Title

Partial replacement of meat by superworm (*Zophobas morio* larvae) in cooked sausages: effect of heating temperature and insect:meat ratio on structure and physical stability.

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Abstract

The effect of different heating temperatures and different insect:meat ratios on the structural properties and water and fat stabilization of (hybrid) cooked sausages was studied. Structural properties were substantially reduced upon partial replacement of meat by insect, even when only 5 or 10 % meat was replaced. Only for sausages with low insect contents (5 or 10 % replacement) heated at 90 °C, similar viscoelastic properties compared to a standard cooked sausage could be obtained. However, textural properties remained inferior in all hybrid cooked sausages, even at high heating temperatures. The cooking loss was generally positively affected by the partial replacement of meat by insect, indicating good water and fat stabilization in hybrid cooked sausages during heating and cold storage. However, the hybrid cooked sausages destabilized more easily when subjected to an external force immediately after heating, resulting in an increased fluid release (both water and fat).

Industrial relevance: This study provides important insights with regards to the processing and composition of hybrid cooked sausages. Results showed that even the replacement of only 5 % meat by insect negatively impacted the structure and physical stability (with the exception of cooking loss) of cooked sausages. Even though applying higher temperatures considerably improved some characteristics (e.g. amount of structure formation and structure stability), the hybrid cooked sausages remained inferior to a standard cooked sausage. Therefore, more research is needed to further improve their structural properties and water and fat stabilization.

Keywords

Edible insect; Hybrid meat product; Viscoelastic properties; Textural properties; Cooking loss; Emulsion stability

1 Introduction

As meat is rich in high-quality proteins, as well as in minerals and vitamins, such as iron, zinc and vitamin B12, meat consumption undeniably holds health benefits ([Huang, Wang, Sivendiran, & Bohrer, 2018](#); [McAfee, et al., 2010](#); [Salter, 2018](#)). However, overconsumption of meat may be linked to an increased risk of certain diseases (e.g. colorectal cancer) ([Salter, 2018](#)). Furthermore, livestock production contributes to climate change through greenhouse gas emissions and requires a lot of natural resources ([Grossi, Goglio, Vitali, & Williams, 2019](#)). Meanwhile, the global demand for high-quality protein continues to increase, due to a rapidly growing world population and rising incomes and urbanization in developing countries. Increasing meat production efficiency and partially substituting meat with alternative protein sources will therefore be crucial for ensuring food security in an environmentally-friendly manner ([Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017](#)).

With regard to alternative protein sources, insects may be an interesting option. Compared to livestock production, insect rearing is generally more environmentally friendly, requires less feed and water and occupies less land ([Rumpold & Schlüter, 2013](#); [van Huis & Oonincx, 2017](#)). Furthermore, *Zophobas morio* larvae, for example, also have a favorable nutritional composition. On a fresh weight basis, [Soares Araújo, dos Santos Benfica, Ferraz, and Moreira Santos \(2019\)](#), [Finke \(2002\)](#) and [Barker, Fitzpatrick, and Dierenfeld \(1998\)](#) reported similar crude protein contents (16.6 and 19.7 %) for these larvae compared to meat (16 to 22 %) ([Huff-Lonergan, 2010](#)). *Z. morio* larvae also contain considerable amounts of iron and zinc ([Barker, et al., 1998](#); [Soares Araújo, et al., 2019](#)) and the presence of vitamin B12 in these insects has been established ([Finke, 2002](#)).

An increasing number of studies is being published on the incorporation of insects in different food products, such as bread ([Cappelli, Oliva, Bonaccorsi, Lorini, & Cini, 2020](#); [de Oliveira, Lucas, Cadaval, & Mellado, 2017](#); [Gonzalez, Garvon, & Rosell, 2019](#); [Osimani, et al., 2018](#); [Roncolini, et al., 2019](#)), cake ([Indriani, Bin Ab Karim, Nalinanon, & Karnjanapratum, 2020](#)), pasta ([Duda, Adamczak, Chelminska, Juszkievicz, & Kowalczewski, 2019](#); [Jakab, Tormási, Dhaygude, Mednyánszky, Sipos, & Szedljak, 2020](#)), meat analogues ([Azzollini, Wibisaphira, Lakemond, & Fogliano, 2019](#); [Smetana, et al., 2018](#)) and hybrid meat products, in which a portion of the meat fraction is replaced by insect

([Choi, et al., 2017](#); [Kim, Setyabrata, Lee, Jones, & Kim, 2017](#); [Kim, Setyabrata, Lee, Jones, & Kim, 2016](#); [Park, et al., 2017](#); [Walkowiak, 2019](#)). The unrecognizable incorporation of insects into familiar food products (e.g. hybrid meat products) may be a useful strategy to improve consumer acceptance of edible insects ([Caparros Megido, et al., 2016](#); [Gmuer, Nuessli Guth, Hartmann, & Siegrist, 2016](#); [Hartmann, Shi, Giusto, & Siegrist, 2015](#)).

