Title

The effect of temperature on structure formation in three insect batters

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Abstract

Since insects are a promising alternative protein source, the application potential of three insect larvae (*Alphitobius diaperinus*, *Tenebrio molitor* and *Zophobas morio*) for food purposes was explored. To this end, the effect of isothermal heating at 5 different temperatures (70 °C-90 °C) on structure formation in insect batters was studied rheologically. Meat batters (with the same protein content as insect batters), isothermally heated at 70 °C, were also studied for comparison. Cryo-SEM imaging was used to visualize the microstructure of raw and heated insect batters. These images showed that a network was formed in the heated batters, as well as in the raw batters. However, no clear effect of temperature or insect larva on the microstructure was observed. Rheologically, both the heating temperature applied and the insect larva used were shown to have a significant effect on the viscoelastic properties of the insect batters. Generally, batters containing *Z. morio* larvae showed both higher storage moduli (G') and longer linear viscoelastic regions (LVRs) compared to the other insect larvae, indicating that these larvae had the best structure forming capacities. Furthermore, both G' and the length of the LVR increased with increasing isothermal heating temperature, indicating more structure formation and structure stability in insect batters heated at higher temperatures. Compared to the meat batters, however, the insect larvae were shown to have inferior structure forming capacities. Even at the highest heating temperature (90 °C) the viscoelastic properties of the insect batters only approached those of meat batters heated at 70 °C. Therefore, it was concluded that higher heating temperatures may need to be employed in insect-based food products compared to meat products in order to obtain sufficient structure formation and the desired textural properties.

Keywords

Tenebrio molitor; *Alphitobius diaperinus*; *Zophobas morio*; edible insects; isothermal heating; viscoelastic properties; microstructure; cryo-SEM

1 Introduction

Due to a growing global population and increasing welfare in developing countries, the demand for meat products is expected to increase drastically in the near future ([van Huis & Oonincx, 2017](#)). Because of the limited land available for livestock production and its negative impact on the environment, the use of sustainable alternative protein sources is being explored. In this respect, insects have gained increasing interest recently ([van Huis, 2015](#)). This is primarily due to their nutritional and ecological advantages. Edible insects have been reported to be rich in high quality protein, in unsaturated fatty acids and in micronutrients, such as iron and zinc ([Rumpold & Schlüter, 2013a](#)). Furthermore, insects are more efficient in converting feed into edible biomass compared to more conventional livestock ([Rumpold & Schlüter, 2013b](#)). Insect farming also requires less water and land and results in less greenhouse gas emissions. These factors contribute to the environmental advantages of insect farming over livestock production ([van Huis & Oonincx, 2017](#)).

In spite of the advantages of producing and consuming insects, consumer acceptance is still very low in Western societies, where edible insects are not a customary part of the diet. However, the willingness to eat insects may be increased when insects are processed into familiar food items ([Hartmann, Shi, Giusto, & Siegrist, 2015](#)). Furthermore, consumers deem savory insect products more appropriate compared to sweet insect products, which is due to the fact that insects have mainly been promoted as an alternative to meat and are expected to be used in products similar to meat products ([Tan et al., 2015](#); [Tan, van den Berg, & Stieger, 2016](#)). By incorporating insects in appropriate food products, with good sensory quality, consumers may be persuaded to regularly eat insects ([Tan, Verbaan, & Stieger, 2017](#)).

From a physical point of view, many protein-rich food products behave as gels, which is why gelling properties of food proteins are important for food quality ([Matsumura & Mori, 1996](#)). Gelation causes the material to convert from a sol to a gel with viscoelastic properties ([Banerjee & Bhattacharya, 2012](#)). In processed meat products, emulsion sausages for example, meat proteins form such gels upon heating of the meat batter ([Tornberg, 2005](#)). The heating temperature applied during the heating of such batters has been shown to have a considerable effect on the viscoelastic properties ([Ferris, Sandoval, Barreiro, Sánchez, & Müller, 2009](#); [Glorieux, Steen, De Brabanter, Foubert, & Fraeye, 2018](#)) and the

microstructure of the gel, which in turn has implications for the physical properties of the food product, including textural and water binding properties ([Barrett, 2002](#)).

