Title

Structure and physical stability of hybrid model systems containing pork meat and superworm (*Zophobas morio* larvae): the influence of heating regime and insect:meat ratio

Authors

Jana Scholliers[a], Liselot Steen[a], Ilse Fraeye[a,*]

Affiliations

jana.scholliers@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

* Corresponding author. Tel: +3293316617 - Fax: +3292658638
E-mail address: Ilse.Fraeye@kuleuven.be

Abstract

Hybrid model systems, containing pork shoulder meat and *Zophobas morio* larvae in different insect:meat ratios, were subjected to isothermal heating at 70 or 80 °C to investigate how this would affect the rheological behavior, water holding properties and textural properties of these model systems. Meat and insect model systems were also studied for comparison. Rheological and textural characteristics were significantly higher in meat model systems compared to insect and hybrid model systems. Furthermore, insect:meat ratio had little to no effect on the studied attributes of the hybrid model systems. It was also demonstrated that heating the hybrid model systems at 80 °C resulted in similar viscoelastic and water holding properties compared to meat model systems heated at 70 °C. However, even when heated at this higher temperature, the maximum force measured during penetration of the hybrid model systems was still approximately 3 times lower compared to meat model systems.

Industrial relevance: This study investigated the effect of insect:meat ratio and heating temperature on structure formation and water holding in hybrid model systems. The results offer important insights with regard to the composition and processing of hybrid meat products. They indicate that similar viscoelastic and water holding properties compared to meat products may be obtained by applying higher heating temperatures. However, results showed that obtaining the desired texture may pose an important challenge when developing hybrid meat products.

Keywords

Edible insect; Hybrid meat products; Isothermal heating; Viscoelastic properties; Water holding; Texture

1 Introduction

While the global demand for high-quality protein continues to increase, the available agricultural land for meat production remains limited. When also taking into account the strain the meat producing industry puts on the environment, it is clear that measures need to be taken. The demand could be met in a more environmentally friendly way, by increasing the sustainability of conventional protein source production and simultaneously introducing alternative protein sources (Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017). In this regard, insects have emerged as an interesting alternative protein source, due to their nutritional, ecological and even potential economic advantages (Patel, Suleria, & Rauf, 2019).

Insects are already a part of the diet and are even considered delicacies in many countries (van Huis, 2013). In the Western world, however, consumers are reluctant to eat insects, since they often consider insects to be unsafe and disgusting (Looy, Dunkel, & Wood, 2013). Research suggests that this poor consumer acceptance may be improved by processing insects in an unrecognizable form into familiar food products (Caparros Megido, et al., 2016; Gmuer, Nuessli Guth, Hartmann, & Siegrist, 2016; Hartmann, Shi, Giusto, & Siegrist, 2015). In this respect, insects could be incorporated into hybrid meat products, in which a fraction of the meat is replaced by insects. Neville, Tarrega, Hewson, and Foster (2017) already suggested that, in general, hybrid meat products may be used to persuade predominantly meat eaters to reduce their meat consumption.

Since sensory attributes similar to those of meat are expected by meat eaters in meat substitutes (Hoek, Luning, Weijzen, Engels, Kok, & de Graaf, 2011), hybrid meat products should possess meat-like properties. In addition to taste, important technological and quality characteristics of meat products include water holding and texture, which are largely determined by the meat proteins. Structure formation of these meat proteins in meat products is already well-understood. They denature and aggregate upon heating, forming a protein network which holds the water, mainly through capillary forces. This protein gelation plays an important role in the final structure of meat products (Tornberg, 2005). Regarding insects, many research articles focus on gelling properties of insect flour (Adebowale, Adebowale, & Oguntokun, 2005; Mishyna, Martinez, Chen, Davidovich-Pinhas, & Benjamin, 2019;

Omotoso, 2006, 2018; Osasona & Olaofe, 2010; Torruco-Uco, Hernández-Santos, Herman-Lara, Martínez-Sánchez, Juárez-Barrientos, & Rodríguez-Miranda, 2018) or extracted insect proteins (Lee, Kim, Ji, & Lee, 2019; Purschke, Tanzmeister, Meinlschmidt, Baumgartner, Lauter, & Jager, 2018; Yi, Lakemond, Sagis, Eisner-Schadler, van Huis, & van Boekel, 2013; Zhao, Vazquez-Gutierrez, Johansson, Landberg, & Langton, 2016). However, knowledge regarding structure formation in hybrid meat products, containing both meat and insect, is limited. Some technological and quality characteristics (including texture and cooking loss) of hybrid meat products have been studied by Kim, Setyabrata, Lee, Jones, and Kim (2017), Kim, Setyabrata, Lee, Jones, and Kim (2016), Park, et al. (2017) and Choi, et al. (2017). However, they did not provide insight into the structure formation during heating. In addition, a dry, pretreated insect flour was added in these studies, which has implications for the insect protein functionality and the composition, for example the moisture content, of the hybrid meat products.

During heating, the applied temperature has a considerable impact on the rheological behavior of meat products, as shown by Glorieux, Steen, De Brabanter, Foubert, and Fraeye (2018) and Ferris, Sandoval, Barreiro, Sánchez, and Müller (2009). Furthermore, Scholliers, Steen, Glorieux, Van de Walle, Dewettinck, and Fraeye (2019) established a similar impact of temperature for insect model systems. This study also showed that the insects had inferior structure forming capacities compared to meat and that higher heating temperatures needed to be applied to the insect model systems in order to approach the viscoelastic properties of meat model systems. These studies all applied isothermal heating to insect or meat model systems to study the effect of temperature. This facilitates an easier allocation of a certain rheological behavior to the applied isothermal heating temperatures compared to non-isothermal heating. Furthermore, by continuing the isothermal heating sufficiently long, insights can be obtained into all changes in the viscoelasticity that can occur at a certain temperature (Glorieux, Steen, Paelinck, Foubert, & Fraeye, 2017). Even though the effect of temperature on either meat model systems or insect model systems has been studied, none of the previously mentioned studies regarding hybrid meat products investigated the effect of temperature on hybrid model systems or hybrid meat products.