Conventional cooked sausages, contain meat, fat, water and additives, which are finely comminuted into a homogeneous batter. In these products, the meat proteins are the main functional constituents ([Barbut, 1995](#)). Through the addition of salt, the meat proteins are solubilized during meat batter preparation and construct a protein film around the fat globules. During the subsequent heating step, the proteins in the film and the continuous aqueous phase coagulate, forming a gel matrix in which water and fat are entrapped. This determines crucial product characteristics, such as textural properties and fat and water stabilization ([Gordon & Barbut, 1992](#)). In hybrid meat products, these characteristics are determined by the insect and meat proteins and how they interact with each other.

However, current studies regarding hybrid meat products ([Choi, et al., 2017](#); [Kim, et al., 2017](#); [Kim, et al., 2016](#); [Park, et al., 2017](#); [Walkowiak, 2019](#)) used dry insect flours. This affects both the hybrid meat product composition (e.g. moisture content) and the insect proteins' functional properties, as producing the flours requires a pretreatment of the insects. Therefore, whole *Z. morio* larvae were used in the current study. In addition to their favorable nutritional composition (see above), out of 3 insect larvae, *Z. morio* was shown to possess the best gelling properties ([Scholliers, Steen, Glorieux, Van de Walle, Dewettinck, & Fraeye, 2019](#)). Furthermore, the current studies on hybrid meat products containing insects only investigated the effect of different formulations, but not of processing conditions, such as heating temperature. In our recent study ([Scholliers, Steen, & Fraeye, 2020b](#)), we investigated the effect of different isothermal heating temperatures and insect:meat ratios on hybrid model systems. Results showed that the textural properties of the hybrid model systems were significantly lower compared to meat model systems, even at the lowest insect content studied (insect:meat ratio 25:75) and the highest heating temperature (80 °C). Therefore, the current study included even lower insect:meat ratios (5:95 and 10:90) and higher temperatures (up to 90 °C).

Furthermore, while the previous study focused on model systems (diluted pH-stabilized systems) at isothermal heating conditions, the current study included realistic (hybrid) cooked sausages produced at pilot scale and representative dynamic heating processes (with heating to core temperatures of 70, 80 or 90 °C), in order to create insight into the structure formation and water and fat stabilization.

2 Materials and methods

2.1 Raw materials

Z. morio larvae (ZM) were purchased alive and in one large batch from Nusect (Deerlijk, Belgium). After sieving and vacuum packing, the larvae were stored at -18 °C. A large batch of pork shoulder (PS) and a large batch of pork back fat were obtained from a local supplier (Norbert Impens, Melle, Belgium). Most fat and connective tissue was removed from the meat, after which it was chopped, vacuum packed and stored at -18 °C. The fat was also chopped, vacuum packed and stored at -18 °C.

2.2 Proximate composition

The *Z. morio* larvae and pork shoulder were characterized by determining their moisture, free fat and nitrogen contents (as a measure for protein content), in triplicate, according to ISO procedures 1442-1973, 1444-1996 and 937-1978, respectively.

2.3 Preparation of (hybrid) cooked sausage batter

The (hybrid) cooked sausages were composed as shown in Table 1, with 40 % protein source (pork shoulder, whether or not in combination with *Z. morio* larvae), 32.3 % pork back fat, 25 % ice, 1.8 % nitrite curing salt and 0.9 % herbs and other additives. The insect and meat contents were varied to obtain insect:meat ratios, on a fresh weight basis, of 0:100 (100PS), 5:95 (5ZM:95PS), 10:90 (10ZM:90PS), 25:75 (25ZM:75PS) and 50:50 (50ZM:50PS) in the (hybrid) cooked sausages. The day before preparation, the fat was thawed, ground through a 3 mm plate, vacuum packed and stored overnight at 3 °C. Pork shoulder was thawed overnight at 3 °C. The meat was then minced, under vacuum, in a bowl cutter (Stephan Cutter UM12, Hameln, Germany) with 500 g of ice, the nitrite curing salt and, for preparation of hybrid cooked sausages, the insect larvae. The latter were first pre-minced at 3,500 rpm (Grindomix GM200, Retsch, Haan, Germany) into a homogeneous paste (3 °C)

before addition to the bowl cutter. Mincing was continued (± 3 -5 min) until a lean batter with a temperature of approximately 6 °C was obtained. Subsequently, the fat, the remaining 500 g of ice and the remaining herbs and additives were added. After further mincing (± 4 -6 min) under vacuum, the final batter, with a temperature of 12-14 °C, was obtained.

A part of the batter was vacuum packed, stored at 15 °C for 1.5 h and then subjected to rheological analysis, as described in section 2.4. In addition, immediately after each batter preparation, 3 cans (diameter 70 mm, height 55 mm) were completely filled with batter (approximately 260 g). Cans were used rather than casings to ensure constant product dimensions. The cans were heated (Rational Climaplus Combi CPC 61, Claes Machines, Paal, Belgium) at 74, 84 or 94 °C for 1h10, after which a core temperature of 70, 80 or 90 °C, respectively, was reached. They were then kept at 70, 80 or 90 °C for another 20 minutes. Afterwards, the cooked sausages were rapidly cooled in an ice bath (30 min) and then stored at 3 °C for a week before further analysis (cooking loss and textural properties), as described in sections 2.5 and 2.6. Furthermore, the core temperature of the samples in these cans was monitored during heating and cooling. Based on the measured temperature profiles, the heating and cooling processes could then be simulated during the temperature sweeps (rheological measurement, see section 2.4). Finally, batter was also transferred to centrifuge tubes, immediately after each batter preparation, for analysis of emulsion stability, as described in section 2.7.

