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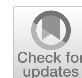
RESEARCH



# Impact of High-Pressure-Assisted Extraction of *Alphitobius diaperinus* and *Tenebrio molitor* on Protein Characteristics

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## Abstract

The effectiveness of high pressure-assisted extraction (HPAE) in improving insect protein extraction and functionality was studied. Defatted insect powders of *Tenebrio molitor* and *Alphitobius diaperinus* were pressure treated at 100–300 MPa for 5 min at 25 °C. Protein content, molecular weight distribution (MWD) of protein fractions, emulsifying activity (EA), and modifications in the secondary structure of the proteins were assessed for the different fractions. In comparison to control, HPAE at 100, 250, and 300 MPa enhanced protein extraction by approximately 18% for both insect species. A 22% increase in protein extraction was observed for *A. diaperinus* at 200 MPa compared to the control. A clear decrease in EA was noted for both *T. molitor* and *A. diaperinus* proteins extracted at 150, 200, 250, and 300 MPa. The MWD analysis indicated that HPEA increased the intensity of protein bands between 10–20 kDa and around 37 kDa for both insect species. This study demonstrates the potential of high pressure to not only enhance protein extraction by up to 22% but also to modify the emulsifying activity of insect proteins.

**Keywords** Insect proteins · High-pressure extraction · Protein solubility · Emulsifying activity · Secondary structure

## Introduction

Global population growth and rising consumer demand for proteins that support a healthy lifestyle prompted the food researchers to evaluate the use of insect proteins as food and feed. Insect proteins exhibit high feed conversion efficiency and can be reared on organic side-streams (such as food and agricultural by-products). Insect farming requires significantly less land and water than cattle farming and produces fewer greenhouse gases and less ammonia than cattle or pigs (van Huis & Oonincx, 2017). The European Food Safety Authority (EFSA) Panel on Nutrition, Novel Foods, and Food Allergens has issued positive opinions on several insect-based foods as novel foods under regulation (EU) 2015/2283. These include dried mealworm (*Tenebrio molitor*), frozen and dried formulations of migratory locust

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(*Locusta migratoria*), whole house crickets (*Acheta domesticus*), and various forms of lesser mealworm (*Alphitobius diaperinus*), including frozen, paste, dried, and powdered. The edible insect proteins have potential applications for formulating nutrient-rich food materials in diverse market segments, including human food, pet food, aquaculture, animal feed, fertilizer, and pharmaceuticals and cosmetics.

Despite being a rich source of proteins, lipids, fiber, and minerals such as calcium, iron, and magnesium (van Huis, 2013), most western consumers find them more acceptable if they are integrated in the food formulation as whole milled powders or as functional fractions, such as proteins. Insect protein has a relatively high biological value and bioavailability, and its content can vary depending on the insect species and the development stage. Alkaline extraction followed by isoelectric point precipitation is the conventional method for isolating protein fractions (Melgar-Lalanne et al., 2019). Alkaline extraction followed by isoelectric point precipitation selectively solubilizes insect proteins at an alkaline pH, resulting in approximately 70% crude protein (Melgar-Lalanne et al., 2019). Protein fractions extracted and precipitated at the isoelectric point from black soldier fly (*Hermetia illucens*) and lesser mealworm (*Alphitobius diaperinus*) demonstrate emulsifying properties similar to those of whey protein isolate (Wang, et al., 2021a & 2021b).

High-pressure processing (HPP), gamma irradiation, pulsed electric field, and ultrasound are some examples of novel technologies that have been employed to improve extraction efficiency by minimizing both extraction time and solvent usage (Rashwan et al., 2025). These nonthermal lethal agents could potentially disrupt insect tissues, cell walls, and enhance the mass transfer of soluble materials (such as proteins) into solvents. Since extraction is typically carried out at or near ambient temperature over a short duration, thermal degradation of constituents is also minimised. Physical methods of extraction, such as high-pressure-assisted extraction (HPAE) have been used to successfully enhance the extraction of bioactive compounds, fat, and proteins from various plant proteins and herbs (Ağçam et al., 2021; Kumar et al., 2021; Okur et al., 2023). Extraction efficiency of pressure is likely matrix dependent.