Even though structure formation is a defining factor for food quality, knowledge regarding gelation of insect biomass and its derivatives is scarce. In a few research articles this was investigated by studying gel forming abilities of insect flour solutions, with the insect flours being prepared from whole insects ([Adebowale, Adebowale, & Oguntokun, 2005](#); [Kim, Setyabrata, Lee, Jones, & Kim, 2017](#); [Kim, Setyabrata, Lee, Jones, & Kim, 2016](#); [Omotoso, 2006, 2018](#); [Osasona & Olaofe, 2010](#)). However, due to the pretreatment of the insects, necessary for flour preparation, native protein structures may have already been altered, affecting their gelling properties. Furthermore, the previously mentioned studies used rather basic methods, such as visual evaluation of gel formation, but did not offer deeper insight into structure formation throughout heating and cooling steps or into gel microstructure. Gel forming capacities of extracted insect proteins ([Purschke et al., 2018](#); [Yi et al., 2013](#); [Zhao, Vazquez-Gutierrez, Johansson, Landberg, & Langton, 2016](#)) have also been studied, with [Zhao et al. \(2016\)](#) and [Yi et al. \(2013\)](#) providing insight into insect protein gelation through rheological measurements. However, to the best of our knowledge, there are currently no studies describing the effect of different heating temperatures on insect protein gelation or structure formation.

Therefore, the objective of this work was to investigate structure formation in insect batters of different insect larvae. To that end, viscoelastic properties of the batters were studied through rheological measurements. By using insect batters, wherein whole insect larvae are incorporated, the contribution of not only the insect proteins, but also other constituents, such as chitin, on structure formation were taken into account. In addition, as fresh, non-pretreated insect biomass was used, the entire intrinsic gelling potential of the biomass was assessed. The effect of different (isothermal) heating temperatures (70-90 °C) on structure development was also studied, since, in meat batters for example, this has been proven to be an important variable during heat treatment, as discussed above. Furthermore, if insects are to be incorporated in meat analogues, properties similar to those of meat products should be envisaged. Therefore, viscoelastic properties of meat batters, heated at 70 °C, were compared to those of the insect batters. Finally, the microstructures of raw and heated insect batters were visualized through cryo-SEM.

Insight into structure development of insect batters is needed to further improve existing insect-based food products and develop new ones.

2 Materials and methods

2.1 Materials

Three different insect larvae from the Tenebrionidae family, more specifically *Tenebrio molitor*, *Alphitobius diaperinus* and *Zophobas morio*, were purchased from Nusect, Sint Elooïs Winkel, Belgium. The larvae were fed with GMP certified wheat products (*T. molitor* and *A. diaperinus*) or cornmeal (*Z. morio*), complemented with apples and carrots. Live larvae were sieved and vacuum packed before being stored in the freezer at – 18 °C until analysis. Pork shoulder was purchased from a local supplier (Norbert Impens, Melle, Belgium). First, visible fat and connective tissue were removed. The meat was then cut into small dices, vacuum packed and, finally, stored in the freezer at -18 °C.

2.2 Proximate composition

Frozen insects as well as pork shoulder were ground for determination of moisture, free fat and crude protein content. Moisture content was determined by drying the ground meat and insects overnight in a drying oven at 103 °C. Free fat content was determined gravimetrically after extraction using hexane (VWR international, Leuven Belgium) in a soxhlet apparatus. Extraction continued for at least 6 hours, after which all hexane was removed by using a rotary evaporator (Laborota 4000 efficient, Heidolph instruments, Schwabach, Germany) and subsequent drying at 103 °C. Crude protein content was determined through the Kjeldahl method (Digest System K-437 and Distillation Unit K-350, Büchi, Flawil, Switzerland), using a conversion factor of 6.25. Moisture, free fat and crude protein content were determined at least in triplicate.

2.3 Batter preparation

The insect batters were prepared by grinding whole insects with sodium phosphate buffer (pH 6, 0.3 M) at 3,500 rpm using a Grindomix GM200 (Retsch, Haan, Germany). The composition of the batters (insect-to-buffer ratio) was adjusted for each insect larva, so that all insect batters contained 10 % protein. The phosphate buffer also contained NaCl, of which the concentration in the buffer was also

adjusted for each insect larva to ensure a salt concentration of 0.6 M in all batters. Grinding was continued until a final temperature of 12-14 °C was obtained in the batters, which resulted in sufficiently long grinding times (approximately 10 minutes) while preventing premature protein denaturation (Zayas, 1997). Finally, the batters were vacuum packed and stored at 15 °C for 1h30 before further analysis. In order to be able to compare the structure forming capacities of the insects with that of meat, meat batter was also prepared in a similar manner. Pork shoulder was thereby used as a meat source.

