermal heating at different
98 temperatures (50-80 °C) for 60 min, subsequent cooling to 7 °C and isothermal holding at 7 °C for 60
99 min via dynamic oscillatory rheology. By simultaneously studying the impact of these three factors
100 (breast vs. leg meat, animal fatty acid composition and temperature), their individual contribution to the
101 viscoelastic properties as well as their interactions could be evaluated. In addition, to further distinguish
102 the contribution of the meat and fat fraction within the meat batters separately, the dynamic viscoelastic
103 properties of chicken breast or leg meat model systems (containing no fat fraction) were also studied, as
104 well as the thermal properties of the two types of lard via differential scanning calorimetry.

2. Material and methods

2.1. Raw materials and lard preparation

In case of the meat, 30 kg fresh chicken breast meat ($\pm$ 120 chickens) and 60 kg fresh chicken leg meat ($\pm$ 240 chickens) were bought from a local cutting plant (Nollens, Kruishoutem, Belgium). Visible fat, connective tissue, skin and bones were removed. Cleaned breast and leg meat was chopped, ground, homogenized to generate 1 large homogeneous batch (approximately 20 kg per type of meat), vacuum-packed and stored at -18°C until further analysis. In case of the fat, 35 kg ($\pm$ 23 pigs) fresh Belgian pork backfat (which generally has a lower degree of saturation) and 54 kg ($\pm$ 14 pigs) fresh Italian pork backfat (generally having a higher degree of saturation) was respectively provided by De Lausnay (Zelee, Belgium) and Spekindustrie Van Maele (Diksmuide, Belgium), trimmed of visible meat and grounded. Lard was rendered from the grounded backfat by heating the backfat for 30 minutes at 120°C (12 kg lard per type of fat). The obtained liquid fraction was filtered and stored at -18°C until further analysis. Due to their different degree of saturation, lard from Belgian pork backfat will be further referred to as ‘unsaturated’ (U) fat. Consequently, lard from Italian pork backfat will be further referred to as ‘saturated’ (S) fat.

2.2. Raw material characterization

Moisture, protein and fat content of breast and leg meat were analyzed according to ISO procedure 1442-1973, 937-1978 and 1443-1973 respectively. pH of breast and leg meat was measured with a Meat Portable pH Meter (HANNA, HI 99163, Netherlands). Moisture, protein, fat and pH were measured in triplicate. Fatty acid composition of lard from the U and S fat (Belgian and Italian pork backfat, respectively) was determined by gas chromatography according to the method of Steen et al. (2015). Only palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) will be presented, as these were the four main fatty acids and accounted for about 90% of the total fatty acids. In addition, the overall degree of saturation was also calculated (based on all fatty acids measured). Fatty acid composition was measured in quadruplicate.

2.3. Meat batter preparation

Meat (chicken breast or leg meat) was comminuted together with salt (1.8%), ice and lard (U or S fat) at 3500 rpm in a Grindomix GM200 cutter (VWR International, Leuven, Belgium) until a final temperature of 12 °C was reached.

Based on the raw materials characterization, meat batters containing chicken breast meat were prepared with 40% meat, 25% ice and 35% lard, whereas meat batters containing chicken leg meat were prepared with 43% meat, 23% ice and 34% lard, in order to achieve a constant amount of water (55%), protein (9%) and fat (36%) for both types of meat batters.

2.4. Lean meat model system preparation

A weighed portion of chicken breast or leg meat was mixed well during 60 s (Ultra-Turrax homogenizer, model T25, IKA-Werke, GmbH, Staufen, Germany) with sodium phosphate buffer (50 mM, pH 6.0, VWR International, Leuven, Belgium) containing 0.71 M or 0.72 M NaCl (Solina, Eke-Nazareth, Belgium) depending of the moisture content of the meat, in order to obtain a lean meat model system containing a final concentration of 4% protein and 0.6 M NaCl (approximately 1:4 w/w, meat/buffer).

2.5. Dynamic oscillatory rheology

Rheological measurements were performed using an AR2000ex stress controlled rheometer (TA instruments, New Castle, USA) equipped with a 40-mm parallel plate geometry. A crosshatched upper and lower plate were used to prevent slippage of the sample. The gap was set at 500 µm (lean meat model systems) or 1000 µm (meat batters). A cap was used to cover the samples to prevent dehydration. The AR2000ex was supplemented with an efficient Peltier temperature control system and upper heated plate to control the sample temperatures accurately.

