

# Exploring the Potential of *Acheta domesticus* Protein Extracts for the Future of Meat Analogues

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This study explores the potential of *Acheta domesticus* (house cricket) protein extracts as a sustainable and functional ingredient for food production, particularly in the development of meat analogues. The objective was to evaluate the techno-functional properties of these proteins, focusing on solubility, gelation, and rheological behaviour. Protein extracts, obtained by alkaline treatment coupled with isoelectric precipitation, showed a solubility at pH 7 of  $59.69 \pm 0.30 \text{ gSoluble Protein}/100 \text{ gTotal Protein}$ . The minimum concentration at which gelation was observed was 15 % w/v. The resulting gels exhibited a final elastic modulus ( $G'$ ) reaching  $3.26 \cdot 10^5 \text{ Pa}$  at the end of the thermal treatments performed through temperature sweeps, a limited viscoelastic region (yield strain of  $0.07 \pm 0.01 \%$ ) and an overall predominance of  $G'$  over  $G''$  in all the rheological tests performed. These results suggest that *Acheta domesticus* protein extracts possess promising gel-forming capabilities and viscoelastic properties, underscoring their potential as a sustainable and functional protein source for diverse food applications.

## 1. Introduction

The growing global demand for sustainable and functional protein sources has spurred interest in alternative ingredients for food applications. Conventional protein sources, such as soy, pea, and wheat, are widely used but have raised environmental concerns due to intensive land use, greenhouse gas emissions, and water consumption. On the contrary, edible insects are emerging as a more efficient and eco-conscious alternative. Among them, *Acheta domesticus* (house cricket) has gained significant attention due to its high nutritional value, particularly its protein content, which ranges from 60 % to 70 % on a dry basis, surpassing many conventional animal protein sources (Raubenheimer et al., 2013). Additionally, house crickets are abundant in unsaturated fatty acids, dietary fiber, vitamins, and minerals, further solidifying their position as a valuable ingredient for human consumption (Udomsil et al., 2019). As for their environmental advantages, crickets have a feed conversion ratio of 2.1, which is twice as efficient as chickens and 12 times more efficient than cattle (van Huis, 2013). These benefits highlight their potential role in novel food products, including meat analogues (products designed to replicate the textural, sensory, and nutritional properties of animal-derived meat). Meat analogues are commonly produced starting from processes like High-Moisture Extrusion (HME), which requires a protein source with specific techno-functional and rheological properties, including discreet solubility to facilitate protein interactions, strong gelation ability to form a cohesive network, and balanced viscoelastic behaviour that allows for elasticity and structural integrity while maintaining resistance to deformation during processing (Wu et al., 2019). To the best of the authors' knowledge, while plant proteins have been extensively studied for their suitability in high-moisture extrusion, the applicability of *Acheta domesticus* proteins remains underexplored. Scholliers et al. (2020) studied gelation properties of a combination of protein extracts of insect (*Zophobas morio*) and pork, at different ratios and heating conditions. They found comparable gel strength in both pure insect and pork protein extracts, but a sensible decrease upon the combination of the two, highlighting a possible antagonistic effect between the different sources. Chia et al. (2024) focused on the heat-induced gelation and rheological properties of *Hermetia illucens* larvae protein, demonstrating that higher temperatures enhance protein aggregation, structural reorganization, and gel elasticity, providing insights into its potential applications

in food texturization and formulation. Overall, previous research has primarily focused on the nutritional composition of house crickets (Raubenheimer et al., 2013), with limited studies addressing their gelation and viscoelastic properties, critical parameters in food processing. In view of all the above, this study aims to expand the knowledge on *Acheta domesticus* protein extracts by exploring their rheological and techno-functional properties, with a specific focus on their potential for texturization in meat analogue production.

## 2. Materials and Methods

### 2.1 Materials

Cricket Flour (CF) was purchased from Small Giants SRL (Milan, Italy). All the analytical-grade chemicals were obtained from Merck KGaA (Darmstadt, Germany). The Pierce™ Bradford Protein Assay Kit was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