2.4 Dynamic viscoelastic properties

The dynamic viscoelastic properties of the insect batters and meat batters (used for comparison) were studied using a stress-controlled rheometer (AR 2000ex, TA instruments, New Castle, USA). A 40 mm parallel plate system (both plates crosshatched) and a gap of 1000 µm were employed to perform the measurements. By using both upper-heated plate and a Peltier temperature control system, accurate temperature control in the sample was obtained. Since high temperatures were applied during the measurements, the samples were covered by a cap in order to prevent dehydration of the samples. During all rheological measurements, the storage modulus (G'), the loss modulus (G''), the complex modulus (G^*) and the phase angle (δ) were monitored. G' is a measure for the elastic properties of the sample, while G'' is a measure for the viscous properties. From these moduli, G^* can be calculated as follows: $|G^*| = \sqrt{(G')^2 + (G'')^2}$. The phase angle relates to the ratio of the viscous character (G'') to the elastic character (G') of a material. For an ideally elastic material, the phase angle is 0 °, while an ideally viscous material shows a phase angle of 90 ° (Rao, 2007). The rheological measurements were performed at least in triplicate, each time on a freshly prepared batter.

2.4.1 Temperature sweep

Immediately after loading the sample and trimming the excess, a temperature sweep was initiated. During the temperature sweep, isothermal heating was first applied for 1 hour (phase 1). Five different isothermal heating temperatures were employed: 70 °C, 75 °C, 80 °C, 85 °C and 90 °C. Subsequently, in phase 2, the sample was cooled to 20 °C at 2 °C/min, followed by phase 3, where it was held at 20 °C for 1 hour. Isothermal steps provide information on all changes that may occur if the sample is kept at a certain temperature sufficiently long. Therefore, isothermal heating at different temperatures allows

allocation of a certain gelling behavior to a specific temperature. During heating, cooling and holding at 20 °C, a constant strain of 0.025, within the linear viscoelastic region (LVR), and a constant frequency of 1 Hz were applied. For comparison, meat batters were subjected to the same temperature sweep, with an isothermal heating temperature of 70 °C. This heating temperature was selected since this is approximately the core temperature that is employed during pasteurization of cooked meat products ([Feiner, 2006](#)).

2.4.2 Frequency sweep

The temperature sweep was immediately followed by a frequency sweep. Hereby, a frequency range of 0.1-10 Hz was applied. The frequency sweep was performed at a constant temperature (20 °C) and at a constant stress within the LVR (0.5 Pa for batters heated at 70 °C and 75 °C, 7 Pa for batters heated at 80 °C, 85 °C and 90 °C) with 15 measuring points per decade.

2.4.3 Stress sweep

Immediately following the frequency sweep, a stress sweep was performed on the insect and meat batters. Oscillatory stress was varied from 1 to 5000 Pa at a constant temperature (20 °C) and a constant frequency (1 Hz). The stress sweep was used to determine the LVR, as a measure of structure stability. Within the LVR, the properties of the formed structure are independent of the applied stress ([Steffe, 1996](#)). However, when stress increases beyond the upper limit of the LVR, structure breakdown occurs, causing a drop in the complex modulus. More stable structures can withstand a higher stress before breakdown and, thus, have a longer LVR ([Steen et al., 2014](#)). The end of the LVR was calculated as the oscillatory stress where the resulting G^* differs more than 5 % from the average G^* of the 5 previous measuring points (70 measuring points per decade). This results in an estimate of the LVR, which allows for a comparison of the effect of different isothermal heating temperatures and different insect larvae on the LVR of the structures formed in the insect batters.