Meat batters or lean meat model systems were loaded between the parallel plates after the lower and upper plate were equilibrated at the preset isothermal temperature. The time needed to load the sample before the start of the analysis was approximately 45 seconds. The following time-temperature profiles were applied: (1) isothermal heating for 60 minutes at 4 different temperatures (50-60-70-80 °C); (2) cooling to 7 °C at a controlled cooling rate (2 °C/min); (3) isothermal for 60 minutes at 7 °C. Oscillation

measurements were performed at a fixed frequency of 1 Hz and a maximum strain of 0.025. Preliminary strain sweep tests indicated that a strain of 0.025 was within the linear viscoelastic region so that the integrity of gel networks was ensured. Viscoelastic properties of the lean meat model systems and meat batters were described in terms of storage modulus (G' , a measure of elastic property) directly obtained from the software (Rheology Advantage, TA version 5.7). Analysis were performed at least in triplicate. For every analysis, at least three independent preparations of fresh meat batters or lean meat model systems were prepared.

2.6. Differential scanning calorimetry (DSC)

The thermal properties of lard from the U and S fat (Belgian and Italian pork backfat, respectively) were measured using a differential scanning calorimeter Q2000 DSC with refrigerated cooling system and auto sampler (TA Instruments, New Castle, USA). Prior to analysis, the instrument was calibrated with indium. Nitrogen was used to purge the system (50 mL/min). Lard (± 10 mg) samples were hermetically sealed in aluminum pans. An empty hermetically sealed pan identical to the sample pans was used as reference.

From the 4 time-temperature profiles applied to the meat batters (see section 2.5), the two most extreme profiles were selected, namely isothermal heating for 60 min at 50 and 80 °C (lowest and highest heating temperature), cooling to 7 °C and isothermal for 60 min at 7 °C, to elucidate the effect of isothermal temperature on the thermal properties of both types of lard. Since there was no effect of isothermal heating temperature, only one time-temperature profile was selected (50 °C) to further study the effect of animal fatty acid composition on the thermal properties of both types of lard. The following time-temperature profile was applied in the DSC: (1) isothermal heating for 60 minutes at 50 °C (isothermal heating temperature did not affect crystallization characteristics); (2) cooling to 7 °C at a controlled cooling rate (2 °C/min); (3) isothermal for 60 minutes at 7 °C; (4) heating to 80 °C (20 °C/min) to melt all lipids that crystallized during processing. The melting peak area of the resulting DSC profiles was then calculated for both types of lard with the TA Universal Analysis 2000 software (version 4.5A). Analysis was performed in triplicate.

2.7. Statistical analysis

Results are expressed as mean $\pm$ standard error. Statistical analysis was performed using IBM SPSS Statistics 22). A balanced fixed effects design was chosen for this study (D. C. Montgomery, 2012).

Student's t-tests were performed to indicate significant differences in pH, moisture, protein and fat content between chicken breast and leg meat, significant differences in the fatty acid composition between lard from the U and S fat, and to evaluate the effect of animal fatty acid composition on the melting peak area (DSC analysis) of lard from the U and S fat (Belgian and Italian pork backfat, respectively).

For the meat batters, the effect of meat type, animal fatty acid composition (lard) and isothermal temperature on G' at the end of the process (G'_{end}) was evaluated by three-way ANOVA. Two-way interactions that were significant were further interpreted, as well as the significant main effects. At least three independent preparations of fresh meat batters were prepared.

For the lean meat model systems, the effect of isothermal temperature and meat type on G'_{end} was evaluated by two-way ANOVA. There was a significant interaction between the two factors, and therefore, the interaction term was further interpreted via one-way ANOVA. At least three independent preparations of fresh lean meat model systems were prepared.

Analyses were performed on the logarithmic values of G'_{end} for both the meat batters and the lean meat model systems.

Tukey's *post hoc* test was performed and a significance level of $P < 0.05$ was used to identify significant differences for all statistical analyses. Actual p-values are reported throughout the text.

3. Results and discussion

3.1. Raw material characterization

Moisture, protein content, fat content and pH of chicken breast and leg meat are presented in **Table 1**. Similar moisture content for chicken breast and leg meat were found ($P = 0.749$). However, chicken breast meat was lower in fat ($P = 0.008$) and pH ($P < 0.001$), and higher in protein content ($P = 0.018$) compared to chicken leg meat. The results agree with the composition of meat in general (Tornberg, 2005) and are comparable with Wattanachant, Benjakul, and Ledward (2004) who analyzed biceps femoris (leg) and pectoralis (breast) muscle of Thai indigenous chickens.