In summary, a full experimental setup (5 insect:meat ratios x 3 temperatures = 15 treatments) was conducted. For each treatment, batter preparations were performed at least in triplicate ($n \geq 3$).

2.4 Dynamic viscoelastic properties

The rheological behavior of the (hybrid) cooked sausages was studied according to [Scholliers, et al. \(2019\)](#), with some modifications. Samples were subsequently subjected to a temperature sweep, a frequency sweep and a stress sweep, as described below. Throughout these analyses, the storage modulus (G'), the loss modulus (G''), the complex modulus (G^*) and the phase angle (δ) were measured. Measurements were performed on each of the (at least) in triplicate prepared fresh batters per treatment.

2.4.1 Temperature sweep

After loading and trimming the samples, they were conditioned (15 °C, 2 minutes) to ensure a uniform temperature. The samples were then subjected to a temperature sweep during which the heating and cooling process of the (hybrid) cooked sausages in the cans (see section 2.3) was simulated. Different temperature profiles were implemented for samples heated to 70, 80 and 90 °C, respectively. The temperature was first increased from 15 °C to 55 °C (1.31 °C/min), 65 °C (1.64 °C/min) or 75 °C (1.97 °C/min). It was then further increased to 70, 80 or 90 °C (0.38 °C/min), respectively, and kept at the respective temperature for 20 minutes. Next, the samples were cooled to 30 °C at 1.31, 1.64 or 1.97 °C/min, respectively, after which the temperature was further decreased to 20 °C at 0.38 °C/min and, finally, kept at 20 °C for 30 minutes. Throughout the temperature sweep, a constant strain (0.025, within the linear viscoelastic region (LVR)) and frequency (1 Hz) were maintained. G' values at the end of heating at 70, 80 or 90 °C for 20 min ($G'_{\text{end heating}}$) and after keeping the samples at 20 °C for 30 min ($G'_{\text{end temperature sweep}}$) were used for further statistical analysis (see section 2.8).

2.4.2 Frequency sweep

Immediately following the temperature sweep, the samples were subjected to a frequency sweep. During this frequency sweep, the frequency was increased from 0.1 to 10 Hz (15 measuring points per decade) at a constant oscillatory stress (within the LVR) and temperature (20 °C).

2.4.3 Stress sweep

Subsequent to the frequency sweep, a stress sweep was performed during which the oscillatory stress was increased from 1 to 15,000 Pa (70 measuring points per decade). A constant frequency (1 Hz) and temperature (20 °C) were employed. The length of LVR was calculated as described by [Scholliers, et al. \(2019\)](#).

2.5 Cooking loss

After heating and 1 week of cold storage, the cooking loss (CL) of (hybrid) cooked sausages was studied according to [Glorieux, Steen, Van de Walle, Dewettinck, Foubert, and Fraeye \(2019\)](#). The mass of the samples before cooking and the mass of the separated fat and/or jelly (drip loss) were thereby determined and cooking loss was calculated as follows:

$$CL (\%) = (mass \text{ drip loss} / initial \text{ sample mass}) * 100 \quad (1)$$

The CL was determined in triplicate per preparation. These results were then averaged for each of the (at least) triplicate preparations per treatment.

2.6 Textural properties

Textural properties of (hybrid) cooked sausages, 1 week after processing, were studied according to [Scholliers, et al. \(2020b\)](#). Samples were thereby subjected to a puncture test, using a Lloyd Texture Analyzer (Model LF plus, Lloyd Instruments) equipped with a cylindrical probe (diameter 12 mm), which was connected to a 100 N load cell. A penetration of 1.5 cm ($\pm 30\%$ of sample height) at 100 mm/min was applied. The maximum force (F_{max}) measured during penetration was used to characterize the textural properties. Per preparation, the F_{max} of 3 samples was determined, each time in duplicate. The results of these 6 measurements were then averaged for each of the (at least) triplicate preparations per treatment.