2.5 Cryo-SEM imaging

Cryo-SEM imaging was used in order to visualize the microstructure of raw insect batters and insect batters heated isothermally at 70 °C and 90 °C, for each insect larva. In preparation of cryo-SEM imaging of heated batters, the raw batters, prepared as described in section 2.3, were transferred to a

petridish (diameter 50 mm, height 8 mm), which was packaged to keep the sample waterproof. The sample was then transferred to a temperature-controlled water bath (Julabo, Seelbach, Germany), which was set to the required heating temperature (70 °C or 90 °C). Since the come-up time (CUT, the time needed for the center of the sample to reach the heating temperature) was 10 minutes, the samples were heated for 1h10 (CUT + 1h isothermal heating). Afterwards, the heated samples were stored at 4 °C until cryo-SEM imaging the following day. The raw batters were also transferred to a petridish and kept at 4 °C, without being heated in the water bath.

In preparation of cryo-SEM imaging, the sample was first vitrified with liquid nitrogen and then transferred to the stage in the cryo-preparation chamber (PP3010T cryo-SEM preparation system, Quorum Technologies, UK) at -140 °C. The sample was then freeze-fractured and subsequently sublimated at -90 °C for 1 hour under controlled vacuum conditions. Finally, the sample was sputter-coated with platinum and examined using a JEOL JSM 7100F SEM (JEOL Ltd, Tokyo, Japan). An accelerated voltage of 3 kV was used.

2.6 Statistical analysis

All results are expressed as mean $\pm$ standard error. Statistical analysis was performed using IBM SPSS 24. Two-way ANOVA was performed to study the effect of different isothermal heating temperatures and different insect larvae on G' at the end of phase 1 (G' end heating), G' at the end of phase 3 (G' end temperature sweep) and the length of the LVR. Statistical processing was performed on the logarithmic values of these variables. Tukey's post hoc test was performed to determine significant differences ($p < 0.05$).

3 Results and discussion

3.1 Proximate composition

Moisture, free fat and crude protein contents of the insect larvae and of the pork shoulder, expressed on a fresh weight basis, are shown in Table 1. All insect larvae showed similar crude protein contents, ranging between 17.7 % and 18.3 %. More variation was observed in the moisture and free fat content of the insect larvae, with moisture content ranging from 61.0 % to 72.0 %, while free fat content ranged

from 5.7 % to 15.5 %. Compared to the pork shoulder, the insect larvae showed comparable protein contents and, overall, a lower moisture content and a higher fat content. The chemical compositions of the insect larvae found in the current study are comparable to those reported by [Yi et al. \(2013\)](#) (for all larvae) and [Finke \(2002\)](#) (for *Z. morio* and *T. molitor*).

3.2 Dynamic viscoelastic properties

3.2.1 Temperature sweep

In Fig. 1, the viscoelastic properties (G' and δ) during isothermal heating at 70 °C and 90 °C (phase 1), cooling to 20 °C (phase 2) and the second isothermal step at 20 °C (phase 3) are displayed for batters containing the three insect larvae. The batters heated at the intermediate temperatures (75 °C, 80 °C and 85 °C) showed intermediate G' and δ profiles and are therefore not shown. In all batters, δ was consistently smaller than 45 °, indicating a predominately elastic character of all insect batters. Furthermore, it was observed that δ only underwent small changes throughout the entire temperature sweep for all insect batters. As for the course of G' throughout the temperature sweeps, all batters showed a sharp increase in G' at the start of phase 1, indicating an initial surge of structure build-up upon heating. Furthermore, G' decreased during the rest of phase 1 for all batters heated at 70°C and the *A. diaperinus* batters heated at 90 °C. This may indicate that the interactions that were initially formed were rather weak and dissociated upon further heating. However, for the *T. molitor* and *Z. morio* batters heated at 90 °C, G' kept increasing slightly throughout phase 1, indicating that not only more structure was formed in these batters, but that the formed interactions may also be stronger. As seen in Fig. 1, a distinct rise in G' occurred in all insect batters during phase 2, suggesting that additional structure was formed during cooling. During phase 3, G' remained relatively constant compared to phases 1 and 2, for all insect batters. Rheological properties during temperature sweeps were also studied for extracted proteins of the same insect larvae by [Yi et al. \(2013\)](#). Although they applied non-isothermal heating from 20 °C to 90 °C, after which they held the proteins at 90 °C for 5 minutes, they also found that G' gradually increased during heating and holding at 90 °C, which is similar to the results obtained in the current study. Furthermore, under similar conditions of pH and sample concentration as used by [Yi et al. \(2013\)](#), comparable results were also obtained by [Zhao et al. \(2016\)](#), who also applied non-isothermal

heating (20 °C to 90 °C and holding at 90 °C for 30 minutes) to extracted *T. molitor* proteins. Furthermore, both [Yi et al. \(2013\)](#) and [Zhao et al. \(2016\)](#) observed an increase in G' during cooling, which was also seen in the current study. [Yi et al. \(2013\)](#) ascribed this additional structure formation to the formation of hydrogen bonds.