The fatty acid composition of the two types of lard is shown in **Table 2** and was in line with other studies (Steen et al. 2015; Marrikar & Yanty, 2014; Nur Illiyin, Marikkar, Shuhaimi, Mahiran, & Miskandar, 2013). Variation in the fatty acid composition can be ascribed to differences in rearing factors such as age, sex, breed, feeding and physiological state (Gandamer, 2002).

3.2. Dynamic viscoelastic properties of meat batters

3.2.1. Changes in G' of meat batters during the different time-temperature profiles

The dynamic viscoelastic properties of the meat batters heated at different isothermal temperatures (50, 60, 70 and 80 °C), subsequently cooled to 7 °C and held at that temperature during 60 minutes are presented in **Fig. 1**.

Irrespective of the effect of meat type, animal fatty acid composition and isothermal temperature, G' strongly increased during the isothermal heating step at 50, 60, 70 and 80 °C (approximately ten- to one hundredfold) (**Fig. 1A-D**). This increase in G' is probably due to the gelation of the myofibrillar proteins from the meat (Glorieux et al. 2017; Tornberg, 2005). In addition, connective tissue proteins (collagen) are known to contract at higher temperatures (65-80 °C), thereby forming a denser material (Tornberg, 2005). This could also, to some extent, contribute to the observed increase in gel elasticity, especially at 70 and 80 °C, although the collagen content in poultry meat is rather low (0.75 to 2% of the muscle dry weight) compared to beef and pork meat (Listrat et al. 2016). In contrast, lipids are not expected to contribute to structure formation during heating, as the lipids from the lard are liquefied at temperatures

above 40 °C, as discussed below. Sarcoplasmic proteins in meat could also contribute to gel formation during heating, however, their contribution is rather limited (Tornberg, 2005; Xiong, 1993).

Notably, at temperatures lower than 25 °C, another sharp increase in G' was observed, presumably attributed to the crystallization of the lipids from the lard. No clear changes in G' were observed during the isothermal step at 7 °C for 60 minutes.

3.2.2. Effect of meat type, animal fatty acid composition and isothermal temperature on G'_{end} of meat batters

Table 3 illustrates the effect of meat type, animal fatty acid composition (further called ‘fat type’) and isothermal temperature on G' at the end of the process (G'_{end}). Statistical analyses were performed on the logarithmic values of G'_{end} of meat batters.

Statistical analysis of G'_{end} revealed no three-way interaction ($P = 0.902$) but an overall effect of temperature ($P < 0.001$) and fat type ($P < 0.001$) but not of meat type ($P = 0.658$), and a two-way interaction for temperature $\times$ meat type ($P = 0.002$) and temperature $\times$ fat type ($P = 0.012$) but not for fat type $\times$ meat type ($P = 0.408$).

The first two-way interaction, temperature $\times$ meat type (**Table 3**, italicized text) revealed that, independent of the type of fat, the effect of isothermal temperature on G'_{end} was dependent of the meat type. A general increase in G'_{end} with increasing temperature was observed for both meat types, however, within the same temperature, differences in G'_{end} between chicken breast and leg meat were rather limited (**Table 3**, italicized text). This observation is confirmed by the fact that the main effect of meat type was also not significant. Thus, although the interaction term temperature $\times$ meat was statistically significant ($P = 0.002$), the relevance of this interaction could be questioned.

Meat type has no effect, despite the fact that different muscle fiber types are present in the chicken breast and leg meat. Type I muscle fibers (also called red or slow-twitch muscle fiber types and predominantly found in chicken leg meat) are most different from those of the type IIb muscle fibers (also called white

or fast-twitch muscle fiber types and present in chicken breast meat) (Listrat et al. 2016; Xiong, 1994). Differences in gelation properties between muscle fiber types have been extensively studied and bundled (Sun & Holley, 2011; Lesiow & Xiong, 2001; Xiong, 1994) and it is widely accepted that discrepancies in gelation between white and red muscle fibers are mainly attributed to isoforms of myofibrillar proteins, particularly myosin, and more immediately, to their different protein aggregation patterns during gel formation. In general, at low protein concentrations ($< 2.5\%$) and under similar processing conditions, myofibrillar proteins derived from poultry white muscles exhibit better gelation properties when compared with poultry red muscle myofibrillar proteins (Lesiow & Xiong, 2001). In meat batters however, protein levels are much higher. Meat batters also represent a much more complex system, in which other components such as sarcoplasmic and connective tissue proteins and lipids are present. Other functional properties of myofibrillar proteins such as viscosity, protein solubility and emulsification, are related to the viscoelastic properties and depend on the muscle fiber type, as well as slight differences in pH between chicken breast and leg meat. (Xiong, 1994).