The aim of the current study was therefore to gain insight into how combining a meat fraction and an insect fraction, in different insect:meat ratios, affects the structure formation and water holding in hybrid model systems. Since previous research (Scholliers, et al., 2019) showed that, out of 3 studied insect larvae, the *Zophobas morio* larvae exhibited the best structure forming capacities, these larvae were used in the current study. This research also showed that higher heating temperatures needed to be applied to insect model systems in order to approach the viscoelastic properties of meat model systems. Therefore, isothermal heating temperatures of both 70 °C and 80 °C were applied in the current study. The rheological behavior of the hybrid model systems, during and after isothermal heating, was studied and compared to that of insect and meat model systems. Furthermore, the water holding and textural properties of these model systems were characterized.

2 Materials and methods

2.1 Materials

A large batch of living *Z. morio* larvae was obtained from Nusect, Deerlijk, Belgium. They were reared on cornmeal, apples and carrots. After sieving and vacuum packing, the larvae were stored at -18 °C until further analysis. A large batch of pork shoulder was purchased from a local supplier (Norbert Impens, Melle, Belgium). After removing the visible connective tissue and the fat, cutting the meat into small dices and vacuum packing, the pork shoulder meat was also stored in the freezer at -18 °C.

2.2 Proximate composition

Moisture, fat and nitrogen contents of the *Z. morio* larvae and the pork shoulder meat were determined (at least in triplicate) to characterize the composition of these raw materials. For moisture content determination, ground larvae and meat were dried overnight at 103 °C. Soxhlet fat extraction (during at least 6h, using n-hexane as solvent), followed by n-hexane evaporation using a rotary evaporator (Laborota 4000 efficient, Heidolph instruments, Schwabach, Germany) and successive drying at 103 °C, was performed to gravimetrically determine the fat content. Finally, the Kjeldahl method (Digest System K-437 and Distillation Unit K-350, Büchi, Flawil, Switzerland) was used to determine the nitrogen content of the raw materials.

2.3 Model system preparation

During model system preparation, frozen *Z. morio* larvae (ZM) and/or frozen pork shoulder meat (PS) and sodium phosphate buffer (0.3 M, pH 6) were brought together in the grinding container. For each model system, the phosphate buffer contained the amount of NaCl needed to obtain a 0.6 M NaCl concentration in the respective model system. Different insect:meat ratios were applied, to ensure that the insect- and meat-derived nitrogen contents shown in Table 1 were obtained, with a total nitrogen content of 1.6 % in all model systems. Using a conversion factor of 6.25, this corresponds to a protein content of 10 %. As seen in Table 1, 3 different hybrid model systems (75ZM:25PS, 50ZM:50PS and 25ZM:75PS) were prepared, as well as an insect model system (100ZM) and a meat model system (100PS). The grinding container, containing the frozen larvae, frozen meat and buffer, was kept at 3 °C for 2 hours before grinding for defrosting and temperature equilibration. Grinding was performed at 3,500 rpm using a Grindomix GM200 **(**Retsch, Haan, Germany**)** until a final temperature of 12-14 °C was obtained in the model systems. The model systems were then vacuum packed and kept at 15 °C for 1.5 h. Subsequently, they were analyzed through dynamic oscillatory rheology (section 2.4). In addition, model systems were transferred to centrifuge tubes and heated, as described in sections 2.5 and 2.6 for analysis of water holding capacity (WHC) and textural properties, respectively.

2.4 Dynamic viscoelastic properties

The rheological behavior of the hybrid, insect and meat model systems was studied according to Scholliers, et al. (2019). An AR 2000ex stress-controlled rheometer (TA instruments, New Castle, USA), equipped with an upper-heated plate and a Peltier temperature control system to accurately control the temperature of the samples, was employed. Crosshatched parallel plates with a diameter of 40 mm (1,000 µm gap) were used and the samples were covered with a cap to prevent dehydration. The viscoelastic properties were characterized through the storage modulus (G’), the loss modulus (G”), the complex modulus (G*) and the phase angle (δ) and the measurements were carried out at least in triplicate (n≥3). For each measurement, a model system was freshly prepared as described in section 2.3.

2.4.1 Temperature sweep

Following the loading and trimming of the samples, they were first subjected to a temperature sweep during which the samples were isothermally heated for 1 h at 70 °C or 80 °C (phase 1), cooled to 20 °C at 2 °C/min (phase 2) and kept at 20 °C for 1 h (phase 3). Throughout this temperature sweep, a constant frequency (1 Hz) and strain (0.025), within the linear viscoelastic region (LVR), were applied. G' values at the end of phase 1 ($G'_{\text{end heating}}$) and at the end of phase 3 ($G'_{\text{end temperature sweep}}$) were used for further statistical analysis (see section 2.7).

2.4.2 Frequency sweep

Subsequent to the temperature sweep, the frequency dependence of the structures formed after heating, cooling and holding the samples at 20 °C was studied through a frequency sweep. The frequency was thereby increased from 0.1 to 10 Hz at a constant temperature of 20 °C and an oscillatory stress within the LVR (15 measuring points per decade).

2.4.3 Stress sweep

Finally, the frequency sweep was followed by a stress sweep to determine the length of the LVR, which was used to characterize the structure stability. At a constant temperature (20 °C) and frequency (1 Hz), the oscillatory stress was increased from 1 to 15,000 Pa (70 measuring points per decade). The stress at which the corresponding G* deviated more than 5 % from the average G* of the previous 5 measuring points was then calculated and used to determine the length of the LVR.