In order to further study the effect of the different isothermal heating temperatures and the different insect larvae on the viscoelastic properties, G' values at the end of phase 1 (G' end heating) and G' values at the end of phase 3 (G' end temperature sweep) were statistically compared (Table 2). Both isothermal heating temperature and insect larva had a significant effect ($p<0.05$) on G' end heating and G' end temperature sweep. The results in Table 2 also seem to indicate an interaction between heating temperature and insect larva. However, even though the interaction term approached significance for both G' end heating ($p=0.073$) and G' end temperature sweep ($p=0.079$), a significance level of $p<0.05$ was not reached. Therefore, only the main effects of isothermal heating temperature and insect larva were studied.

Regarding the overall effect of the different insect larvae on the structure formation at the end of phase 1 (G' end heating, upper half Table 2), it was observed that most structure formation occurred in batters containing *Z. morio*. Significantly lower G' values were obtained in batters containing *A. diaperinus*. Similar results were obtained when the effect of the different insect larvae on G' end temperature sweep (at the end of phase 3) was statistically studied (lower half Table 2). Here, batters containing *A. diaperinus* and *T. molitor* generally showed the lowest G' values, with no significant differences between these two insect larvae, while significantly higher G' values were again found for *Z. morio* batters. This indicates that more and/or stronger interactions were formed in *Z. morio* batters upon isothermal heating, cooling and holding at 20 °C. These results are in accordance with [Yi et al. \(2013\)](#), who also obtained higher G' values for gels formed by extracted *Z. morio* proteins compared to gels formed by extracted *A. diaperinus* and *T. molitor* proteins, after non-isothermal heating to 90 °C and cooling to 20 °C. However, the extracted protein fraction studied by [Yi et al. \(2013\)](#) only contained approximately 20 % of all proteins, while in the current study the complete, non-pretreated insect biomass was used. The results obtained in the current study indicate that the *Z. morio* larvae have better

structure forming capacities compared to the *T. molitor* and *A. diaperinus* larvae. This is an important finding with regard to the application of insect larvae in insect-based food products, since structure forming capacity has an impact on different quality characteristics, including textural properties ([Barrett, 2002](#)).

As for the overall effect of the different isothermal heating temperatures on structure formation at the end of phase 1 (G' end heating, upper half Table 2), it was found that the G' values gradually increased when the heating temperature increased. The same trend was observed for the G' values at the end of phase 3 (G' end temperature sweep, lower half Table 2), after the batters were heated, cooled and held at 20 °C. However, here, the differences between the batters heated at the different isothermal heating temperatures were more distinct than at the end of phase 1. Results showed that heating the batters at 70 °C and 75 °C generally resulted in the lowest G' values, indicating that the least structure was formed in these batters after heating, cooling and holding at 20 °C. Applying an isothermal heating temperature of 80 °C resulted in significantly higher G' values at the end of phase 3. G' significantly increased further when batters were heated at 85 °C. However, a further increase from 85 °C to 90 °C did not significantly affect G'. These results indicate that more and/or stronger interactions are formed during heating and cooling when the insect batters are heated at higher temperatures. To the best of our knowledge, no literature regarding the effect of different heating temperatures on gelation of insect proteins or structure formation in insect batters exists. However, [Bußler et al. \(2016\)](#) did study the effect of dry thermal treatment at different temperatures (20-140 °C) on proteins in dry *T. molitor* flour through, amongst others, fluorescence measurements. The results showed that fluorescence intensity decreased with increasing temperature, indicating more protein unfolding and, thus, protein denaturation. Since denatured proteins can form interactions and aggregate, resulting in structure formation, this may explain the results obtained in the current study. Furthermore, a temperature effect, similar to the one found for the insect batters in the current study, has been observed in meat batters, with G' also increasing when higher isothermal temperatures were applied ([Ferris et al., 2009](#); [Glorieux et al., 2018](#)).