Despite its proven potential, the application of HPAE to edible insects remains largely unexamined. Systematic studies on the impact of HPAE on different insect species are needed to identify HPAE treatment conditions to maximize protein yield and quality. Very limited insights are available to elucidate the impact of HPAE on extracted protein structure, solubility, emulsification, and amino acid profiles. Accordingly, the objective of this study was to evaluate the impact of HPAE treatment on edible powders of *A. diaperinus* and *T. molitor* in enhancing protein extraction, as well as to assess how this treatment affects the functional properties of the extracted proteins.

## Materials and Methods

### Defatting Process

*Tenebrio molitor* (TM) and *Alphitobius diaperinus* (AD) powders were purchased from DeliBugs (Lelystad, the Netherlands). AD and TM powders were defatted following the method provided by Wang et al. (2021a). Hexane (250 mL) was added to 50 g of insect powder and mixed (600 rpm, 22 °C; Isotemp, Fisher Scientific, Pittsburgh, USA) for 1 h. The mixture was left to stand until complete phase separation occurred, after which the solvent layer was carefully decanted. The solid fraction was mixed again with hexane (250 mL), and the separation–extraction process was repeated twice. The defatted insect powder was dried at 35 °C overnight.

### Sample Preparation

Defatted insect fractions (0.8 g) were suspended by transferring into sterile higher barrier polyethylene pouches (5 by 3.5 cm; Fisher Scientific) and mixed with 4.2 mL of 0.15 M NaCl solution (pH 8.3, Sigma–Aldrich, St Louis, USA). The alkaline condition combined with low ion concentration (<0.15 M NaCl) were selected to enhance protein extraction. These conditions have been reported to be optimal for extracting proteins in the control sample (Jiang et al., 2021). The sample pouches were sealed using a heat sealer (American International Electric, Whittier, USA), minimizing air retention, and stored at 4 °C overnight prior to treatment.

### High-Pressure-Assisted Extraction

HPAE was carried out using a bench scale high-pressure processor (PT-1, Avure Technologies Inc., Kent, USA) (Ağçam et al., 2021). Food-grade propylene glycol (Brenntag, Reading, PA) was employed as pressure-transmitting medium. K-type thermocouple sensors (model KMQSS-040U-7, Omega Engineering, Stamford, USA) were used to record the temperature of the bath, sample, and the pressure chamber, and the data were logged by a data acquisition computer.

Insect sample pouches (see the “Sample Preparation” section) were loaded inside a 10-ml polypropylene syringe (Becton Dickinson and Company, Franklin Lakes, USA). The remaining space in the syringe was filled with water. This helped to ensure that the area surrounding the sample pouch had similar thermal characteristics to the sample itself during pressurization (Patazca et al., 2007). The syringe was preconditioned for 2 min using an ice-water bath. Then it was loaded into the pressure chamber, and the vessel was

closed. Samples were treated at pressures of 100, 150, 200, 250, and 300 MPa, with a final process temperature of 20 °C and a pressure holding time of 5 min. All high-pressure experiments were carried out at least 3 times.

Previous studies have reported that such certain HPP treatment conditions promote protein solubilization and denaturation (Yang, 2016). However, the extent of protein unfolding will depend on the pressure level applied. Temperature increases from 30 to 40 °C have been reported to produce a decrease in the protein solubility in insect powders (Bolat et al., 2021). Table 1 summarizes representative pressure-thermal treatment conditions during HPAE studies. The final process temperature under pressure during HPAE was kept <25 °C. HPAE treatment time did not include pressure come-up time (~30 s) or depressurization time (2 s). Similarly, untreated control samples were preconditioned for 2 min using an ice-water bath and then kept at room temperature for 5 min.

HPAE-treated samples were first filtered with medical gauze and then centrifuged at 10,000 rpm for 10 min (room temperature). The pellets were discarded, and the supernatants were kept at 4 °C until further analysis. HPAE and control samples were kept in a refrigerator (<5 °C) until further analysis.

## Determination of Nitrogen Content in Insect Powders

Crude protein content of *T. molitor* and *A. diaperinus* powders and their defatted powders were determined following the Dumas method (rapid N exceed, Elementar, Langenselbold, Germany) in duplicated using L-Aspartic acid (Sigma–Aldrich, CAS nr. 56-84-8) as standard.

## Determination of Amino Acid Composition

The amino acid composition of the untreated insect powder was analyzed according to the International Standards ISO 13903:2005 and ISO 13904:2005. The total protein content was calculated based on the overall amino acid content. Kp values were determined using the ratio of the sum of amino acid residue weights to nitrogen content (Janssen et al., 2017).