2.7 Emulsion stability

The emulsion stability was studied based on the procedure of [Hughes, Cofrades, and Troy \(1997\)](#). Approximately 40 g of batter was transferred to a centrifuge tube (height=106 mm, diameter=38 mm and sample height= ± 50 mm) (VWR International, Leuven, Belgium) immediately after batter preparation. The samples were heated at 70, 80 or 90 °C for 30 minutes, after which they were immediately centrifuged at 4,226 g for 1 min 30 (Hettich Universal 320R, Newport Pagnell, UK). The total expressible fluid (TEF), the supernatant after centrifuging, was separated and weighed. It was then dried (at 103 °C) overnight and weighed again. The percentage of TEF and the water and fat part of TEF were then calculated as follows:

$$TEF (\%) = (mass \text{ supernatant} / initial \text{ sample mass}) * 100 \quad (2)$$

$$Water \text{ part of TEF} (\%) = ((mass \text{ TEF}_{before \text{ drying}} - mass \text{ TEF}_{after \text{ drying}}) / mass \text{ TEF}_{before \text{ drying}}) * 100 \quad (3)$$

$$Fat \text{ part of TEF} (\%) = 100 - Water \text{ part of TEF} (\%) \quad (4)$$

The emulsion stability was analyzed in duplicate per batter preparation. These results were then averaged to obtain a single value for each of the (at least) triplicate batter preparations per treatment.

2.8 Statistical analysis

All results are expressed as mean $\pm$ standard error, with $n \geq 3$ for all analyses. IBM SPSS 25 software was used to perform two-way ANOVA on the studied parameters. For the rheological properties, logarithmic values were used. Significant interactions were found for all studied properties, which were further interpreted. Tukey's *post hoc* tests were performed and a significance level of $p < .05$ was used.

3 Results and discussion

3.1 Proximate composition

The moisture, fat and nitrogen contents of the pork shoulder and *Z. morio* larvae are shown in Table 2. The *Z. morio* larvae showed a slightly higher nitrogen content compared to pork shoulder. Nitrogen contents were used as a measure of protein content due to a lack of standard conversion factor for insects. However, *Z. morio* larvae contain chitin (6 % on dry weight basis ([Adamkova, et al., 2017](#))), which also contains nitrogen and which may lead to a slight overestimation of the protein content. Still, these results indicate similar protein contents in the insects compared to the meat. While the fat content of the larvae was over 10 % higher than that of the meat, larvae contained considerably less moisture. Partially replacing meat by insect will therefore have affected the composition of the cooked sausages. Similar compositions of both pork shoulder and *Z. morio* larvae were found by [Scholliers, et al. \(2020b\)](#) and [Scholliers, et al. \(2019\)](#).

3.2 Structural properties

3.2.1 Dynamic viscoelastic properties

3.2.1.1 Temperature sweep

Fig. 1 shows the course of G' and δ throughout the temperature sweeps for cooked sausages 100PS (meat reference) and 50ZM:50PS heated to 70, 80 and 90 °C. The hybrid cooked sausages with intermediate insect contents (5ZM:95PS, 10ZM:90PS and 25ZM:75PS) are not shown as intermediate

G' and δ profiles were found. During these temperature sweeps, the heating and cooling process of the (hybrid) cooked sausages in the cans (see section 2.3) was simulated. For all (hybrid) cooked sausages, there were no substantial changes in δ throughout the temperature sweeps. Furthermore, δ was considerably lower than 45 ° at all times, indicating that all samples showed predominantly elastic characteristics throughout the process. G', however, did show some remarkable changes. After an initial decrease, G' sharply increased in all hybrid samples when 40-45 °C was reached during the first rapid heating step. The same was observed for 100PS, containing only meat. During meat protein gelation, myofibrillar proteins start to unfold at 30-32 °C, which may explain the initial decrease in G'. This is followed by protein-protein association at 36-40 °C and, then, protein gelation at 45-50 °C ([Tornberg, 2005](#)), resulting in the sharp increase in G'. For 100PS, at all heating temperatures, G' continued to increase during heating, though the increase was less sharp during the second heating step (with a lower heating rate) and the isothermal heating step. In 50ZM:50PS samples, however, G' started to decline once a temperature of approximately 60 °C was reached. In the samples heated to 70 °C, this gradual decline continued throughout the rest of the heating process. However, in the 50ZM:50PS samples heated to 80 and 90 °C, G' increased again upon further heating. This increase was initiated at $\pm$ 70-75 °C and endured throughout the remainder of the heating process. As similar behavior was observed in the other hybrid cooked sausages (even at insect:meat ratio 5:95), this may be caused by the denaturation (resulting in the decline in G') and subsequent aggregation of the insect proteins in these hybrid cooked sausages. Furthermore, it may be possible that in the samples heated to 70 °C the temperature was not sufficiently high to induce substantial aggregation, causing G' not to rise, in contrast to the samples heated at 80 and 90 °C. Previous research ([Scholliers, Steen, & Fraeye, 2020a](#)) showed that a considerably higher temperature was required for *Z. morio* protein aggregation compared to meat protein aggregation. Extracted meat proteins already started to aggregate after isothermal (1 h) heating at 40 °C, while the vast majority of the extracted *Z. morio* proteins only started to aggregate during isothermal heating (1 h) at 70 °C. During cooling and keeping the samples at 20 °C for 30 min, G' decreased for all samples heated to 70 °C. As for the samples heated to 80 °C, 100PS also showed this decrease, while G' rose for 50ZM:50PS samples. The latter indicates that additional structure was formed in these hybrid cooked sausages during cooling. Although this