From the second two-way interaction, temperature $\times$ fat type (**Table 3**, underlined text) it was observed that, independent of the meat type, the effect of isothermal temperature on G'_{end} was dependent on the fat type. As lard is completely liquid at high temperatures ($>40\text{ }^{\circ}\text{C}$), the lipids are not assumed to contribute to the elastic properties of the gel network formed during the heating step, which makes this interaction term difficult to explain. It is hypothesized that the increase in G'_{end} is especially codependent of the cooling phase and its important effect on the physical properties (crystallization and therefore increase in viscoelastic properties) of the different types of lard during processing. It was also seen that G'_{end} of meat batters prepared with the S fat type yielded higher G'_{end} within each temperature (except at $70\text{ }^{\circ}\text{C}$). In addition, the main effect of fat type was found to be significant ($P < 0.001$). Fat in meat batters does not form a continuous three-dimensional crystal network but is in contrast discontinuously dispersed in a myofibrillar protein matrix, yet the fat particles can contribute to overall gel strength. This is explained by the fact that fat particles are coated by a protein membrane which interacts with the continuous protein matrix. Therefore, these fat globules act as active filler particles, contributing to overall gel rigidity (Wu, Xiong, Chen, Tang, & Zhou, 2009). Furthermore, the rigidity of the filler

particles can affect overall gel strength (Dickinson, 2012). The S fat in our study contained more saturated fatty acids compared to the U fat (**Table 2**), which has been correlated with a higher solid fat content (SFC) and hardness (Campos, Narine, & Marangoni, 2002; Gläser, Wenk, & Scheder, 2004). Consequently, the higher degree of saturation for the S fat type presumably explains why meat batters prepared with the S fat yielded higher G'_{end} values compared to meat batters prepared with the U fat.

Liu and Lanier (2016), studied the viscoelastic properties (G' , G'' and δ) of meat batters prepared with chicken meat and pork fat or soybean oil, rapidly heated via a two-step process (firstly to 50 °C and held isothermally for 30 min, secondly to 70 °C and held for 20 min) after cooling to 25 °C via frequency sweeps. They observed small, but not significant differences in G' between the meat batters prepared with the different types of fat. This is in contrast with our results and rather unexpected. However, a possible explanation why the effect of fat type (pork fat vs. soybean oil) did not affect G' of the meat batters in their study, is lacking.

It was also clear from the two-way interactions and the main effects that G'_{end} of meat batters was significantly affected by temperature ($P < 0.001$), in which G'_{end} increased with increasing temperature. This was also seen in a former study and is presumably attributed to a more complete denaturation and aggregation of the myofibrillar proteins from the meat, namely myosin, and actin at higher temperatures (70 and 80 °C) (Glorieux et al. 2017). Also, the denaturation of other meat proteins such as sarcoplasmic proteins together with collagen may slightly contribute to the increase in G' (Tornberg, 2005).

Using frequency sweeps, Ferris et al. (2009) measured G' of imitation-mortadella emulsions prepared with chicken meat and pork/chicken skin after isothermal heating in a thermal bath at different temperatures (60, 70 or 80 °C) for different periods of time (50 to 350 seconds) and subsequent cooling in an ice bath. They also concluded that a higher temperature during isothermal heating led to an increase in G' and attributed this to network formation by protein aggregation.

3.3. Dynamic viscoelastic properties of lean meat model systems

Viscoelastic properties of lean meat model systems prepared with chicken breast and leg meat were evaluated to clarify the contribution of the meat fraction to the viscoelastic properties of the meat batters during the same time-temperature profiles (3.3.1), and to verify the effect of temperature on G'_{end} of lean meat model systems (3.3.2.).