## Analysis of Total Soluble Protein Content

The total soluble protein content of control and HPAE supernatants was quantified using a colorimetric method with the Pierce™ bicinchoninic acid (BCA) protein assay kit (ThermoScientific, Rockford, IL, USA). Bovine serum albumin (BSA) was used as the standard for protein quantification in the BCA assay. The results are expressed as BSA-equivalent values (mg/mL).

## Molecular Weight Distribution

After the HPAE treatment, protein samples were centrifuged and the supernatant solution (with protein concentration around 10–12 mg/mL-determined by BCA analysis) were diluted 1:6 v/v in water. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Laemmli, 1970) was used to determine the molecular weight distribution of the control and HPAE samples. Ten µL of protein solution at 1% (wt/v) were loaded onto a 4–15% Mini-PROTEAN® TGX™ pre-cast gel (Bio-Rad, Hercules, CA, USA), and electrophoresed at 200 V for 35 min. The gel was stained with Coomassie blue G250 (Bio-Rad) for 45 min, followed by destaining for 2–3 h. A 250 kDa to 10 kDa molecular weight marker (Precision Plus Protein Dual Color Standard, Bio-Rad) was used as the standard. Images from SDS-PAGE gels were analyzed using ImageJ (Schneider et al., 2012) to obtain density data.

## Emulsifying Activity

The emulsifying activity of protein supernatants (from control and HPAE treated) was evaluated at 1 g/L protein concentration (Purschke et al., 2018). In brief, 0.250 mL of protein solution and 0.250 mL of sunflower oil were homogenised in a graduated vial at 12,000 rpm for 2 min using a vortex (due to the low volume of sample). The sample was centrifuged at 3250 g for 10 min at room temperature. Duplicates were performed for each treatment. The height of the

**Table 1** Representative pressure, thermal processing conditions during 5 min pressure holding time[a] of insect power samples

| Target treatment pressure (MPa) | Average initial T (Ti, °C) | Average processing T (Tp) during 5 min pressure holding time (°C) | Average processing pressure (P, MPa) |
|---|---|---|---|
| 100 | 7.0 ± 1.5 | 14.9 ± 2.5 | 97.4 ± 2.1 |
| 150 | 7.7 ± 1.4 | 17.7 ± 1.9 | 151.4 ± 1.8 |
| 200 | 6.5 ± 1.5 | 18.9 ± 1.7 | 199.5 ± 3.0 |
| 250 | 7.5 ± 1.3 | 21.3 ± 1.5 | 253.2 ± 1.8 |
| 300 | 9.5 ± 2.1 | 20.5 ± 1.7 | 298.9 ± 3.9 |

[a]Pressure holding time does not include pressure-come up time (30 s) and depressurization time (2 s)

emulsified layer was measured, and the emulsifying activity was calculated using the following equation: [1].

$$\%EA = \frac{H_{el}}{H_{tot}} \times 100 \quad (1)$$

where $H_{el}$ is the height of the emulsified layer, and $H_{tot}$ is the total height of the emulsion in the vial.

### Spectral Data Acquisition

Spectral acquisition was performed using an Agilent 4500 portable spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with an attenuated total reflectance (ATR) diamond crystal (3-bounce) and a thermoelectrically cooled deuterated triglycine sulfate (dTGS) detector. An aliquot of 5 µL of the supernatant was placed onto the ATR crystal and vacuum-treated to remove water interference. A background spectrum was acquired before each reading, and 128 scans were co-added with a resolution of 8 $cm^{-1}$ to improve the signal-to-noise ratio. The spectral data were recorded using Agilent MicroLab PC software (Agilent Technologies Inc., Danbury, CT, USA). Spectral acquisition was performed three times under the same conditions.

### Spectra Deconvolution Analysis

Changes in the secondary structure of proteins were analyzed through spectral deconvolution in the 1500 to 1700 $cm^{-1}$ region (Yang et al., 2015) using OriginPro 2024b (OriginLab, Northampton, MA, USA). The spectral data were first normalized, smoothed using a 5-point Savitzky-Golay filter, and then subjected to a 7-point Savitzky-Golay second-derivative transformation. The resulting spectra were fitted with Gaussian band profiles using the Multipeak option (Hackshaw et al., 2023).