increasing G' during cooling was also observed for 25ZM:75PS, this was not the case for the hybrid cooked sausages with lower insect contents (results not shown). All samples heated to 90 °C formed additional structure during cooling, although this was most pronounced for the hybrid cooked sausages with the highest insect contents (25ZM:75PS and 50ZM:50PS). These results indicate that mainly insect protein gelation was important for additional structure formation during cooling. This is in accordance with [Scholliers, et al. \(2020a\)](#), who found that, while meat proteins exhibited most structure formation during heating, insect proteins formed a substantial amount of additional structure during cooling for (mixtures of) extracted insect and meat proteins. [Scholliers, et al. \(2020b\)](#) studied viscoelastic properties of meat (100PS) and hybrid (25ZM:75PS, 50ZM:50PS, 75ZM:25PS) model systems under isothermal conditions followed by cooling, rather than the dynamic process simulations presented here. Still, similar results were obtained.

Statistical analysis showed a significant interaction ($p < .05$) between the effects of heating temperature and insect:meat ratio on both G' at the end of the isothermal heating step ($G'_{\text{end heating}}$) and at the end of the isothermal step at 20 °C ($G'_{\text{end temperature sweep}}$). The results, presented in Fig. 2, showed that a higher insect content resulted in lower G' values and, thus, reduced gel strength both at the end of heating and at the end of the entire heating and cooling process. This indicates that the amount of structure formation in the (hybrid) cooked sausages was diminished upon the partial replacement of meat by insect, even if only 5 or 10 % meat was replaced, though this decrease was not always significant. Furthermore, the heating temperature had little to no effect on the gel strength of the meat reference (100PS), while $G'_{\text{end heating}}$ and $G'_{\text{end temperature sweep}}$ both increased with increasing temperature in the hybrid cooked sausages. This effect was most pronounced for 25ZM:75PS and 50ZM:50PS at the end of the temperature sweep. Due to this effect of temperature, similar final gel strength could be obtained in the hybrid cooked sausages compared to a standard cooked sausage (100PS, which is generally pasteurized at approximately 70 °C) by heating them at higher temperatures (80 or 90 °C, with the exception of 50ZM:50PS at 80 °C). [Scholliers, et al. \(2020b\)](#) found similar results for hybrid and meat model systems. Furthermore, [Bejosano and Corke \(1998\)](#) and [Paulson and Tung \(1989\)](#)

showed that partially replacing meat with plant protein in meat emulsions also resulted in reduced gel strengths.

3.2.1.2 Frequency sweep

The results of the frequency sweeps (Fig. 3) indicate that weak gels with mostly elastic characteristics were formed during the heating and cooling process, as $G' > G''$ and as both G' and G'' were slightly frequency dependent ([Rao, 2007](#); [Tabilo-Munizaga & Barbosa-Cánovas, 2005](#)).

The results also show that both G' and G'' decreased with increasing insect content in the cooked sausages. It is remarkable that, upon replacing only 5 or 10 % meat by insect, G' and G'' were already reduced (most pronounced at 70 and 80 °C). Furthermore, G' and G'' were higher for hybrid cooked sausages which were heated at higher temperatures. For the meat reference (100PS), however, this effect of temperature was negligible. These results are consistent with the results from the temperature sweep (see section 3.2.1.1) and those from [Scholliers, et al. \(2020b\)](#), who studied hybrid and meat model systems, although the effect of temperature on these model systems was more pronounced.

3.2.1.3 Stress sweep

The (hybrid) cooked sausages were subjected to stress sweeps to further characterize the structures that were formed during heating and cooling. The stress on the formed structures was thereby increased until the structures yielded, marking the end of the LVR. The length of this LVR was used as a measure for structure stability, since higher stress is required to break structures with high stability. A significant interaction ($p < .05$) between the effects of temperature and insect:meat ratio was found.

As seen in Fig. 4, the length of LVR was already significantly reduced when only 5 % (at 70 and 80 °C) or 10 % (at 70, 80 and 90 °C) meat was replaced by insect. Further increasing the insect content also further reduced the length of the LVR. This indicates that the partial replacement of meat by insect not only negatively affected the amount of structure formation in the (hybrid) cooked sausages (see section 3.2.1.1), but also the stability of the formed structures. The structure stability of the (hybrid) cooked sausages increased, however, with increasing heating temperature. This effect became

more outspoken at higher insect contents, while it was not significant for the meat reference (100PS). While, as discussed above, heating the hybrid cooked sausages at higher temperatures (80 or 90 °C, with the exception of 50ZM:50PS at 80 °C) resulted in similar final gel strength compared to the standard cooked sausage (100PS at 70 °C), the gel stability of most samples remained significantly lower. In the hybrid cooked sausages with the lowest insect contents (5ZM:95PS and 10ZM:90PS) similar lengths of LVR compared to 100PS at 70 °C could be obtained, but only when heated at 90 °C. Similar effects of temperature and insect:meat ratio on the length of LVR of hybrid and meat model systems were found by [Scholliers, et al. \(2020b\)](#).