3.3.1. Changes in G' of lean meat model systems during the different time-temperature profiles

The viscoelastic properties of lean meat model systems containing chicken breast or leg meat are presented in **Fig. 2**. Complex changes in G' were observed during the isothermal heating step at 50 °C for both lean meat model systems (**Fig. 2A**). The sharp increase in G' during the first 3 minutes of heating, reduction after reaching a maximum and renewed increase in G' were probably due to denaturation of heavy meromyosin (HMM) of myosin with resulting temporarily newly formed bonds, denaturation of light meromyosin (LMM) of myosin with dissociation of the actin-myosin complex and formation of more permanent, irreversible filaments or complexes respectively (Xiong, 1994). During cooling and the isothermal step at 7 °C, G' further decreased, probably due to the stabilization of the formed filaments.

Changes in G' during the isothermal heating processes of lean meat model systems containing chicken breast and leg meat at 60-80 °C were rather similar (**Fig. 2B-D**). G' sharply increased during the isothermal heating step and further increased during the cooling phase, however to a lesser extent, and then slightly decreased during the isothermal step at 7 °C. The increase in G' was probably due to formation of a permanent gel network at these high temperatures (Xiong & Blanchard, 1994).

The same changes in G' were observed in a recent study of Glorieux et al. (2017) in which myofibrillar proteins (2%) from chicken breast and leg meat were isothermally heated. Changes in viscoelastic properties of lean meat model systems (**Fig. 2**), although lower in G' values compared to the meat batter systems because of the lower protein content in lean meat model systems, thus suggest the same events (gelation of meat proteins, especially myofibrillar proteins) with regard to the results of the viscoelastic properties of meat batters as seen in **Fig. 1**. However, the second clear increase in G' upon cooling below

25 °C was not seen in the lean meat model systems, which strengthens the hypothesis that this was due to the crystallization of the lipids from the lard.

3.3.2. Effect of meat type and isothermal temperature on G'_{end} of lean meat model systems

Two-way ANOVA revealed a significant interaction ($P = 0.041$) between meat type and temperature on G'_{end} of the different lean meat model systems. Statistical analyses were performed on the logarithmic values of G'_{end} of lean meat model systems. The logarithmic values of G' and significant differences are indicated in **Table 4**.

Globally, G'_{end} increased when temperature increased. This increase could again probably be attributed to a more complete denaturation and aggregation of the myofibrillar proteins from the meat (Glorieux et al. 2017). It is important to mention that only small differences in G'_{end} between chicken breast and leg meat within the same temperature could be observed in the lean meat model systems (**Table 4**), which was also seen for the meat batters (**Table 3**). Thus, it could be concluded that the meat type does only affect G'_{end} of lean meat model systems to a slight extent, which is similar for the meat batters.

3.4. Thermal properties of lard

Differential scanning calorimetry was applied to evaluate the thermal properties of the two types of lard in order to clarify the contribution of the fat fraction to the viscoelastic properties of meat batters (3.4.1). Next, the effect of animal fatty acid composition on the melting peak areas of both types of lard, was compared (3.4.2.).

3.4.1. Thermal properties of lard during different time-temperature profiles

Lard is expected to be completely liquid above 40 °C (Keller, Lavigne, Loisel, Ollivon, & Bourgaux, 1996). With this underlying thought, the thermal properties of the two types of lard were analyzed using

the time-temperature profiles of the two extreme heating temperatures (50 and 80 °C) applied in this study. If the lipids were liquefied during the 60 min heating phase even at the lowest temperature (50 °C), then the lipids should also be liquefied at 60 or 70 °C.

Fig. 3A shows that, irrespective of the type of lard, no melting peaks were observed during the 60 min at high temperatures (50 °C, which was also true at 80 °C, results not shown), which suggests that the fat was completely liquid during the entire heating phase. One main exothermic peak was seen during the second part of the time-temperature profile (cooling from high temperatures to 7 °C), and could be related to the crystallization of the more saturated lard triglycerides (Santacatalina, Garcia-Perez, Corona, & Benedito, 2011). The S fat started to crystallize earlier ($\pm$ at 76 min) compared to the U fat ($\pm$ at 77 min). The absence of an exothermic peak during the final isothermal step at 7 °C also suggested that the lipids from the lard especially crystallized during the second (cooling) step of the time-temperature process.

3.4.2. Effect of animal fatty acid composition on the melting peak area of lard

Preliminary results showed that isothermal temperature had no effect on the thermal properties of the two types of lard. Therefore, one time-temperature profile was selected (50 °C), and the effect of animal fatty acid composition on the melting peak areas (**Fig. 3B**) of both types of lard was evaluated via a student's t-test.