2.5 Water holding properties

As water holding capacity is an important technological characteristic, the effect of temperature and insect:meat ratio on the WHC of the model systems was also determined. The method for WHC determination was based on the procedure of Hughes, Cofrades, and Troy (1997) and was performed at least in quadruplicate, each time on a fresh model system (prepared as described in section 2.3). Approximately 40 g of raw model system was transferred to a centrifuge tube (height=106 mm, diameter=38 mm and sample height=± 50 mm) (VWR International, Leuven, Belgium) and subsequently heated in a cooking chamber (Rational Climaplus Combi CPC 61, Claes Machines, Paal, Belgium). The model systems were first heated at a chamber temperature of 74 °C or 84 °C for 30 minutes, after which a sample core temperature of 70 °C or 80 °C, respectively, was reached. Then, the chamber temperature was decreased to 70 °C or 80 °C, respectively, at which the model systems were heated for another hour. For each of the (at least) 4 model system preparations, 2 samples were analyzed for WHC immediately after heating (warm WHC), while 2 samples were first stored at 3 °C for 1 week before analysis (cold WHC). WHC was determined by first removing and weighing the cooking loss from the centrifuge tubes. The samples were then centrifuged for 15 minutes at 4,146 g (Hettich Universal 320R, Newport Pagnell, UK). Similar to the cooking loss, the drip loss (supernatant after centrifuging) was removed from the centrifuge tubes and weighed. The percentages of cooking loss (CL) and Total Expressible Fluid (TEF) were determined as follows:

$$CL\ (\%) = (mass\ of\ cooking\ loss\ /\ initial\ sample\ mass) * 100 \qquad (1)$$

$$TEF\ (\%) = (mass\ of\ drip\ loss\ /\ initial\ sample\ mass) * 100 \qquad (2)$$

For both warm WHC and cold WHC, the results for CL and total drip loss (CL+TEF) for the 2 samples per model system preparation were averaged for each of the (at least) 4 model system preparations ($n \geq 4$).

2.6 Textural properties

Textural properties of the model systems were also analyzed, at least in quadruplicate, each time on a freshly prepared model system. Samples were prepared as is described in section 2.5, by transferring 40 g of raw model system to a centrifuge tube (height=106 mm, diameter=38 mm and sample height=± 50 mm). The samples were then heated to a core temperature of 70 °C or 80 °C (30 min at a chamber temperature of 74 or 84 °C), kept at that core temperature for 1 h (chamber temperature of 70 or 80 °C) and, finally, stored at 3 °C for 1 week. After cooled storage, a puncture test was performed, using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments), to assess the textural properties. The samples were axially penetrated for 1.5 cm (corresponds to approximately 30 % of sample height) using a cylindrical probe (diameter 12 mm). The probe was connected to a 100 N load cell and penetrated the sample at a speed of 100 mm/min. During penetration of the sample, the force-distance curve was recorded and from that curve the maximum force (F_{max}) was obtained (based on Glorieux, Steen, Van de Walle, Dewettinck, Foubert, and Fraeye (2019)). This analysis was performed in triplicate per model system preparation. These values were averaged to obtain a single result for each of the (at least) 4 model system preparations (n≥4).

2.7 Statistical analysis

Results are expressed as mean ± standard error (n≥3 or n≥4, as described above). The effect of isothermal heating temperature and insect:meat ratio was statistically analyzed through two-way ANOVA using IBM SPSS 25. For the rheological properties the logarithmic values were used. The two-way ANOVA showed significant interactions for all analyzed attributes, which were further interpreted and Tukey's *post hoc* tests were performed. A significance level of $p < 0.05$ was employed.

3 Results and discussion

3.1 Proximate composition

In Table 2, the proximate compositions, on a fresh weight basis, of the pork shoulder meat and *Z. morio* larvae are shown. While both had similar nitrogen contents, their moisture and fat contents differed considerably. Pork shoulder meat showed a high moisture content, but contained only a small percentage of fat. *Z. morio* larvae, on the other hand, contained more fat, which was compensated through a lower moisture content. Yi, et al. (2013) found a comparable composition for *Z. morio* larvae. However, the composition of the insect larvae found in the current study differs slightly from the ones found in the studies of Soares Araújo, dos Santos Benfica, Ferraz, and Moreira Santos (2019) and Scholliers, et al. (2019), which both found higher moisture contents (± 60-65 %) and lower nitrogen contents (± 2.65-2.92 %). The composition of insects can be affected by their diets and environment (van Huis, 2016), which may have caused these slight differences. The moisture, fat and nitrogen contents of the pork shoulder meat found in the current study are in accordance with the average composition of meat (Huff-Lonergan, 2010).

3.2 Dynamic viscoelastic properties

3.2.1 Temperature sweep

The course of G' and δ throughout the temperature sweep is shown in Fig. 1 for all model systems at both heating temperatures. In all cases, δ did not undergo any remarkable changes during the temperature sweep and remained well below 45°, indicating that the model systems were more elastic than viscous.

Regarding G’, a sharp increase at the beginning of heating (phase 1) can be seen for all model systems, indicating a rapid development of more elastic characteristics due to structure formation upon heating. It is remarkable that, during the remainder of phase 1, G’ slowly declined in all model systems containing insect (100ZM, 75ZM:25PS, 50ZM:50PS and 25ZM:75PS) heated at 70 °C, regardless of insect:meat ratio. In the meat model systems (100PS) heated at 70 °C and all model systems heated at 80 °C, however, G’ continued to increase. This might indicate that, when heated at a lower temperature (70 °C), the presence of insect biomass results in the formation of weaker interactions which dissociate upon further heating. During cooling (phase 2), G’ increased in all model systems, while it decreased slightly during phase 3. The results are in accordance with a previous study regarding the effect of isothermal heating temperature on structure formation in insect model systems (Scholliers, et al., 2019). There, a decline of G’, following the initial sharp increase, in *Z. morio* model systems during isothermal heating at 70 °C was also observed. Furthermore, similar to the current study, a rise in G’ during cooling was detected, which was attributed to the formation of hydrogen bonds, resulting in additional structure formation. An increasing G’ during both heating and cooling was also observed under non-isothermal conditions for insect powder by Mishyna, et al. (2019) and for extracted insect proteins by Yi, et al. (2013) and Zhao, et al. (2016) (under similar conditions of concentration and pH as Yi, et al. (2013)). Regarding meat model systems, Glorieux, et al. (2018) also observed a rise in G’ during isothermal heating and cooling and a slight decrease during a second isothermal step (7 °C) in lean meat model systems containing chicken meat. To the best of our knowledge, no rheological studies have been performed on hybrid model systems, containing both insect and meat.