3.2.2 Textural properties

Puncture tests were performed on (hybrid) cooked sausages after heating and cold storage for 1 week (see sections 2.3 and 2.6). Pictures of such cooked sausages can be found in Figure A.1 (supplementary material). The maximum force measured during sample penetration ($F_{\max}$, see Table 3) was used to characterize the textural properties of the (hybrid) cooked sausages. Statistical analysis showed a significant interaction ($p < .05$) between the effect of temperature and insect:meat ratio. The results clearly show that replacing increasing amounts of meat by insect resulted in decreasing textural properties. $F_{\max}$ was drastically reduced in the hybrid cooked sausages compared to the meat reference (100PS), even when only 5 or 10 % meat was replaced. Even though the $F_{\max}$ of hybrid cooked sausages increased by applying higher heating temperatures, these $F_{\max}$ values were still substantially lower compared to a standard cooked sausage (100PS at 70 °C). This indicates that the textural properties of the hybrid cooked sausages were inferior to those of a standard cooked sausage, even at very low insect contents and high heating temperatures. As for the effect of temperature on the meat reference (100PS), no clear trend was observed, although the $F_{\max}$ was lowest after heating to 90 °C. [Scholliers, et al. \(2020b\)](#) also measured drastically lower $F_{\max}$ for hybrid model systems compared to meat model systems. Furthermore, an increase in $F_{\max}$ at higher heating temperatures was also found for the hybrid model systems, while an opposite trend was observed for meat model systems. Similar to the results in the current study, [Choi, et al. \(2017\)](#) found lower hardness, measured through Texture Profile Analysis (TPA), in hybrid meat products (containing insect flour) compared to

a meat reference. However, an opposite effect was observed by [Kim, et al. \(2016\)](#), [Kim, et al. \(2017\)](#) and [Park, et al. \(2017\)](#). It must be noted, however, that the hybrid meat product composition and insect protein functionality may have been affected by the use of dry insect flour in these four studies, which in turn could have influenced the textural properties. Partial replacement of meat with plant proteins also resulted in lower hardness (determined through TPA), according to [Kamani, Meera, Bhaskar, and Modi \(2019\)](#), although puncture tests showed similar penetration forces in the hybrid samples compared to the all-meat samples. Also through TPA, [Wang, et al. \(2019\)](#) and [Youssef and Barbut \(2010\)](#) found that partially replacing meat with *Lentinula edodes* (an edible fungus) and milk proteins, respectively, resulted in reduced hardness of meat batters, while the opposite was observed by [Youssef and Barbut \(2011\)](#) for whey and certain soy proteins.

From these results, it can be concluded that the structure of a standard cooked sausage remained superior to that of hybrid cooked sausages, as replacing even only 5 % of meat by insect resulted in a drastic decrease in the amount of structure formation (see section 3.2.1.1), the structure stability (see section 3.2.1.3) and the textural properties. Although viscoelastic and textural properties were improved by heating at higher temperatures and for some hybrid cooked sausages similar viscoelastic properties compared to standard cooked sausages were even obtained, the textural properties remained significantly lower (even at the lowest insect content and highest heating temperature).

3.3 Water and fat stabilization

3.3.1 Cooking loss (CL)

Table 3 shows CL of (hybrid) cooked sausages after heating and 1 week of cold storage (see sections 2.3 and 2.5). Statistical analysis revealed a significant interaction ($p < .05$) between the effects of temperature and insect:meat ratio.

The hybrid cooked sausages with the highest insect contents (25ZM:75PS and 50ZM:50PS) showed the lowest CL, indicating best water and fat stabilization in these samples. Contrary to the effect on the structural properties (see section 3.2), cooking losses were, in most cases, positively influenced by partially replacing meat with insect. Furthermore, CL generally increased with increasing temperature, which may be due to more internal pressure build-up at higher heating temperatures, resulting in more

fluid being expelled from the matrix ([Barbut & Youssef, 2016](#)). Similarly, [Scholliers, et al. \(2020b\)](#) found no cooking loss for insect and hybrid model systems, while meat model systems did show minimal or high cooking losses at 70 and 80 °C, respectively. [Kim, et al. \(2016\)](#), [Kim, et al. \(2017\)](#) and [Park, et al. \(2017\)](#) also found lower cooking loss in hybrid meat products containing insect flour compared to a meat reference, while [Choi, et al. \(2017\)](#) found increased cooking losses. However, as mentioned before, these four studies employed pretreated, dry insect powders, which may have influenced the results. Partially replacing meat with different plant and milk proteins in meat products has also been reported to result in a reduction of cooking loss ([Bejosano, et al., 1998](#); [Kamani, et al., 2019](#); [Youssef, et al., 2010, 2011](#)). [Wang, et al. \(2019\)](#), however, found that partial replacement of meat with *Lentinula edodes* negatively affected cooking losses of sausages.

3.3.2 Emulsion stability

In order to further study the water and fat stabilization in the (hybrid) cooked sausages, the emulsion stability, established through forced destabilization immediately after heating, was characterized by determining the amount of TEF and the parts of water and fat in the TEF (see Table 3). A significant interaction ($p < .05$) between the effects of temperature and insect:meat ratio was established.