### 2.2 Protein extracts preparation

CF was dispersed in n-hexane at a 1:5 (w/v) ratio, stirred for 180 min at room temperature and then centrifuged (SL 8, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 4800 rpm for 20 min. The obtained Defatted Cricket Flour (DCF) was then left overnight under a fume hood. Subsequently, DCF was subjected to alkaline protein extraction by addition to 0.25 M NaOH solution at a 1:15 (w/v) ratio. The mixture was stirred for 120 min at 40 °C and then centrifuged at 5100 rpm for 20 min. The precipitate was collected and a second extraction was repeated under the same conditions. The supernatants from the first and second extractions were combined and acidified with HCl (2 M) to reach the isoelectric point (pH 4.5) and induce protein precipitation. Finally, the Cricket Protein Extract (CPE) was neutralized to pH 7.0 and then freeze dried using a Small Pro Freeze Dryer (Harvest Right, Salt Lake City, UT). The extraction yield was evaluated through the equation (1):

$$\text{Extraction yield (\%)} = \frac{m_{CPE}(g)}{m_{DCF}(g)} \cdot 100 \quad (1)$$

where  $m_{CPE}$  is the mass (g) of CPE obtained and  $m_{DCF}$  is the mass (g) of defatted cricket flour sample. The protein content of CPE was determined using total nitrogen analysis (Kjeldahl method) with 6.25 as conversion factor (Fernández et al., 2022). Finally, the protein extraction yield was calculated using the equation (2):

$$\text{Protein extraction yield (\%)} = \frac{m_{CPE}(g) \cdot P_{CPE}(g/g)}{m_{DCF}(g) \cdot P_{DCF}(g/g)} \cdot 100 \quad (2)$$

where  $P_{CPE}$  represents the amount of protein in CPE while  $P_{DFC}$  represent the amount of protein in DCF.

### 2.3 Solubility

The amount of soluble proteins in CPE was measured according to Zielińska et al. (2018), with minor modifications. CPE samples were dispersed in Phosphate Buffer Solution pH 7 at a concentration of 0.1 % (w/v). The mixtures were stirred for 60 min at room temperature and then centrifuged at 4900 rpm for 20 min. 50 µL of the mixture was mixed with 1.5 mL of the Pierce™ Bradford Protein Assay reagent, and the absorbance was read at 595 nm using a UV-6300PC spectrophotometer (VWR International Europe BVBA, Leuven, Belgium). The amount of soluble protein was determined by a calibration curve performed by different concentration of bovine serum albumin as a standard. Finally, the solubility was determined through the equation (3):

$$\text{Solubility (} g_{\text{Soluble Protein}}/100 g_{\text{Total Protein}}) = \frac{P_{SP} (\mu g/mL)}{P_{TP} (\mu g/mL)} \cdot 100 \quad (3)$$

where  $P_{SP}$  represents the soluble proteins contained in the supernatant and  $P_{TP}$  is the total protein content in the sample.

### 2.4 Least Gelation Concentration

The gelation properties of the extracted protein were evaluated according to Lawal et al. (2007), with some modifications. Suspensions of the protein extract were prepared in PBS pH 7 at 5 different concentrations, ranging from 10 to 30 % w/v, and stirred for 60 min at room temperature. The suspensions were transferred to conical test tubes, then heated at 90 °C for 30 min in a water bath (WB-M5, Falc, Treviglio, Italy) and immediately cooled in a beaker containing cold water (4 °C). The samples were then stored overnight at 4 °C and the least gelation concentration was determined as the minimum concentration at which the suspension did not exhibit fluidity when inverted.

## 2.5 Rheological Analysis

### 2.5.1 Temperature sweep

Temperature sweeps tests were conducted to evaluate the gelation capacity of CPE by mimicking heat-induced gelation. For this purpose, a modular compact rheometer (MCR 102, Anton Paar, Austria) equipped with a Peltier temperature device and a plate-plate geometry, was used. Suspensions of CPE (15 % w/v) were prepared using PBS at pH 7 as above described. Right after preparation, samples were placed between the plates of the rheometer, with a final gap of 1 mm. First, the samples were exposed to equilibrium (5 min, 20 °C), then heated up to 90 °C (heating interval) with a rate of 3 °C/min, held at this temperature for 10 min (holding interval), cooled down to 20 °C (cooling interval) with a rate of 5 °C/min and then kept at this temperature for 5 more min. Deformation and frequency were maintained constant throughout the test at 0.01 % and 0.5 Hz respectively. The storage modulus ( $G'$ , Pa), the loss modulus ( $G''$ , Pa), the loss factor ( $\tan(\delta)$ , dimensionless) and the complex viscosity ( $\eta^*$ , Pa s) were measured using the RheoCompass® software (Anton Paar, Austria). Moreover, the onset temperature ( $T_{\text{onset}}$ ), the temperature at which the  $G'$  and  $\eta^*$  increase significantly, was determined (Nnyigide and Hyun, 2020).