Through statistical analysis of G’ at the end of phase 1 ($G'_{\text{end heating}}$) and at the end of phase 3 ($G'_{\text{end temperature sweep}}$), more insight into the effect of insect:meat ratio and temperature on the viscoelastic properties was obtained, as presented in Fig. 2. For both $G'_{\text{end heating}}$ and $G'_{\text{end temperature sweep}}$ an interaction between the effects of insect:meat ratio and temperature ($p < .05$) was obtained. Increasing the isothermal heating temperature from 70 to 80 °C resulted in higher G’ values, both after heating ($G'_{\text{end heating}}$) and after heating, cooling and holding the model systems at 20 °C ($G'_{\text{end temperature sweep}}$), for all insect:meat ratios. This indicates that heating at a higher temperature results in more structure formation in the insect, meat and hybrid model systems. Scholliers, et al. (2019) also found higher G’ values with increasing isothermal heating temperatures for insect model systems, while Glorieux, et al. (2018) and Ferris, et al. (2009) established the same for meat model systems.

Both at the end of heating and at the end of the temperature sweep, highest and lowest G’ values were found for the meat (100PS) and insect (100ZM) model systems respectively. This is in accordance with Scholliers, et al. (2019), who found that the structure forming capacities of insects were inferior to those of meat. Intermediate gel strengths were found for the hybrid model systems (75ZM:25PS, 50ZM:50PS and 25ZM:75PS), both at the end of heating and at the end of the temperature sweep. However, increasing the meat content in the hybrid model systems did not result in significantly higher gel strengths, as no significant differences were found between the G’ values of the different hybrid model systems, neither at 70°C, nor at 80 °C. Furthermore, it is remarkable that heating all hybrid model systems, including 75ZM:25PS where 75 % of nitrogen was insect-derived, at 80 °C resulted in a similar $G'_{\text{end temperature sweep}}$ compared to meat model systems heated at 70 °C. The latter can be considered as a reference for cooked meat products, as many meat products are pasteurized at a core temperature of approximately 70 °C (Feiner, 2006). These results are therefore promising with regard to the production of hybrid meat products, as heating these products at higher temperatures may result in similar viscoelastic properties as those found in common meat products.

3.2.2 Frequency sweep

Immediately following the temperature sweep, the model systems were subjected to a frequency sweep. As shown in Fig. 3, for all insect:meat ratios and at both temperatures G’ > G”, indicating that all model systems had a predominantly elastic character after heating, cooling and holding at 20 °C (Tabilo-Munizaga & Barbosa-Cánovas, 2005). As G’ and G” also showed a slight frequency dependence in the studied frequency range (0.1-10 Hz), the structures formed in the model systems can be characterized as weak gels (Rao, 2007).

Model systems that were heated at 80 °C showed higher G’ and G” values throughout the frequency sweeps compared to those heated at 70 °C for all insect:meat ratios. Furthermore, the meat model systems (100PS) clearly showed the highest G’ and G” values at both temperatures, even though the effect of temperature seems less outspoken compared to the insect and hybrid model systems (100ZM, 75ZM:25PS, 50ZM:50PS and 25ZM:75PS). These results are in accordance with the results from the temperature sweep (see section 3.2.1).

3.2.3 Stress sweep

Subsequent to the frequency sweep, a stress sweep was performed in order to determine the length of the LVR as a measure for structure stability, as more stable structures are able to withstand more stress before breakdown occurs, which translates into a longer LVR (Steen, Fraeye, De Mey, Goemaere, Paelinck, & Foubert, 2014). The length of the LVR was calculated as described in section 2.4.3 and the results are shown in Fig. 4. Similarly to the temperature sweep, statistical analysis showed a significant interaction between the effect of temperature and insect:meat ratio ($p < .05$).

As seen in Fig. 4, increasing the temperature from 70 to 80 °C did not only lead to higher G' values (see section 3.2.1), but also resulted in longer LVRs and, thus, higher structure stability in the insect, meat and hybrid model systems. This effect was also observed for insect model systems in a previous study (Scholliers, et al., 2019).

As for the effect of insect:meat ratio, longest and shortest LVRs at both temperatures were found for the meat (100PS) and insect (100ZM) model systems respectively, which is in accordance with the results from Scholliers, et al. (2019). There, structure stability of meat model systems was also superior to that of insect model systems. At both heating temperatures, the hybrid model systems (75ZM:25PS, 50ZM:50PS and 25ZM:75PS) showed intermediate structure stability, with no significant differences in length of LVR between the different hybrid model systems. This indicates that similar structure stability was obtained in hybrid model systems, regardless of insect and meat content. Similarly to what was observed for the G' values at the end of the temperature sweep, the results in Fig. 4 show that heating any of the hybrid model systems at 80 °C leads to similar LVR lengths as the meat model systems heated at 70 °C, which were considered as a reference for meat products. As mentioned before, these insights may be helpful in the development of hybrid meat products with properties similar to those of meat products.

3.3 Water holding properties

WHC immediately after heating (warm WHC) and after heating and subsequent cooled storage (cold WHC) were characterized by first determining the cooking loss (CL) and then centrifuging the samples to determine the amount of Total Expressible Fluid (TEF). Fig. 5 shows CL (upper graphs) and total drip loss (CL + TEF, lower graphs) for warm WHC (left) and cold WHC (right). Statistical analysis showed a significant interaction between the effect of temperature and insect:meat ratio ($p < .05$) for both CL and CL + TEF of cold and warm WHC.

As seen in Fig. 5A, meat model systems (100 PS) heated at 80 °C showed high cooking losses immediately after heating (warm WHC), while all other model systems showed very low cooking losses in comparison. This indicates that, during heating, model systems containing (only) insect larvae had good WHC. From a technological point of view, this is an interesting observation, since low cooking losses result in economically favorable high yields. After centrifugation (Fig. 5C), however, high TEF values were found for the insect and hybrid model systems (100ZM, 75ZM:25PS, 50ZM:50PS and 25ZM:75PS), resulting in high total drip losses (CL+TEF). Hence, when an external force was applied, these model systems easily released the held water, suggesting that a substantial amount of the remaining water was rather weakly bound during heating.