In general, partially replacing meat with insect negatively affected the emulsion stability of the cooked sausages, resulting in higher amounts of TEF, even when only 5 or 10 % meat was replaced. It was remarkable that increasing the heating temperature affected the (hybrid) cooked sausages differently, depending on their insect content. The meat reference (100PS) and the hybrid cooked sausages with the lowest insect content (5ZM:95PS) showed increasing amounts of TEF with increasing temperature. The emulsion stability of 10ZM:90PS samples, on the other hand, was not significantly affected by the heating temperature, while the amount of TEF decreased with increasing temperature for the hybrid cooked sausage with the highest insect contents (25ZM:75PS and 50ZM:50PS). As for the water and fat contents in the TEF, 100PS, 5ZM:95PS and 10ZM:90PS all showed similar compositions of TEF at all heating temperatures. The hybrid cooked sausages with the highest insect contents (25ZM:75PS and 50ZM:50PS), however, showed higher fat contents in TEF at 70 °C compared to the other (hybrid) cooked sausages, which declined again upon heating at higher

temperatures. This indicates that, at high insect contents and a heating temperature of 70 °C, fat binding was negatively affected, although it could be improved by increasing the heating temperature. Although water and fat stabilization in cooked sausages (after heating and 1 week of cold storage) was positively affected by partial replacement of meat by insect (see section 3.3.1), water and fat were more easily expelled from the matrix upon subjecting the samples to an external (centrifugal) force immediately after heating. Similar trends were observed by [Scholliers, et al. \(2020b\)](#), who found that hybrid model systems retained water and fat in the matrix during heating, while a substantial amount of fluid was expelled from the formed structure when an external force was applied. It can be hypothesized that the lower structure stability in the hybrid cooked sausages (see section 3.2.1.3) was the result of weaker interactions, which yielded more easily under the influence of the external force, thereby releasing water and fat. Regarding the effect of temperature, it is possible that TEF increased for 100PS and 5ZM:95PS at higher temperatures due to a higher build-up of pressure in the gel network ([Barbut & Youssef, 2016](#)). However, in 10ZM:90PS, 25ZM:75PS and 50ZM:50PS, no effect or even an opposite effect of temperature was observed. Results in Fig. 4 showed that structure stability increased at higher temperatures and this effect was more pronounced at higher insect contents. Therefore, it may be possible that in these hybrid cooked sausages, the formed structures were able to better withstand the external force, resulting in lower TEF.

4 Conclusions

In this study, it was investigated how the partial replacement of meat by insect (at different insect:meat ratios) and different heating temperatures affected the structural properties and water and fat stabilization of cooked sausages.

Structural properties were substantially reduced, even when only 5 or 10 % meat was replaced by insect. These properties could be improved by applying higher temperatures. This resulted in similar viscoelastic properties in some hybrid cooked sausages (5ZM:95PS and 10ZM:90PS at 90 °C) compared to a standard cooked sausage. Their textural properties, however, remained inferior, even at the lowest insect content and highest heating temperature.

In most cases, water and fat stabilization during heating and cold storage (cooking loss) was positively affected by the partial replacement of meat by insect, although cooking losses increased at higher temperatures for all (hybrid) cooked sausages. However, when an external force was applied to the hybrid cooked sausages immediately after heating, they destabilized more readily compared to the meat reference, releasing more fluid.

As structural properties and water and fat stabilization are important for the quality of hybrid cooked sausages, it can be concluded that the latter will be negatively affected by a partial replacement of meat with insect, even at very low replacement levels. Therefore, more research is needed in order to further improve the structure and physical stability of these hybrid meat products, for example through the addition of functional ingredients such as hydrocolloids or proteins.

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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: Composition of the different (hybrid) cooked sausages.

Ingredients	100PS	5ZM:95PS	10ZM:90PS	25ZM:75PS	50ZM:50PS
Pork shoulder (g)	1,600	1,520	1,440	1,200	800
<i>Z. morio</i> larvae (g)	-	80	160	400	800
Pork back fat (g)	1,292	1,292	1,292	1,292	1,292
Ice (g)	1,000	1,000	1,000	1,000	1,000
Nitrite curing salt (g)	72	72	72	72	72
Pepper (g)	8	8	8	8	8
Foil (g)	2	2	2	2	2
Coriander (g)	1	1	1	1	1
Cardamom (g)	1	1	1	1	1
Sodium ascorbate (g)	4	4	4	4	4
Dextrose (g)	20	20	20	20	20
Total mass (g)	4,000	4,000	4,000	4,000	4,000

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

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

Raw material	Moisture content (%)	Fat content (%)	Nitrogen content (%)
Pork shoulder	74.82 ± 0.11	4.53 ± 0.04	3.24 ± 0.02
<i>Z. morio</i> larvae	56.64 ± 0.09	17.69 ± 0.47	3.43 ± 0.12

Results are shown as mean ± standard error (n ≥ 3).

Table 3: $F_{\max}$, cooking loss, TEF and water and fat part of TEF of (hybrid) cooked sausages 100PS, 5ZM:95PS, 10ZM:90PS, 25ZM:75PS and 50ZM:50PS heated at 70, 80 or 90 °C.