### 2.5.2 Amplitude sweep

Amplitude sweep tests were conducted immediately after the thermal treatment described in Section 2.5.1. Samples were then subjected to increasing deformation from 0.01 % to 100 % (logarithmic ramp) at a constant frequency of 0.5 Hz and maintaining the temperature constant at 20 °C. The linear viscoelastic region (LVR), commonly defined as the strain level at which the reduction in the initial storage modulus did not exceed 5 % (Kaboarani and Blanchet, 2014), was determined by analysis of the software as the strain range within which  $G'$  deviates by no more than 3 %. The yield strain (%) was identified as the yield point in the LVR.

### 2.5.3 Frequency sweep

Frequency sweep tests were conducted on samples first subjected to the thermal treatment described in Section 2.5.1 and performed over a frequency range of 0.1 – 100 Hz, maintaining constant temperature (20 °C) and deformation (0.01 %), within the LVR.

## 2.6 Statistical Analysis

All analyses were made in triplicate and results were reported as the mean ( $\pm$  standard deviation) of the evaluations. The results were statistically examined by using one-way analysis of variance (ANOVA) while significant differences were identified through Tukey's test at a significance level of  $p < 0.05$ . All statistical computations were carried out using the statistical analysis software JMP Pro 18 (SAS Institute. Inc. Cary, NC, USA).

## 3. Result and Discussion

### 3.1 Protein Extraction

The protein content of the CPE and the efficiency of the extraction method are reported in Table 1.

Table 1: Impact of the extraction method on protein content and extraction yields from *Acheta Domesticus* flour

|     | Protein content<br>$\text{g}_{\text{Protein}}/100 \text{ g}_{\text{CPE}}$ | Extraction yield<br>%       | Protein Extraction yield<br>% |
|-----|---|-----------------------------|-------------------------------|
| CF  | $74.24 \pm 0.51^{\text{d}}$   | -                           | -                             |
| DCF | $77.87 \pm 0.84^{\text{c}}$   | -                           | -                             |
| CPE | $85.65 \pm 0.72^{\text{b}}$   | $55.71 \pm 1.02^{\text{e}}$ | $61.28 \pm 0.82^{\text{a}}$   |

Values followed by different letters (a-e) are significantly different ( $p < 0.05$ ).

As expected, the protein content in DCF samples increased compared to CF, due to the fat removal, while the extract showed a protein increase close to 10 % and 15 % compared to DCF and CF, respectively. The protein content reported across studies for *Acheta domesticus* varies significantly based on defatting and extraction methods used. The data obtained in this study falls within the ranges reported in the literature (Ma et al., 2023), where the nitrogen to protein conversion factor used is 6.25 (thus including chitin nitrogen), since values for protein content are reported from 46 % to 85 % and protein extraction yields between 58.3 – 78.5 %, depending on factors like extraction time, temperature, and pH. Moreover, this data surpasses those reported by Laroche et al. (2019) who found extraction yields in the range of 31.0 – 38.9 % as well as the studies conducted by Amarender et al. (2020) who reported a protein content of 69.69 % with an extraction carried out by ascorbic

acid. The used extraction method is an established method for efficient protein extraction from insects and the results obtained demonstrate the effectiveness for both the defatting procedure, that typically results in protein contents ranging from 65 – 75 % (Hall et al., 2017), and the extraction ones, for which are reported, for similar extraction methods, protein concentrations in the range of 70 – 85 % (Yi et al., 2013).

### 3.2 Solubility

Protein solubility is a critical factor for the production of protein-based food, necessary to achieve uniform texture and prevent undesirable phase separation in food applications (Mokaya et al., 2024). The solubility of CPE at pH 7 was found equal to  $59.69 \pm 0.03 \text{ g}_{\text{Soluble Protein}}/100\text{g}_{\text{Total Protein}}$ . This value is in agreement with Edward et al. (2023) who investigated the solubility of cricket protein isolates obtained by chemical extraction followed by an isolation step with ammonium sulphate.

### 3.3 Least Gelation Concentration

The gelation capability of CPE has been initially investigated by the determination of the minimum protein concentration required to form a gel under specific heating conditions. When globular proteins are thermally denaturated, they lose their native structure and expose reactive sites. This leads to aggregation as proteins interact through covalent and non-covalent bonds, forming a continuous network. The resulting gel traps water within its matrix, giving it solid-like properties. The process is influenced by several factors such as pH and protein concentration (Chia et al., 2024). In this research, it has been individuated the formation of a gel starting from concentrations of 15 % (w/v). Similarly, Yi et al. (2013) only investigated two concentration, 3 % w/v and 30 % w/v and found that, while the lower concentration led to the formation of large aggregates, at 30 % w/v a strong gel was formed.