### Statistical Analyses

One-factor analysis of variance (ANOVA; OriginPro 2024b, OriginLab, Northampton, MA, USA) was performed using the Fisher test, with a significance level set at $p < 0.05$.

## Results and Discussion

### Amino Acid Profile, Kp, and Protein Content of the Insect Powders and Defatted Powders

The protein content in *T. molitor* and *A. diaperinus* powder was calculated based on the quantification of 18 amino acids out of 20 and total nitrogen content. Unlike the conventional nitrogen-to-protein conversion factor ($K_p$) of 6.25 used for most foods, insects require species-specific $K_p$ values due to the presence of non-protein nitrogenous compounds such as chitin. In this study, the calculated $K_p$ values were 4.50 for *T. molitor* and 5.06 for *A. diaperinus* (Table 2), consistent with those reported by Janssen et al., (2017) (4.75 and 4.86, respectively). Using these $K_p$ values, the protein contents of *T. molitor* and *A. diaperinus* powders were calculated to be 37.49% and 45.59%, respectively. These values are slightly lower than those obtained by Janssen et al., (2017), with protein contents of 44.74% for *T. molitor* and 49.58% for *A. diaperinus*.

The discrepancies may be attributed to differences in insect rearing conditions, sample biological variations or extraction methods. In this study, to evaluate the potential benefits of high-pressure extraction on insect proteins, we utilized commercially available powders of *Tenebrio molitor* (TM) and *Alphitobius diaperinus* (AD). These commercial samples may originate from different insect batches, where interspecific phenotypic variation could be a contributing factor. While factors such as diet, rearing conditions, and interspecific phenotypic variation may indeed influence protein composition, investigating these variables was beyond the scope of this study. Given the promising results of this study, future research could this variability by using insect

**Table 2** Amino acid composition, nitrogen content, and calculated Kp factor and protein content of untreated TM (*Tenebrio molitor*) and AD (*Alphitobius diaperinus*) larvae powders

| Type aa | TM powder (%) | AD powder (%) |
|---|---|---|
| Asp | 3.95 | 4.19 |
| Glu | 5.91 | 6.55 |
| Ala | 3.53 | 5.95 |
| Arg | 2.60 | 3.02 |
| Cys | 0.51 | 0.47 |
| Phe | 1.66 | 1.83 |
| Gly | 2.54 | 3.29 |
| His | 1.48 | 1.33 |
| Ile | 2.24 | 2.36 |
| Leu | 3.99 | 4.72 |
| Lys | 2.77 | 3.00 |
| Met | 0.64 | 0.72 |
| Pro | 3.72 | 3.70 |
| Ser | 2.03 | 2.25 |
| Tyr | 2.90 | 3.65 |
| Thr | 1.84 | 2.18 |
| Trp | 0.58 | 0.47 |
| Val | 3.12 | 3.72 |
| Nitrogen content | 8.33 ± 0.04 | 9.01 ± 0.22 |
| Kp | 4.60 | 5.06 |
| Protein content | 37.49 | 45.59 |
| Non protein content | 14.57 | 10.72 |

powders prepared from specimens reared under controlled laboratory conditions.

It has been reported that temperature significantly influences the fat and protein content of *T. molitor and A. diaperinus* (Bjørge et al., 2018). Diet plays a significant role in shaping the protein and amino acid composition of *T. molitor* larvae. For instance, a wheat bran-based diet supplemented with pea and rice protein has been shown to yield the highest protein levels in the larvae, along with a reduced fat content. In contrast, cassava-based diets resulted in the highest concentrations of total and essential amino acids (Kröncke & Benning, 2023). Generally, substrates with higher protein content tend to produce larvae with correspondingly higher protein levels (Rumbos et al., 2020). While it is evident that diet influences the amino acid composition of *T. molitor*, no clear or consistent pattern has emerged linking the amino acid content of the diet directly to the amino acid profile of the larvae (Zhang et al., 2019).

Defatting is typically the first step in producing insect-derived ingredients with enhanced protein content and functionality (Choi et al., 2017; Gravel et al., 2021). The removal of lipids increased the nitrogen content of the insect powder from 8.33% to 12.63% for *T. molitor* and from 9.01% to 13.03% for *A. diaperinus*. The same Kp factor was used for full-fat and defatted insect powders, resulting in calculated protein contents of 56.83 and 65.94% for *T. molitor* and *A. diaperinus*, respectively.