If insects are to be incorporated into insect-based meat analogues, these meat analogues should possess similar properties compared to meat, since this will appeal to more consumers, more specifically current meat consumers ([Hoek et al., 2011](#)). Therefore, the viscoelastic properties of the insect batters were compared to those of meat batters, which were subjected to the same temperature sweep as the insect batters, using an isothermal heating temperature of 70 °C. The G' end heating and G' end temperature sweep values obtained from these measurements were $19,663 \pm 635$ Pa and $33,100 \pm 5,079$ Pa, respectively. These G' values are approximately 100 times greater than those obtained in insect batters heated at the same isothermal heating temperature. Applying a heating temperature of 90 °C to the *Z. morio* batters resulted in G' end temperature sweep values that approached, but were still considerably smaller than, those obtained in the meat batters heated at 70 °C. Therefore, it can be concluded that structure formation in the insect batters was inferior to that in the meat batters. The application of higher heating temperatures may be needed to ensure sufficient structure formation in foodstuff containing these insect larvae. This will be crucial in obtaining the desired texture in, for example, insect-based meat analogues.

3.2.2 Frequency sweep

Immediately following the temperature sweep, the insect batters were subjected to a frequency sweep. G' and G'' recorded during these frequency sweeps are shown in Fig. 2. For all batters, regardless of isothermal heating temperature or insect larva, G' was consistently higher than G'' in the frequency range of 0.1-10 Hz. This predominance of G' over G'' indicates that the formed structures behaved more solid-like ([Tabilo-Munizaga & Barbosa-Cánovas, 2005](#)). Furthermore, in all batters, G' and G'' increased slightly with increasing frequency. This slight frequency-dependence indicates that, after heating, cooling and holding the batters at 20 °C, a weak gel was formed in all batters ([Rao, 2007](#)).

When comparing the effect of the different insect larvae on the frequency sweeps, the highest G' and G'' values were generally obtained for *Z. morio* batters. This is in accordance with the results from the temperature sweep (Table 2), which showed that, overall, more structure was formed in these batters.

As for the effect of isothermal heating temperature on G' and G'', both G' and G'' were generally higher for batters heated at higher isothermal heating temperatures. This is also in accordance with the results

from the temperature sweeps, where G' increased with increasing temperature (Table 2). This temperature effect was least prominent for *A. diaperinus* batters, where heating at 70 °C, 75 °C and 80 °C resulted in similar G' and G'' values. Only when these batters were heated at 85 °C and 90 °C, a distinct rise in G' and G'' occurred. In contrast, a clear effect of temperature was already observed at lower temperatures for *T. molitor* and *Z. morio* batters and was most distinct in *Z. morio* batters.

3.2.3 Stress sweep: length of LVR

Subsequent to the frequency sweep, a stress sweep was performed. Through the stress sweep, the stress at which the formed structure started to break down and G^* started to drop was determined. This stress was calculated as described in section 2.4.3 and indicates the length of the LVR. The effect of isothermal heating temperature and insect larva on the length of the LVR is shown in Table 3. Both isothermal heating temperature and insect larva had a significant effect ($p < 0.05$) on the length of the LVR. Similar to the statistically analyzed G' values in Table 2, it was found that the interaction between isothermal heating temperature and insect larva closely approached, but did not reach, significance ($p = 0.053$). Therefore, only the main effects of isothermal heating temperature and insect larva were studied.

Regarding the effect of insect larva on the length of the LVR, overall, the batters containing *Z. morio* showed significantly longer LVRs compared to the other insect larvae. Furthermore, no significant differences were found in the length of the LVRs of batters containing *A. diaperinus* and *T. molitor*. Since a longer LVR indicates more stability in the formed structure, these results indicate that highest structure stability was obtained in *Z. morio* batters. This further confirms that *Z. morio* larvae have better structure forming properties compared to the other insect larvae. Strain sweeps, which are similar to stress sweeps, were also performed by [Yi et al. \(2013\)](#), after heating to 90 °C and cooling to 20 °C, in order to determine the maximum linear strain. They found, however, that the gels formed by the extracted *A. diaperinus*, *T. molitor* and *Z. morio* proteins all had a similar maximum linear strain.