		F _{max} (N)	CL (%)	Emulsion stability		
				TEF (%)	Water part of TEF (%)	Fat part of TEF (%)
100 PS	70 °C	22.9 ± 0.5 ^{ab}	4.1 ± 0.3 ^{cde}	1.5 ± 0.2 ^g	90.3 ± 0.3 ^a	9.7 ± 0.3 ^e
	80 °C	23.2 ± 0.8 ^a	6.5 ± 0.6 ^{bc}	3.28 ± 0.05 ^g	89.6 ± 0.4 ^{ab}	10.4 ± 0.4 ^{de}
	90°C	19.8 ± 0.8 ^b	8.9 ± 0.6 ^b	7.3 ± 0.7 ^f	90.4 ± 0.7 ^a	9.6 ± 0.7 ^e
5ZM:95PS	70 °C	7.5 ± 0.5 ^{fg}	2.8 ± 0.7 ^{def}	11.9 ± 0.7 ^{cde}	87.0 ± 1.8 ^{ab}	13.0 ± 1.8 ^{de}
	80 °C	11.9 ± 1.4 ^{de}	5.3 ± 0.7 ^{cd}	14.1 ± 0.6 ^{bcd}	85.7 ± 0.7 ^{abc}	14.3 ± 0.7 ^{cde}
	90°C	16.2 ± 1.2 ^c	12.2 ± 0.4 ^a	16.4 ± 0.2 ^{ab}	88.2 ± 0.4 ^{ab}	11.8 ± 0.4 ^{de}
10ZM:90PS	70 °C	4.5 ± 0.6 ^{gh}	3.5 ± 0.5 ^{cde}	14.6 ± 0.1 ^{bc}	85.2 ± 1.2 ^{abc}	14.9 ± 1.2 ^{cde}
	80 °C	8.0 ± 0.3 ^f	2.7 ± 0.2 ^{def}	14.5 ± 0.3 ^{bc}	84.6 ± 1.6 ^{abc}	15.4 ± 1.6 ^{cde}
	90°C	12.1 ± 0.7 ^d	9.2 ± 1.2 ^{ab}	16.1 ± 0.6 ^{ab}	86.9 ± 0.6 ^{ab}	13.1 ± 0.6 ^{de}
25ZM:75PS	70 °C	3.3 ± 0.4 ^h	1.4 ± 0.4 ^{ef}	18.3 ± 0.9 ^a	73.5 ± 1.9 ^e	26.5 ± 1.9 ^a
	80 °C	6.5 ± 0.2 ^{fgh}	3.3 ± 0.4 ^{de}	17.2 ± 0.4 ^{ab}	77.3 ± 0.9 ^{de}	22.7 ± 0.9 ^{ab}
	90°C	8.7 ± 0.4 ^{ef}	5.7 ± 0.6 ^{cd}	11.0 ± 0.7 ^{cde}	83.9 ± 1.1 ^{bc}	16.1 ± 1.1 ^{cd}
50ZM:50PS	70 °C	3.8 ± 0.2 ^h	0.1 ± 0.1 ^f	18.2 ± 1.3 ^a	74.5 ± 1.8 ^{de}	25.5 ± 1.8 ^{ab}
	80 °C	5.6 ± 0.1 ^{fgh}	1.8 ± 0.3 ^{ef}	10.7 ± 0.7 ^{def}	79.7 ± 1.2 ^{cd}	20.3 ± 1.2 ^{bc}
	90°C	7.4 ± 0.3 ^{fg}	4.0 ± 0.2 ^{cde}	8.9 ± 0.6 ^{ef}	84.6 ± 0.4 ^{abc}	15.4 ± 0.4 ^{cde}

Results are shown as mean ± standard error (n ≥ 3).

Superscripts a-h: different letters within a column indicate significant differences ($p < .05$).

$F_{\max}$: maximum force measured during puncture test; CL: cooking loss; TEF: Total Expressible Fluid;

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

Figure 1: changes in G' and δ of (hybrid) cooked sausages 100PS and 50ZM:50PS during thermal process simulations (with heating to 70, 80 and 90 °C) (G' , the solid black lines; $G' \pm SE$, the dashed black lines; δ , the solid grey lines; $\delta \pm SE$ the dashed grey lines; Temperature, the dotted black lines) ($n \geq 3$). ZM: *Z. morio* larvae; PS: pork shoulder.

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

Figure 3: Frequency sweeps of (hybrid) cooked sausages (A) 100PS, (B) 5ZM:95PS, (C) 10ZM:90PS, (D) 25ZM:75PS and (E) 50ZM:50PS heated at 70, 80 or 90 °C. Confidence intervals are not shown to preserve readability of the graph ($n \geq 3$). ZM: *Z. morio* larvae; PS: pork shoulder.

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

Figure A.1: Pictures of hybrid cooked sausages 50ZM:50PS, 25ZM:75PS, 10ZM:90PS, 5ZM:95PS and 100PS after being heated at 70, 80 or 90 °C and subsequent cold storage for 1 week.