Cooking loss after heating and cooled storage (Fig. 5B, cold WHC) was absent or negligible in all model systems, except for meat model systems heated at 80 °C, which showed rather high cooking losses. Hence, in case of meat model systems, cooking losses were strongly affected by temperature. While Carballo, Fernández, Barreto, Solas, and Jiménez-Colmenero (1996) and Pietrasik and Li-Chan (2002) found no significant effect of temperature on cooking loss of Bologna sausages and meat gels respectively, Tornberg (2005), Glorieux, et al. (2019) and Barbut and Youssef (2016) found increased cooking losses with increasing heating temperature in meat products, which was in accordance with the results in the current study. Higher cooking losses at higher heating temperatures can be attributed to increased protein denaturation (and subsequent aggregation) and network contraction, as well as internal pressure build-up (Barbut, et al., 2016; Glorieux, et al., 2019), both resulting in water being expelled from the matrix. Furthermore, Kim, et al. (2016), Kim, et al. (2017), Park, et al. (2017) and Choi, et al.

(2017) studied cooking losses of hybrid meat products. While Kim, et al. (2016), Kim, et al. (2017) and Park, et al. (2017) found lower cooking losses in hybrid meat products containing insect flour compared to the control (containing only meat), Choi, et al. (2017) established an increasing cooking loss with increasing insect flour content. However, it must be noted that pretreated, dry insect flours were used in these studies, which has an effect on insect protein functionality and on the composition of the hybrid meat products (e.g. moisture content). As seen in Fig. 5D, the insect and hybrid model systems had much lower total drip losses (CL+TEF) compared to the warm WHC, indicating a considerable improvement in WHC after cooled storage compared to the WHC during heating. During cooling and storage, part of the water that was only weakly bound immediately after heating, was better incorporated into the network and therefore could not be removed through centrifugation. As seen in Fig. 1, a substantial amount of additional structure was formed in all model systems during cooling, which may have aided in better water holding. This effect appears to be of importance mainly in model systems containing insect (insect and hybrid model systems). Furthermore, the insect and hybrid model systems all showed similar CL + TEF values, regardless of insect:meat ratio or heating temperature. In addition, it is observed that there were no significant differences between the total drip loss of these model systems and the total drip loss found for meat model systems heated at 70 °C. These results indicate that, even when higher heating temperatures need to be applied to hybrid meat products, in order to obtain viscoelastic properties similar to meat products (see sections 3.2.1 and 3.2.3), good water holding properties can be retained in hybrid meat products.

3.4 Textural properties

Fig. 6 shows the maximum force (F_{max}) measured during penetration of the insect, hybrid and meat model systems after heating and cooled storage. Statistical analysis of these results revealed a significant interaction between the effect of insect:meat ratio and temperature ($p < .05$).

As seen in Fig. 6, increasing the heating temperature resulted in an increased F_{max} for the insect (100 ZM) and hybrid (75ZM:25PS, 50ZM:50PS and 25ZM:75PS) model systems. This effect of temperature was expected, as higher heating temperatures normally lead to more protein denaturation, resulting in a more aggregated protein network (Barbut, et al., 2016). The results for the insect model systems are in accordance with Lee, et al. (2019), who found a higher gel strength, measured through a puncture test, when the heating temperature was increased from 75 to 95 °C for gels made from extracted insect proteins. However, to the best of our knowledge, there is no research regarding the effect of temperature on the texture of hybrid meat products. Furthermore, a different effect of temperature was observed for the meat model systems (100 PS), as F_{max} of these model systems decreased when the temperature was increased from 70 to 80 °C. This is in contrast with findings of Barbut, et al. (2016), Jiménez-Colmenero, Fernández, Carballo, and Fernández-Martín (1998) and Carballo, et al. (1996). Through Texture Profile Analysis (TPA), they all found increasing hardness for meat batters and meat products heated at higher temperatures. However, non-isothermal heating (implying a gradual increase towards the desired core temperature and subsequent cooling) was applied in the previously mentioned studies. In the current study, samples were immediately heated to the desired core temperature and then kept at that temperature for 1 h (isothermal heating). The latter long heating step at high temperature provides the proteins with more time to denature and aggregate, which will therefore affect structure formation.

The results in Fig. 6 clearly show that, for both temperatures, highest F_{max} was found in meat model systems, while for the insect and hybrid model systems considerably lower F_{max} values were observed. This is in accordance with Choi, et al. (2017), who found lower hardness (measured through TPA) in Frankfurters in which a part of the meat was replaced by insect flour. However, TPA of the hybrid meat products studied by Park, et al. (2017), Kim, et al. (2017) and Kim, et al. (2016) showed higher hardness compared to the control (containing only meat). As mentioned above, however, pretreated, dry insect flours were used in these studies, affecting the insect protein functionality and batter composition. Furthermore, Fig. 6 shows no significant differences between the F_{max} values of the insect model systems and those of the different hybrid model systems. This indicates that, even though a high force is needed to penetrate the meat model systems, adding meat to hybrid model systems does not result in a significantly higher F_{max} compared to insect model systems, not even when 75 % of nitrogen is meat-derived. Furthermore, even though the hybrid model systems heated at 80 °C showed similar G’ values compared to meat model systems heated at 70 °C (see section 3.2.1), this did not translate into a similar F_{max}. These results therefore indicate that, although heating the hybrid model systems at higher temperatures showed promising results regarding the viscoelastic and water holding properties, this may not be sufficient to obtain the desired texture in hybrid meat products.

4 Conclusions

In order to gain insight into how the insect and meat fractions may interact in hybrid meat products and how this is influenced by different heating temperatures, the effect of insect:meat ratio and heating temperature on the rheological behavior, water holding properties and textural properties of hybrid model systems was studied. For comparison, the same properties were also determined for insect and meat model systems.

The hybrid model systems showed better viscoelastic properties and similar water holding and textural properties compared to the insect model systems. However, overall, meat model systems still showed the best properties, with the exception of WHC when heated at 80 °C.