As seen in Table 3, heating at higher isothermal heating temperatures generally resulted in a longer LVR. Heating the insect batters at 70 °C and 75 °C resulted in similar LVR lengths, while significantly longer LVRs were obtained in batters heated at 80 °C. Even longer LVRs were obtained by further increasing the heating temperature, with the longest LVRs being found for the batters heated at 90 °C.

These results indicate that heating at higher temperatures not only leads to more structure formation (Table 2), but also to the formation of more stable structures in the insect batters.

The meat batters, which were used for comparison with the insect batters and which were heated at an isothermal heating temperature of 70 °C, were also subjected to a stress sweep. The structures in these batters started to break down at an oscillatory stress of $2,137.6 \pm 368.6$ Pa, meaning the LVR of the meat batters was approximately 100 times longer than the LVRs of the insect batters heated at the same temperature. Even when *Z. morio* batters, which showed highest structure stability out of the studied insect batters, were heated at 90 °C, their LVR was still approximately 3 times smaller than that of meat batter heated at 70 °C. This indicates that not only less structure was formed in the insect batters compared to the meat batter (Table 2), but that the structures in the insect batters also showed inferior stability. This further confirms that higher heating temperatures may need to be applied to obtain the desired texture in food products containing insects compared to meat products.

3.3 Cryo-SEM images

The microstructure of the raw insect batters and the batters isothermally heated at 70 °C and 90 °C was visualized through cryo-SEM. No clear effect of insect larva on microstructure was seen in the cryo-SEM images. Therefore, only the cryo-SEM images of *A. diaperinus* batters are shown in Fig. 3 at magnifications of 1,000 and 5,000. From the cryo-SEM images of the raw insect batters, it is clear that some network already originated after grinding insect biomass with buffer, indicating that structure formation already started before applying heat. The formation of a network in batters prior to cooking has also been observed for meat batters ([Barbut, Gordon, & Smith, 1996](#); [Gordon & Barbut, 1990](#); [Liu, Lanier, & Osborne, 2016](#)). The cryo-SEM images of the heated insect batters showed a more coarse network compared to the raw insect batters. In addition, upon heating, the network in the *A. diaperinus* batters became more dense, with smaller pore sizes and increasing interactions between the protein strands. However, this was not always seen in the cryo-SEM images of the batters containing the other insect larvae (not shown). [Barbut et al. \(1996\)](#) also found a more dense protein network in meat batters heated to 70 °C compared to raw meat batters, which was attributed to reinforcement of the protein matrix in the raw batter through protein gelation during heating. Finally, no clear effect on the

microstructure of the insect batters was observed when the isothermal heating temperature was increased from 70 °C to 90 °C.

4 Conclusions

The effect of different isothermal heating temperatures on structure formation in insect batters was studied for three insect larvae, through both dynamic rheological measurements and cryo-SEM imaging. Results showed that a network was already formed in raw batters and that further structure formation occurred during both heating and cooling of the batters. The structures of all batters that were formed could be characterized as weak gels, exhibiting solid-like behavior.

Cryo-SEM images showed no clear effect of the application of different isothermal heating temperatures and different insect larvae on the microstructure of insect batters. Rheologically, however, both heating temperature and insect larva were shown to have a significant effect on structure formation. Regarding the effect of temperature, it was found that increasing the heating temperature resulted in increased structure formation and structure stability. Furthermore, the *Z. morio* larvae showed better structure forming capacities compared to the other insect larvae, since more structure was formed in *Z. morio* batters and higher structure stability was obtained. As quality characteristics, such as textural properties, are affected by structure formation, it can be concluded that, in that regard, the *Z. morio* larvae show most potential for application in food products compared to the other two larvae.

However, the structure forming capacities of the insect larvae were shown to be inferior to those of meat. For insect batters, an isothermal heating temperature of 90 °C is needed in order to approach the amount of structure and structure stability obtained in meat batters heated at 70 °C. The current findings therefore indicate that insect-based food products may need to be heated at higher temperatures, compared to meat products, in order to obtain sufficient structure formation and the desired texture.