Statistical analysis revealed that the melting peak area was significantly ($P < 0.001$) affected by the fat type. The melting peak area (J/g) of the S fat was higher (47.17 ± 0.27) compared to the U fat (28.06 ± 0.19). Similar results with regard to different enthalpies in agreement with different fatty acid compositions of pork fats were also seen in other studies (Jiménez-Colmenero et al. 2012; Svenstrup et al. 2005).

The melting peak area (**Fig. 3B**) is a measure of the solid fat content (SFC) and consequently, the amount of crystallized (solid) fat during the applied process. The U fat had a lower degree of saturation and

stearic acid proportion (**Table 2**) so consequently, a lower melting peak area and thus SFC (less crystallized lipids) could be expected.

Macroscopic properties (such as texture and sensory attributes) of fat-containing products are influenced by the crystal habits and SFC, which at their turn depend on the chemical composition of the fat. The proportion of saturated fatty acids and the stearic acid content have proved to be suitable predictors for the SFC and hardness of lard (Campos et al., 2002; Gläser et al., 2004), which could explain the lower G'_{end} values for meat batters prepared with the U fat, independent of the type of meat or temperature used during processing. It also needs to be mentioned that a difference in saturation degree of approximately 27 to 45% between pork backfats has been reported (Davenel et al. 1999; Gläser et al. 2004). So, it is striking that the rather 'small' difference in saturation degree in this study (8%) probably affects the viscoelastic properties of meat batters in terms of fat crystallization to such a great extent.

4. Conclusions

Gelation of meat proteins contributed most to the viscoelastic properties of meat batters during heating, while crystallization of the lipids especially contributed to the viscoelastic properties during the cooling phase. Only small differences in terms of G'_{end} in lean meat model systems or meat batters prepared with chicken breast or leg meat were found, leading to the conclusion that the meat type had little effect on the final viscoelastic properties of the meat product. During heating, the lipids of lard were liquefied but they clearly crystallized upon cooling. A different fatty acid composition showed a clear impact on the melting peak area (and therefore SFC, amount of crystallized solid fat) of lard, and subsequently on G'_{end} of meat batters prepared with different types of animal fats, with higher G'_{end} values for the most saturated animal fat. The crystallization of the fat clearly transcends the effect of the meat type with regard to G'_{end} . Since the fatty acid composition of different animal fats, even originating from the same anatomical location, differs widely, these results may be a concern for all manufactures of cooked sausages products with regard to the product structure and final texture. Viscoelastic properties of meat batters also clearly increased with increasing (isothermal) temperature. In conclusion, it can be

summarized that structural properties of heated meat batters mainly depend on the heating temperature and the fatty acid composition, rather than the meat type.

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Author contributions

S. Glorieux collected test data and drafted the manuscript. L. Steen assisted in data interpretation. J. De Brabanter supported the statistical analysis. I. Foubert coordinated analyses related to lipid composition and DSC. I. Fraeye was project coordinator and supported experimental setup development. All co-authors proofread the manuscript.

Conflict of interest statement and exclusivity of work

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no 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. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We also confirm that neither this manuscript nor one with substantially similar content has been published, accepted for publication, or is being considered for publication elsewhere.

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Table 1: Moisture (%), protein content (%), fat content (%) and pH (-) of chicken breast and leg meat.

Meat	Moisture (%)	Protein (%)	Fat (%)	pH (-)
Breast	75.56 ± 0.05 ^a	21.57 ± 0.26 ^a	1.12 ± 0.08 ^b	5.89 ± 0.02 ^b
Leg	75.75 ± 0.46 ^a	20.24 ± 0.20 ^b	3.90 ± 0.58 ^a	6.45 ± 0.04 ^a

Different letters indicate significant differences at $P < 0.05$ within columns. Means and standard errors are presented (n=3).

Table 2: Fatty acid composition (%) of lard from the U and S fat type

Fat type	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	Degree of saturation (%)
U	22.45 ± 0.03 ^b	11.09 ± 0.02 ^b	41.88 ± 0.03 ^a	16.72 ± 0.03 ^a	35.07 ± 0.04 ^b
S	26.28 ± 0.03 ^a	15.76 ± 0.01 ^a	41.64 ± 0.04 ^b	10.02 ± 0.03 ^b	43.66 ± 0.03 ^a

Different letters indicate significant differences at $P < 0.05$ within columns. Means and standard errors are presented (n=3). U = Unsaturated

(lard from Belgian pork backfat) S = Saturated (lard from Italian pork backfat)

Table 3: Effect of meat type, animal fatty acid composition and isothermal temperature on G'_{end} of meat batters.