### 3.4 Rheological Analysis

The gelation capability of CPE (15 % w/v) was further tested through temperature sweeps tests (Figure 1). During the overall cycle (Figure 1a),  $G'$ , which is correlated to the recoverable elastic energy stored during a deformation, remained predominant on  $G''$ , which represents the energy dissipated during a deformation. This predominance is an indication of a largely solid-like characteristic. Moreover, both  $G'$  and  $G''$ , as well as  $\eta^*$  (Figure 1b), increased sharply during the beginning of the heating cycle as an indication of the start of protein network development. This phenomenon can be correlated to the formation of molecular entanglements and subsequently of protein aggregates (Martin et al., 2014). Given that the primary increase in  $G'$  predominantly occurred during the heating phase, it can be inferred that the onset of CPE gelation was primarily driven by the denaturation of proteins with low thermal stability. The onset temperature was measured at  $31.10 \pm 3.70 \text{ }^\circ\text{C}$ , as determined by the software analysis. This temperature, defined as the point at which the structural transition of the protein begins, corresponds to the abrupt increase in the magnitude of the storage modulus and the complex viscosity as well as a significant change in their slopes (Nnyigide and Hyun, 2020). During the holding phase at  $90 \text{ }^\circ\text{C}$ , it can be noticed a slight continuous increase of the viscoelastic moduli, hinting at the establishment of stronger and/or more intermolecular crosslinks within the CPE protein networks. Finally, both  $G'$  and  $G''$  moduli further slightly increased during the cooling phase. In this phase, the continual strengthening of the gel network could be due to short range non-covalent interactions such as hydrogen bonds (Martin et al., 2014) that, reportedly, play an important role in enhancing the elasticity of heat-induced gels of other insect proteins (Scholliers et al., 2020). However, the minimal increase in  $G'$  observed during the cooling phase suggests that its role in determining the final  $G'$  of CPE gels was relatively limited, in contrast to the heating phase, that played a more significant role, as the majority of the gel structure had already developed before the cooling process began. At the end of the temperature sweep test, the value of  $G'$  is  $10^4$  times higher than the starting value, reaching  $3.26 \cdot 10^5 \text{ Pa}$ , comparable to the results of Scholliers et al. (2020), who reported comparable values of  $G'$  at the end of temperature sweep test in both pure *Zophobas morio* larvae and pork protein extracts.

The  $\tan(\delta)$  quantifies the ratio of the energy dissipated through viscous flow to the energy stored via elastic deformation, ranging from 0 in ideal solids to infinity in ideal liquids. Gel-like materials generally have  $\tan(\delta) < 1$ , with solid gels around 0.01 and weaker, paste-like gels exceeding 0.1 (Ross-Murphy, 1994). On that account, the final value of  $\tan(\delta)$  in this study resulted in  $0.28 \pm 0.03$  as indication of a weak gel-like characteristic. This value is in accordance to Chia et al. (2024) who performed similar analysis focusing on heat-induced gelation of *Hermetia illucens* protein.

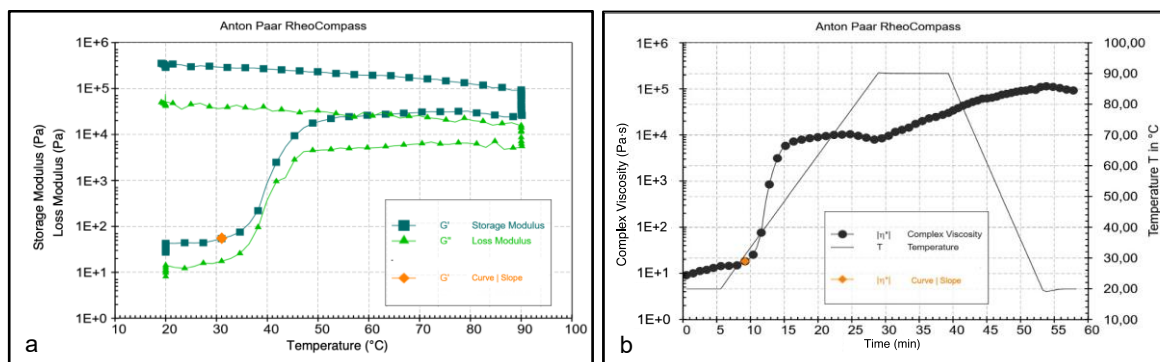


Figure 1: The evolution of  $G'$  and  $G''$  (a) and the complex viscosity ( $\eta^*$ ) (b) during temperature sweep of a Cricket Protein Extract suspension (15 % w/v) at pH 7. The orange rhombuses mark the  $T_{onset}$ .