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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: Proximate composition of *A. diaperinus* larvae, *T. molitor* larvae, *Z. morio* larvae and pork shoulder on a fresh weight basis.

Insect	Moisture content (%)	Free fat content (%)	Crude protein content (%)
<i>A. diaperinus</i>	71.97 ± 0.18	5.73 ± 0.17	17.69 ± 0.07
<i>T. molitor</i>	64.42 ± 0.11	11.74 ± 0.04	18.26 ± 0.06
<i>Z. morio</i>	60.98 ± 0.13	15.50 ± 0.11	18.23 ± 0.13
Pork shoulder	76.50 ± 0.10	2.19 ± 0.11	20.67 ± 0.11

Mean values and standard errors are used to present the results (n≥3).

Table 2: Effect of isothermal heating temperature and insect larva on G' at the end of phase 1 (G' end heating) and G' at the end of phase 3 (G' end temperature sweep).

G' end heating (Pa)				
	<i>A. diaperinus</i>	<i>T. molitor</i>	<i>Z. morio</i>	Average by temperature
70 °C	293 ± 70	315 ± 109	129 ± 18	246 ± 83 ^D
75 °C	270 ± 31	296 ± 82	711 ± 188	426 ± 131 ^{CD}
80 °C	576 ± 73	825 ± 265	2,152 ± 780	1,185 ± 419 ^{BC}
85 °C	940 ± 73	2,686 ± 1,110	3,158 ± 1,076	2,262 ± 927 ^{AB}
90 °C	1,454 ± 348	4,683 ± 850	5,010 ± 1,399	3,716 ± 966 ^A
Average by insect larva	707 ± 145 ^b	1,761 ± 542 ^{ab}	2,232 ± 795 ^a	
G' end temperature sweep (Pa)				
	<i>A. diaperinus</i>	<i>T. molitor</i>	<i>Z. morio</i>	Average by temperature
70 °C	476 ± 114	476 ± 138	177 ± 25	376 ± 116 ^C
75 °C	353 ± 97	333 ± 108	1,250 ± 500	645 ± 327 ^C
80 °C	923 ± 164	1,544 ± 593	4,068 ± 1,366	2,178 ± 785 ^B
85 °C	1,822 ± 114	6,090 ± 2,440	7,922 ± 2,838	5,278 ± 2204 ^A
90 °C	4,253 ± 872	11,649 ± 3,160	17,230 ± 5,127	11,044 ± 3514 ^A
Average by insect larva	1,565 ± 348 ^b	4,018 ± 1446 ^b	6,130 ± 2474 ^a	

Mean values and standard errors are used to present the results (n≥3). Two-way ANOVA was performed on the logarithmic values.

Superscripts A-D: different capital letters indicate significant differences (p<0.05) between different isothermal heating temperatures.

Superscripts a-b: different small letters indicate significant differences (p<0.05) between different insect larvae.

Table 3: Effect of isothermal heating temperature and insect larva on the length of the LVR.

	Length of LVR (Pa)			
	<i>A. diaperinus</i>	<i>T. molitor</i>	<i>Z. morio</i>	Average by temperature
70 °C	22.0 ± 5.0	22.2 ± 6.6	7.6 ± 1.2	17.3 ± 5.3 ^C
75 °C	15.3 ± 4.4	14.6 ± 4.5	55.9 ± 22.0	28.6 ± 14.4 ^C
80 °C	38.3 ± 6.6	71.3 ± 26.3	174.5 ± 57.7	94.7 ± 33.7 ^B
85 °C	81.2 ± 3.7	268.3 ± 110.6	347.5 ± 128.4	232.3 ± 99.8 ^{AB}
90 °C	174.8 ± 38.3	502.5 ± 133.0	730.0 ± 206.3	469.1 ± 143.4 ^A
Average by insect larva	66.3 ± 15.2 ^b	175.8 ± 63.2 ^b	263.1 ± 102.8 ^a	

Mean values and standard errors are used to present the results (n≥3). Two-way ANOVA was performed on the logarithmic values.

Superscripts A-C: different capital letters indicate significant differences (p<0.05) between different isothermal heating temperatures.

Superscripts a-b: different small letters indicate significant differences (p<0.05) between different insect larvae.