T (°C)	Fat type	Log G'_{end} (Pa)		
		Breast meat	Leg meat	T x fat
50	U	4.32 ± 0.05	4.42 ± 0.04	<u>$4.37 \pm 0.04^{\text{F}}$</u>
	S	4.52 ± 0.03	4.68 ± 0.05	<u>$4.59 \pm 0.04^{\text{E}}$</u>
	<i>T x meat</i>	$4.44 \pm 0.05^{\text{d}}$	$4.55 \pm 0.07^{\text{d}}$	
60	U	4.92 ± 0.02	4.92 ± 0.03	<u>$4.92 \pm 0.02^{\text{D}}$</u>
	S	5.07 ± 0.01	5.11 ± 0.05	<u>$5.09 \pm 0.03^{\text{C}}$</u>
	<i>T x meat</i>	$4.99 \pm 0.04^{\text{c}}$	$5.00 \pm 0.05^{\text{bc}}$	
70	U	5.17 ± 0.04	5.13 ± 0.05	<u>$5.15 \pm 0.03^{\text{C}}$</u>
	S	5.33 ± 0.03	5.27 ± 0.05	<u>$5.30 \pm 0.03^{\text{BC}}$</u>
	<i>T x meat</i>	$5.25 \pm 0.05^{\text{ab}}$	$5.20 \pm 0.05^{\text{abc}}$	
80	U	5.25 ± 0.07	5.16 ± 0.04	<u>$5.20 \pm 0.04^{\text{BC}}$</u>
	S	5.55 ± 0.06	5.50 ± 0.01	<u>$5.53 \pm 0.03^{\text{A}}$</u>
	<i>T x meat</i>	$5.43 \pm 0.08^{\text{a}}$	$5.31 \pm 0.08^{\text{a}}$	

Different small letters indicate significant differences at $P < 0.05$ for T x meat (italicized text). Different capital letters indicate significant differences at $P < 0.05$ for T x fat (underlined text). Means and standard errors are presented (n=3). U = Unsaturated (lard from Belgian pork backfat) S = Saturated (lard from Italian pork backfat)

Table 4: Effect of meat type and isothermal temperature on G'_{end} of lean meat model systems.

Log G'_{end} (Pa)		
T (°C)	Breast meat	Leg meat
50	$2.77 \pm 0.08^{\text{de}}$	$2.46 \pm 0.11^{\text{e}}$
60	$3.26 \pm 0.05^{\text{c}}$	$3.24 \pm 0.07^{\text{cd}}$
70	$3.94 \pm 0.07^{\text{b}}$	$3.84 \pm 0.14^{\text{b}}$
80	$4.28 \pm 0.07^{\text{ab}}$	$4.58 \pm 0.06^{\text{a}}$

Different letters indicate significant differences at $P < 0.05$ across means. Means and standard errors are presented (n=3).

Fig 1: Dynamic viscoelastic properties (G' - storage modulus) of meat batters containing chicken breast (grey lines) and leg (black lines) meat and lard from the U (dotted lines) and S (solid lines) fat (Belgian and Italian pork backfat, respectively) during different time-temperature profiles (A=50 °C; B=60 °C; C=70 °C; D=80 °C). Confidence intervals are not included to enhance the readability of the graph.

Fig.2: Dynamic viscoelastic properties (G' - storage modulus) of lean meat model systems containing chicken breast (grey lines) and leg (black lines) meat during different time-temperature profiles (A=50 °C; B=60 °C; C=70 °C; D=80 °C). Confidence intervals are not included to enhance the readability of the graph.

Fig. 3: (A) Thermal properties of lard from the U (Belgian pork backfat, solid line) or S fat (Italian pork backfat, dashed line) as measured by DSC during isothermal heating at 50 °C, cooling to 7 °C and isothermal step at 7 °C. (B) Melting peak area of lard from the U (Belgian pork backfat, solid line) or S fat (Italian pork backfat, dashed line) after heating to 80 °C in order to melt all lipids that crystallized during processing (exo up).