## Protein Content

The protein content of HPAE fractions is shown in Fig. 1. The protein content of *T. molitor* (Fig. 1a) solutions obtained after the HPAE (at 100, 200, 250, and 300 MPa) was significantly higher than the control samples. About a 22% increase in the protein content for those samples treated at 200 MPa was noted. Accordingly, HPAE for *A. diaperinus* resulted in solutions with significantly higher protein content than the control for 150, 200, 250, and 300 MPa HPAE treatment, with the highest protein content (about 11.8 mg/mL) for samples obtained after 200 MPa treatment. Our results point out that regardless of the insect species, HPAE at 200, 250, and 300 MPa lead to a higher protein solubility than the control sample, with the 200 MPa treatment yielding the highest protein extraction.

Kim et al., (2021) reported significantly higher protein content of the pressure-treated *P. brevitarsis seulensis* compared with the control. These authors found that increasing the pressure up to 200 MPa resulted in the highest protein content. Similar results were obtained in the present study with both insect species, but only *T. molitor* showed a clear protein solubility maximum at 200 MPa (Fig. 1a). For *A. diaperinus,* the maximum protein extraction was also obtained at 200 MPa; however, the protein content obtained at 250 and 300 MPa was not significantly different from the one obtained at 200 MPa (Fig. 1b). Moreover, our results showed that the protein solubility for *A. diaperinus* was enhanced by HPAE treatment at 150 MPa or higher, while *T. molitor* showed a clear maximum at 200 MPa. It was noteworthy that protein solutions from *T. molitor* processed at 100 and 150 MPa showed that only 100 MPa gave a significantly higher protein content than the control sample. The observed increase in protein solubility is likely attributed to pressure-induced unfolding of protein structure which facilitates extractability. However, at pressures above 200 MPa, further denaturation may lead to aggregation and a subsequent reduction in solubility. This trend was also described by Zhang et al., (2017). Marcos et al., (2010) reported maximum solubility of myofibrillar and sarcoplasmic proteins at

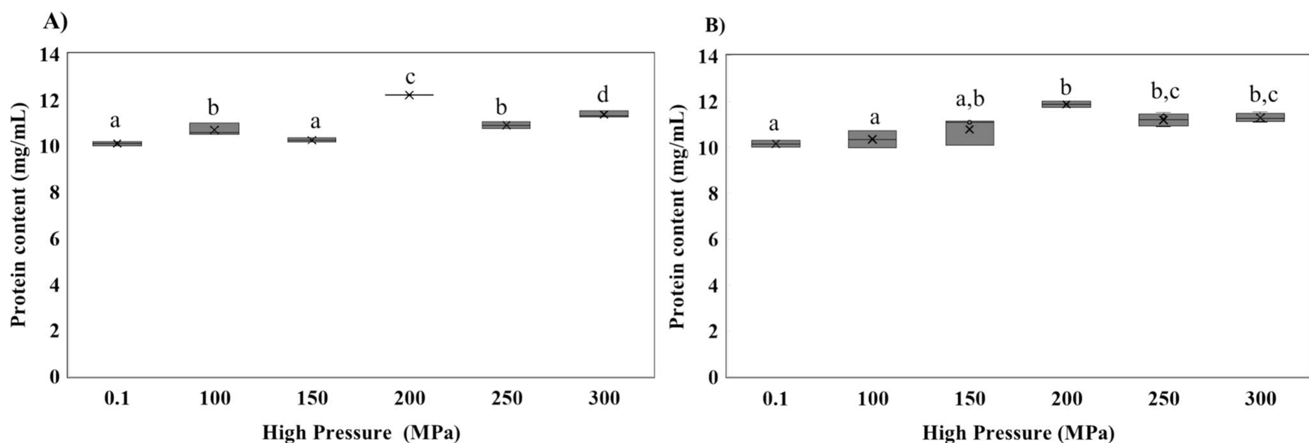


**Fig. 1** Protein content (mg/mL) (estimated using BCA method) of samples extracted from *T. molitor* (**A**) and *A. diaperinus* (**B**) species, by applying HPAE, using various pressures from 0.1 MPa (control) to 300 MPa at 25 °C for 5 min. Means that do not share a letter are significantly different ($p < 0.05$)

intermediate pressures, followed by declines due to aggregation. Yousefi and Abbasi, (2022) similarly suggested that excessive pressure promotes the formation of insoluble protein aggregates. HPAE improves extractability by disrupting cellular structures and facilitating the release of both hydrophilic and hydrophobic compounds (Braspaiboon & Laokuldilok, 2024). This study did not examine the digestibility or potential allergenicity of HPAE-treated proteins. Further research is needed to address these aspects before such insect proteins can be considered for inclusion in food formulations intended for human consumption.