It was also shown that insect:meat ratio had little to no effect on the studied attributes of the hybrid model systems, since for each temperature similar viscoelastic properties, WHC and F_{max} were obtained in all hybrid model systems, regardless of their insect and meat content. Furthermore, similar viscoelastic properties compared to meat model systems heated at 70 °C could be obtained in the hybrid model systems by heating them at a higher temperature (80 °C). In addition, good water holding properties were retained in these hybrid model systems when the heating temperature was increased. Therefore, these results are very promising with regard to the development of hybrid meat products. However, heating at this higher temperature still resulted in poor textural properties in the hybrid model systems, indicating that obtaining the desired texture in hybrid meat products may pose an important challenge in the future. Therefore, further research into the use of, for example, functional ingredients (e.g. hydrocolloids) to improve the textural properties may be useful.

Acknowledgements

The authors acknowledge the financial support from the Research Council of the KU Leuven, in particular the Internal Funds (grant no. STG 16 006).

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.

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Table 1: Insect- and meat-derived nitrogen contents in the studied model systems.

	Model system	Insect-derived nitrogen content (%)	Meat-derived nitrogen content (%)
Insect model system	100ZM	1.6	0
Hybrid model systems	75ZM:25PS	1.2	0.4
	50ZM:50PS	0.8	0.8
	25ZM:75PS	0.4	1.2
Meat model system	100PS	0	1.6

ZM: *Z. morio* larvae; PS: pork shoulder meat

Table 2: Proximate composition of the raw materials on a fresh weight basis.

Raw material	Moisture content (%)	Fat content (%)	Nitrogen content (%)
Pork shoulder meat	76.50 ± 0.10	2.19 ± 0.11	3.31 ± 0.02
Zophobas morio larvae	57.48 ± 0.02	16.17 ± 0.17	3.30 ± 0.04

Results are shown as mean value ± standard error ($n \geq 3$).

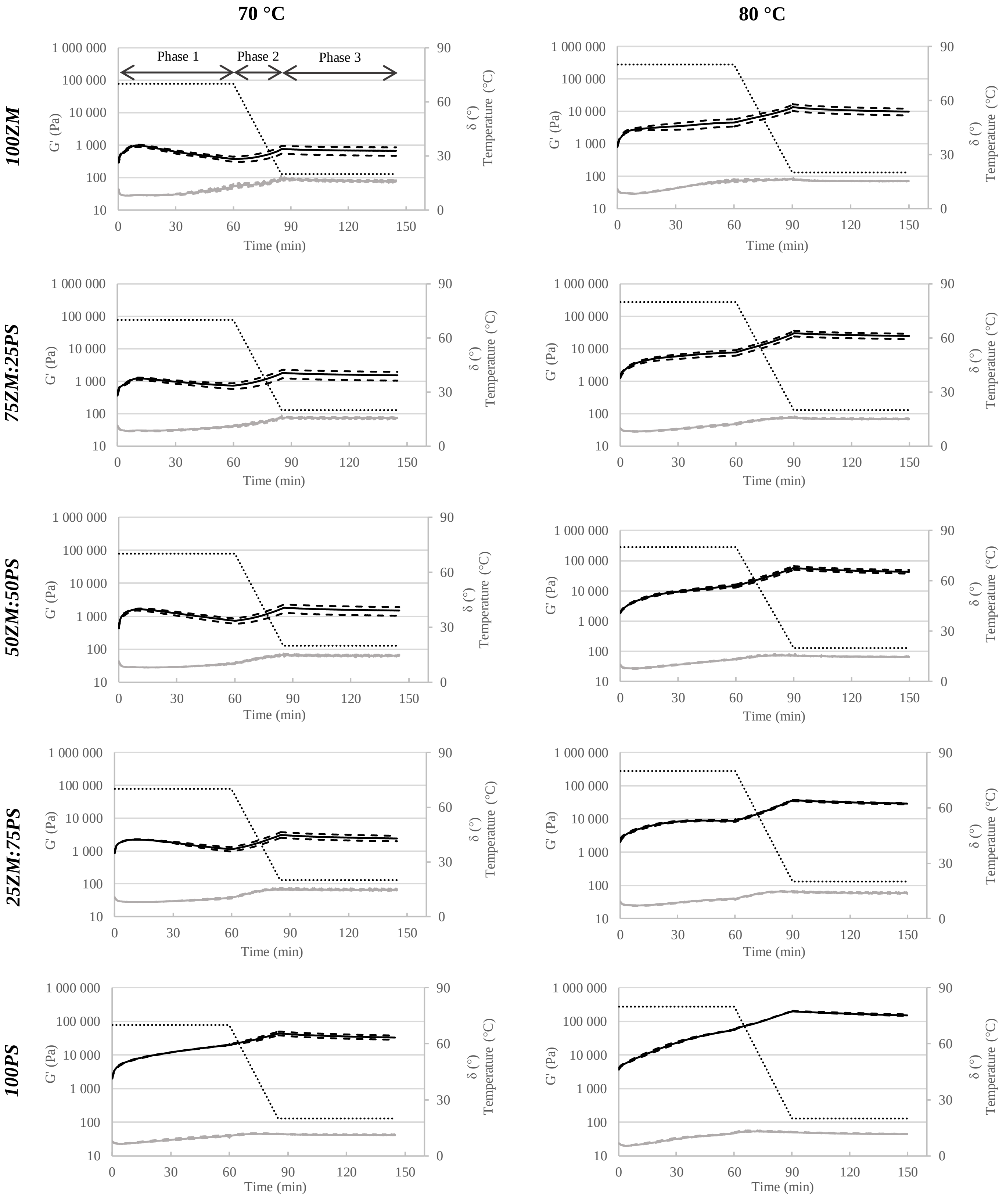

Figure 1: Changes in G' and δ of (hybrid) model systems with different insect:meat ratios during isothermal heating at 70 °C or 80 °C (1 h), cooling to 20 °C (2 °C/min) and holding at 20 °C (1 h) (G', the solid black lines; G' +/- SE, the dashed black lines; δ, the solid grey lines; δ +/- SE the dashed grey lines; Temperature, the dotted black lines) (n≥3). ZM: *Z. morio* larvae; PS: pork shoulder meat.