To obtain more information on the gel structure, firstly, amplitude sweeps were performed (Figure 2a) to detect the LVR as a measure of the gel stability. During the test, the formed structure of the gel is independent from the applied strain amplitude up to a certain value that limits the LVR, beyond which the gel structure breakdowns, resulting in a decrease of the loss modulus. The test performed showed gel characteristic and a weak viscoelastic solid behaviour since  $G'$  remains constantly higher than  $G''$  and  $\tan(\delta)$  is consistently equal to  $0.23 \pm 0.04$  throughout the LVR, with a yield strain of  $0.07 \pm 0.01$  %. This value is relatively low, compared to values reported in the literature for insects (Yi et al., 2013), however the value of  $G'$  in the linear region is significantly higher (Scholliers et al., 2020), hinting at less stable but stronger gels.

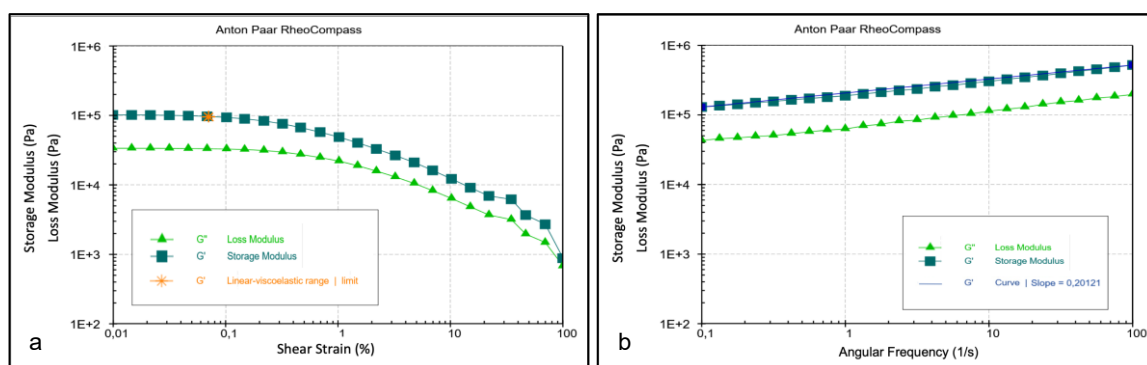


Figure 2: Amplitude sweep (a) and frequency sweep (b) of the Cricket Protein Extract (15 % w/v) gel formed.

Finally, the frequency sweep tests conducted are reported in Figure 2b. As Tunick (2011) reported, protein gels can be classified into three types: entangled biopolymers networks, chemical, or physical gels. Entangled networks form soft gels, exhibiting strong  $G'$  dependence on the frequency and a  $G'-G''$  crossover, indicating liquid-like behaviour at low frequencies and solid-like properties at higher ones. Cross-linked or chemical gels, characterized by permanent covalent networks, are strong and exhibit minimal frequency dependence. Finally, physical gels, intermediate in strength, show some frequency dependence but lack a moduli crossover. The frequency sweep of the CPE gel shows an overall predominance of  $G'$  over  $G''$ . Throughout the test,  $\tan(\delta)$  is consistently equal to  $0.28 \pm 0.02$  and the storage modulus curve slope is found equal to 0.20, indicating minimal dependence of  $G'$  from frequency. This parameter is equal to 0 for chemical cross-linked gels and a positive number for elastic or physical gels (Rao, 2007). These results confirm the formation of physical weak gels with pronounced solid-like behaviour, in accordance with Scholliers et al. (2020), who found similar characteristic while analysing the gelation properties of *Zophobas morio* protein extracts.

#### 4. Conclusions

This study demonstrated that *Acheta domesticus* protein extracts exhibit promising techno-functional properties, making them suitable candidates for food applications. Firstly, the alkaline extraction method has been proved suitable for isolating the protein fraction from commercial *Acheta domesticus* flour, significantly enhancing protein content and extraction yield. Functional properties' evaluation revealed that the protein extract exhibited a discreet amount of soluble protein at neutral pH, while the least gelation concentration was determined to be 15 % (w/v), indicating the formation of a gel network through hydrophobic interactions and disulfide bonds.

Rheological analysis led to the identification of the onset gelation temperature ( $31.10 \pm 3.70$  °C) and a relatively small yield strain. Also, the tests confirmed the formation of weak gels with strong solid-like behaviour ( $G'$  overall predominant on  $G''$ ) and a high final  $G'$  value, comparable to meat proteins. These findings highlight the potential of *Acheta domesticus* protein extracts as a sustainable and functional alternative to conventional protein sources, offering a viable option for meat analogue production and other food systems.

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