## Molecular Weight Distribution (SDS-PAGE)

The molecular weight distribution of the controls and HPAE-treated samples obtained by SDS-PAGE at reducing conditions are shown in Fig. 2 and Fig. S2. Accordingly, irrespective to the pressure intensity of the treatment, *T. molitor* (Fig. 2a) presents protein bands between 10 and 37 kDa, with an intense band between 10 and 15 kDa, which is likely associated to anti-freeze proteins (Li et al., 2023), a fraction below to 20 kDa, and a band slightly above 25 kDa (only for the control sample) which can be linked to cuticle proteins (Gkinali et al., 2022). Another band is present around 37 kDa, which could originate from actine (Gravel et al., 2021); this band becomes more intense and wider for the highest-pressure treatment. *A. diaperinus* (Fig. 2b) presents clear protein bands between 10 and 37 kDa, with an intense fraction below 15 kDa, which is likely associated to anti-freeze proteins (Li et al., 2023), another band between 15 and 20 kDa that is more intense for the elevated-pressure treatments. This has been identified as a cockroach allergen-like protein (Gravel et al., 2021). There are also protein bands between 20 and 37 kDa that may be derived from cuticle proteins, as well as a band at 24kDa from chymotrypsin-like proteinase (Gkinali et al., 2022) and a band at 37 kDa that is as intense and wide as *T. molitor*. Results from the molecular weight distribution after HPAE treatments showed an increase in the intensity of the protein bands between 10–20 kDa and 37 kDa as pressure increases (Fig. 2). For *T. molitor*, the control sample showed two protein bands between 20 and 37 kDa that were not present in the HPAE samples, regardless of pressure. *A. diaperinus* also showed protein bands around 20–25 kDa in the control sample, but for this insect species, after the HPAE treatment, these bands do not fade, on the contrary, they were more intense (Fig. S2). From the molecular weight distribution results based on SDS-PAGE, there was no change in the molecular weight distribution of the protein extracts obtained after the HPAE treatment for *A. diaperinus*. Our results were consistent with the fact that *T. molitor* and *A. diaperinus* proteins are mainly fibrous, therefore possessing a highly ordered structure stabilized with many hydrogen bonds that is less impacted by HPAE treatments due to their compact structure. Boukil et al., (2022) confirmed that *T. molitor* protein structures were modified mainly at 600 MPa. They also found protein bands in the 50–75 kDa region that are not present in our case, while the changes they observed in the 70–275 MPa range align with the results of the present study. Regarding the bands in the 50–75 kDa region, Kim et al., (2020) stated that *T. molitor* water soluble proteins have molecular weights mainly below 25 kDa.