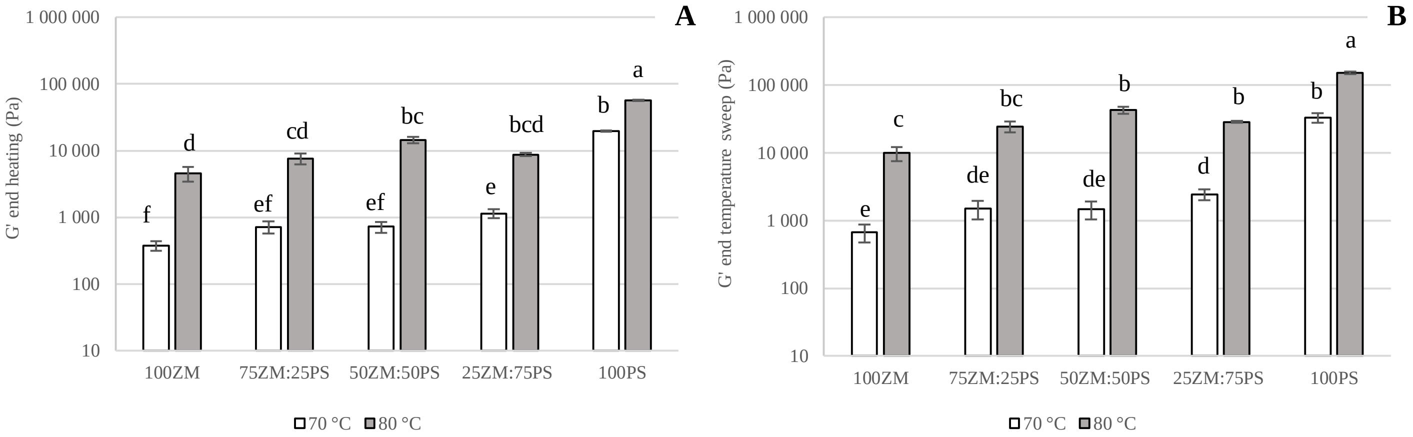

Figure 2: Effect of isothermal heating temperature and insect:meat ratio on (A) G'$_{\text{end heating}}$ and (B) G'$_{\text{end temperature sweep}}$ of insect, hybrid and meat model systems. Different letters indicate significant differences ($p < .05$). Mean values and standard errors are presented (n≥3). ZM: *Z. morio* larvae; PS: pork shoulder meat.

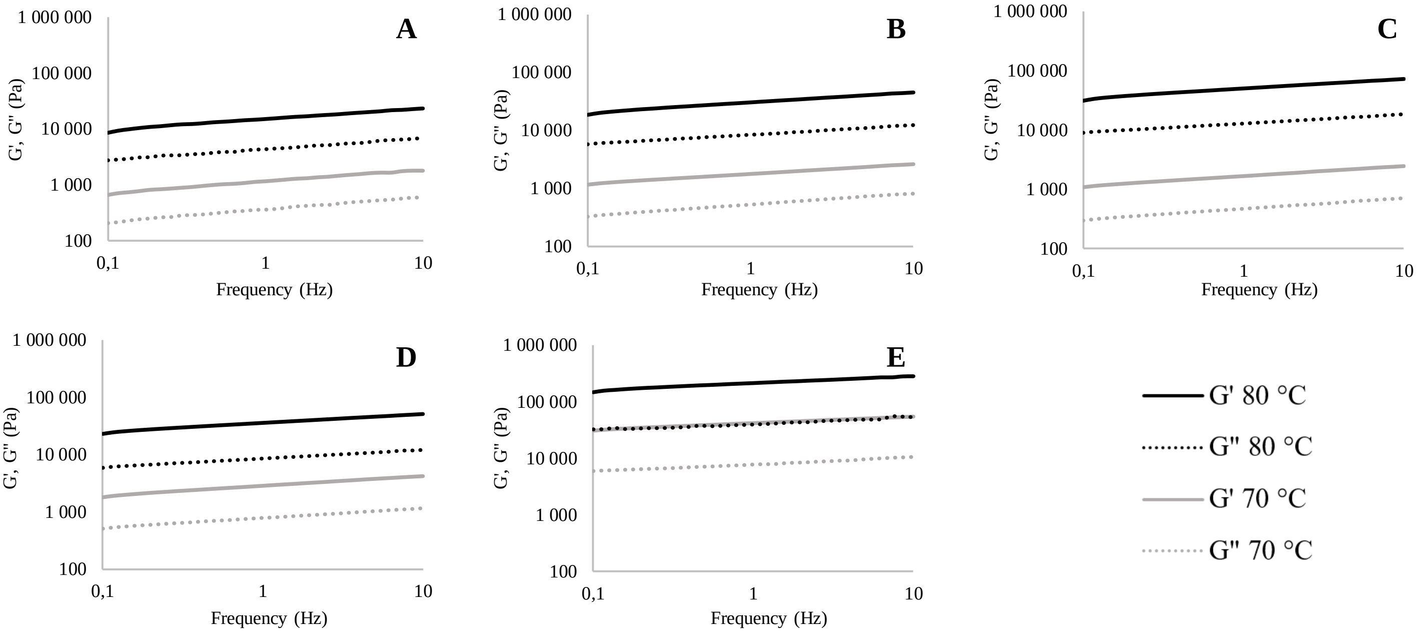

Figure 3: Frequency sweeps of model systems (A) 100 ZM, (B) 75 ZM:25 PS, (C) 50 ZM:50 PS, (D) 25 ZM:75 PS and (E) 100 PS after being heated at 70 or 80 °C, cooled to 20 °C and held at 20 °C. Confidence intervals are not shown to preserve readability of the graph (n≥3). ZM: *Z. morio* larvae; PS: pork shoulder meat.

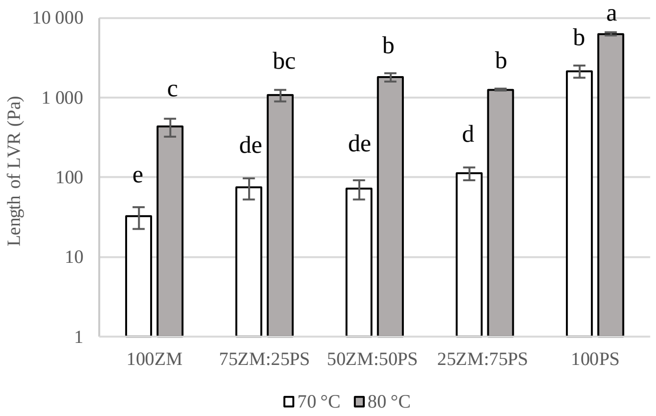

Figure 4: Effect of isothermal heating temperature and insect:meat ratio on the length of LVR of insect, hybrid and meat model systems. Different letters indicate significant differences ($p < .05$). Mean values and standard errors are presented (n≥3). ZM: *Z. morio* larvae; PS: pork shoulder meat.

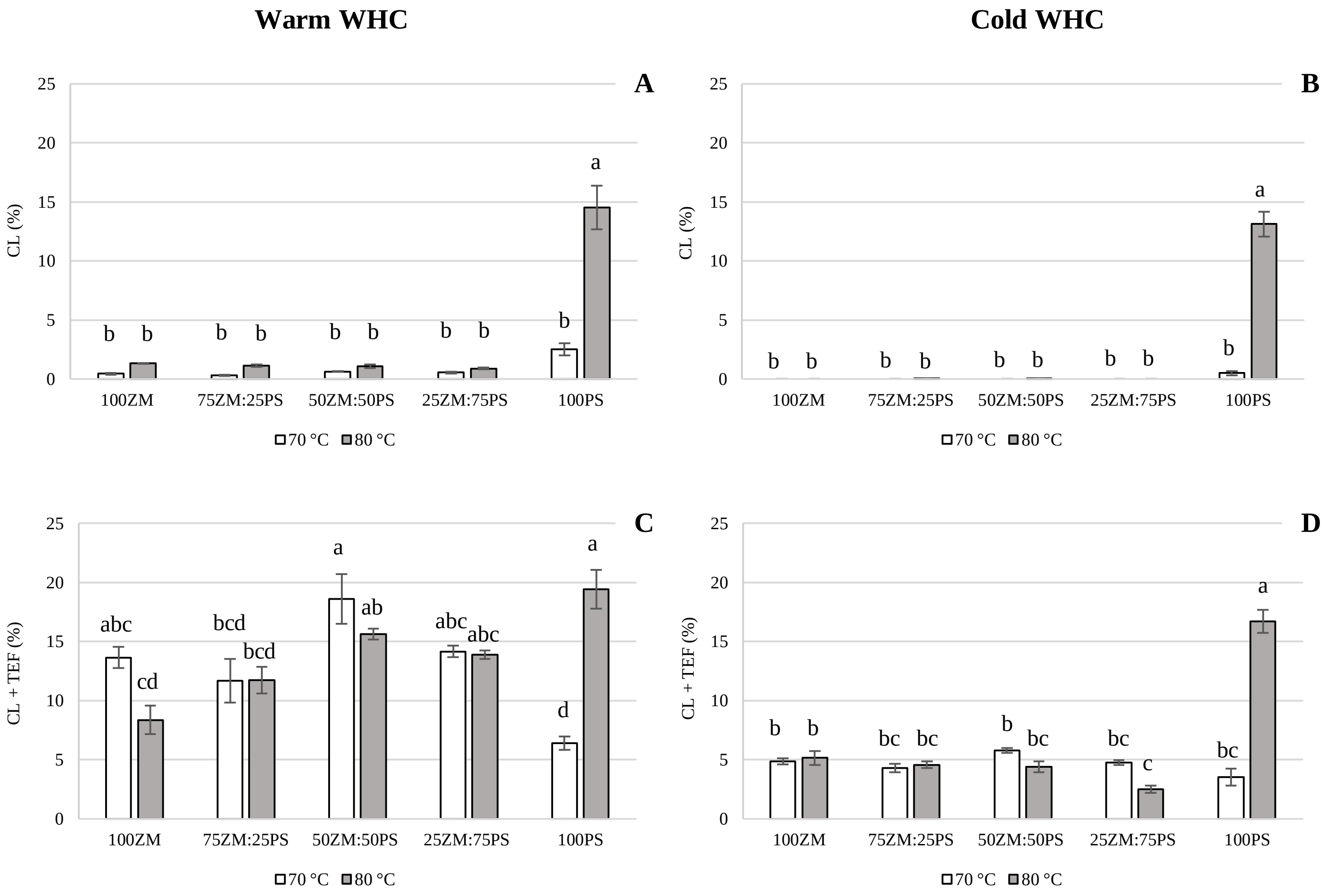

Figure 5: Effect of isothermal heating temperature and insect:meat ratio on warm (left) and cold (right) WHC, with (A) and (B) Cooking Loss (CL) and (C) and (D) total drip loss (CL+TEF) for insect, hybrid and meat model systems. Different letters indicate significant differences (*p* < .05). Mean values and standard errors are presented (n≥4). ZM: *Z. morio* larvae; PS: pork shoulder meat.

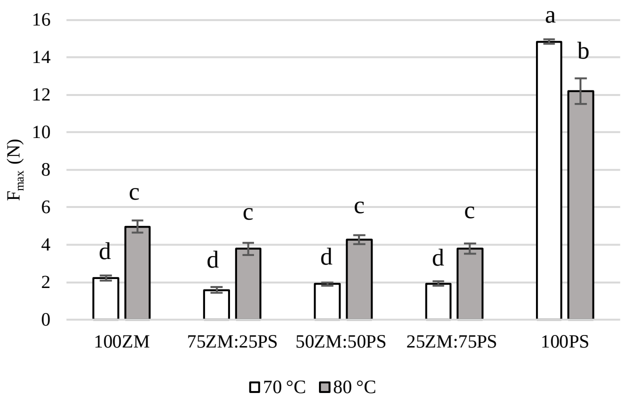

Figure 6: Effect of isothermal heating temperature and insect:meat ratio on the maximum force (F_{max}) measured during penetration of insect, hybrid and meat model systems. Different letters indicate significant differences (*p* < .05). Mean values and standard errors are presented (n≥4). ZM: *Z. morio* larvae; PS: pork shoulder meat.