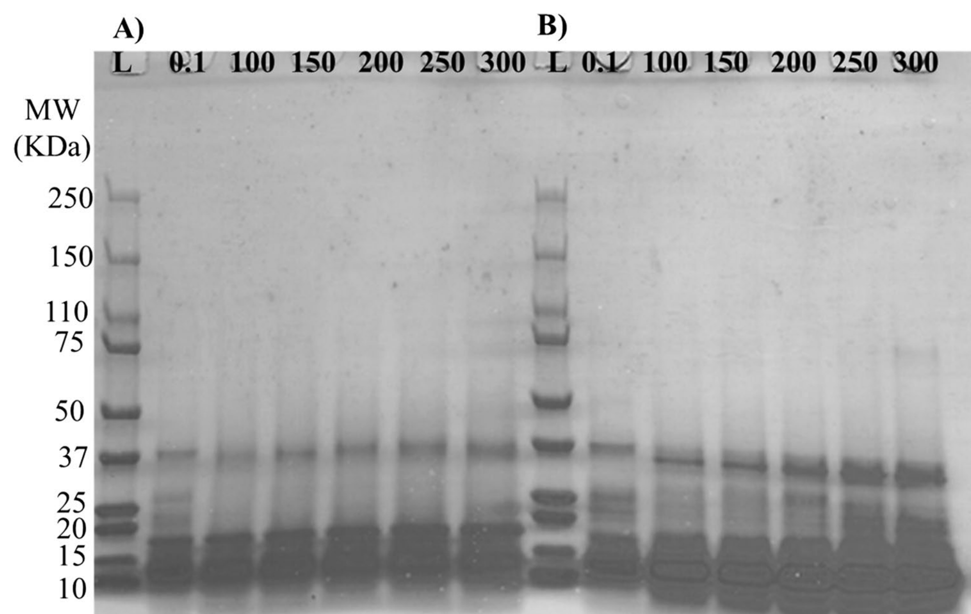


**Fig. 2** Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (4–15% SDS-PAGE; 200 V, 35 min) of proteins extracted from *T. molitor* (A) and *A. diaperinus* (B) species, by applying HPAE using different high pressures from 0.1 MPa (control C), to 300 MPa at 25 °C.L: Ladder

## Effect on Secondary Structure of Proteins

The potential effects of HPAE treatments on the insect protein secondary structure were determined by analysing the protein extracts using Fourier transform spectroscopy in the mid-infrared (Gkinali et al., 2022). Spectral data (Fig. S1) in the amide I region (1600 to 1700 $cm^{-1}$) were initially normalized, smoothed (SG, 5-point), and then subjected to second derivative transformation (SG, 7-point). The data were baseline-corrected, and the absorbance spectra were fitted using Gaussian band profiles with full width at half maximum (FWHM) (Fig. 3).

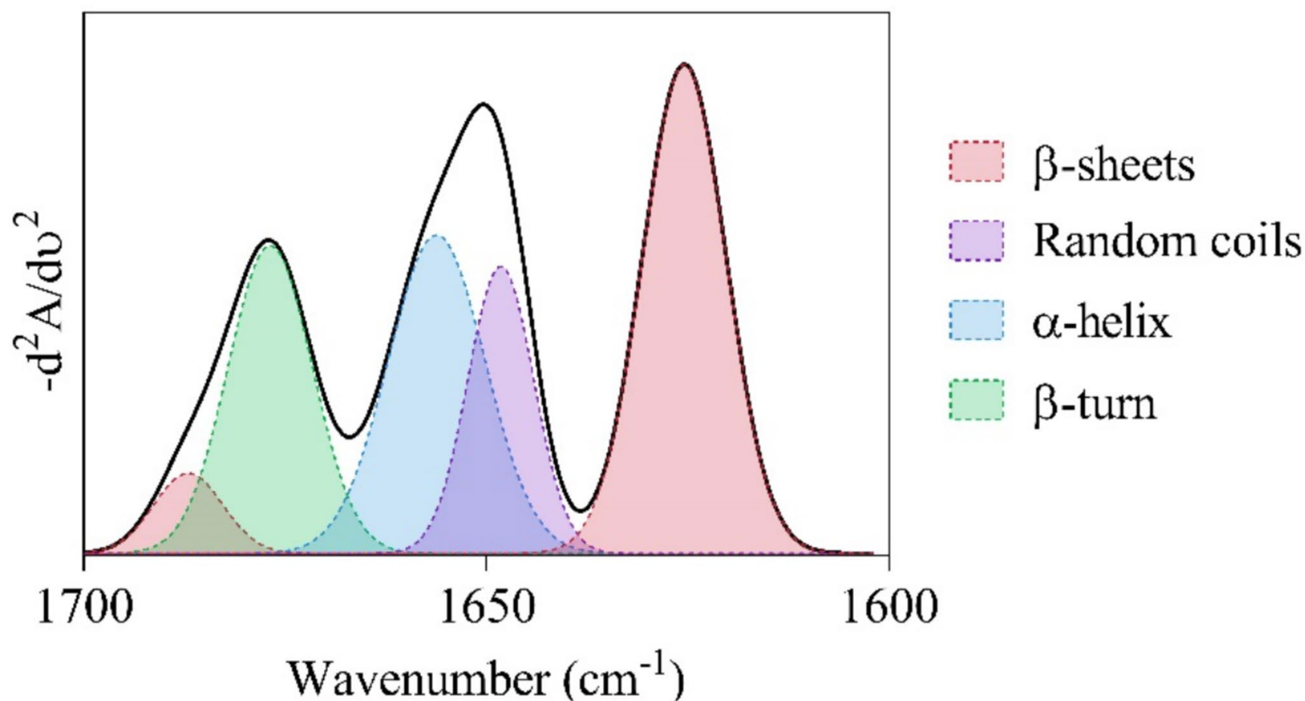


**Fig. 3** Example of curve-fitted inverted second derivative of the amide I region of *T. molitor* Control sample

**Table 3** Percentage of secondary structure of protein extracted from *Alphitobius diaperinus* defatted powder without and with applying different high-pressure extraction treatments

| Functional group | Control (% area) | 100 MPa (% area) | 150 MPa (% area) | 200 MPa (% area) | 250 MPa (% area) | 300 MPa (% area) |
|---|---|---|---|---|---|---|
| Beta-sheets | 34.3 ± 2.0[a,b] | 31.4 ± 0.9[b] | 35.1 ± 2.9[a,b] | 38.1 ± 0.4[a] | 31.8 ± 0.5[b] | 33.7 ± 1.2[b] |
| Random coil | 15.0 ± 1.6[d] | 24.7 ± 2.2[b,c] | 21.0 ± 1.9[c] | 26.0 ± 1.2[b] | 34.3 ± 2.3[a] | 36.3 ± 1.0[a] |
| Alpha-helix | 16.5 ± 3.0[a] | 10.2 ± 1.4[b] | 14.7 ± 1.1[a,b] | 10.1 ± 0.6[b] | 3.7 ± 2.6[c] | 1.6 ± 1.7[c] |
| Beta-turns | 34.1 ± 2.6[a] | 33.7 ± 2.7[a] | 29.1 ± 5.8[a,b] | 25.8 ± 0.2[b] | 24.6 ± 0.5[b] | 28.3 ± 1.9[a,b] |

Different lowercase within the same row indicates significant differences ($p < 0.05$)

**Table 4** Percentage of secondary structure of protein extracted from *Tenebrio molitor* without and with applying different high-pressure extraction treatments

| Functional group | Control (% area) | 100 MPa (% area) | 150 MPa (% area) | 200 MPa (% area) | 250 MPa (% area) | 300 MPa (% area) |
|---|---|---|---|---|---|---|
| Beta-sheets | 40.7 ± 4.2[a] | 39.5 ± 0.6[a] | 41.4 ± 1.9[a] | 41.4 ± 0.2[a] | 43.2 ± 3.0[a] | 42.2 ± 4.0[a] |
| Random coils | 11.4 ± 3.8[b] | 14.4 ± 0.5[b] | 13.8 ± 1.1[b] | 18.9 ± 7.1[b] | 11.1 ± 3.5[b] | 34.4 ± 6.9[a] |
| Alpha-helix | 27.6 ± 3.0[a] | 24.9 ± 1.1[a,b] | 25.0 ± 0.2[a] | 17.6 ± 7.4[b,c] | 26.3 ± 5.3[a] | 12.7 ± 1.1[c] |
| Beta-turns | 20.3 ± 5.0[a] | 21.2 ± 1.1[a] | 19.8 ± 0.7[a] | 22.0 ± 0.6[a] | 19.4 ± 1.2[a,b] | 10.7 ± 9.8[b] |

Different lowercase within the same row indicates significant differences ($p < 0.05$)

The relative percentages of β-sheet (1626 $cm^{-1}$ and 1687 $cm^{-1}$), random coil (1644 $cm^{-1}$), α-helix (1657 $cm^{-1}$), and β-turn (1677 $cm^{-1}$) (Ballon et al., 2024) for *A. diaperinus* and *T. molitor*, Tables 3 and 4, respectively. In the control samples, *A. diaperinus* exhibited higher content β-turn (34.1%) and lower content in β-sheet structures (34.3%) and α-helix (16.5%) than *T. molitor*.

Before HPAE treatment, our insect samples were defatted and suspended in NaCl alkaline solution (salting-in alkaline conditions). Defatting has been reported to favour the formation of β-sheet structures and α-helix for *T. molitor* (Gkinali et al., 2022). Moreover, the salting-in-alkaline procedure produced an increase in β-sheet content compared with the basic alkaline extraction acid precipitation protein (Jiang et al., 2021). The emulsifying properties of proteins are associated with their ability to adsorb at the oil/water interface. Even though protein adsorption is a complex phenomenon, protein structure has been identified as one of its controlling factors. Therefore, the conformational studies via changes in the secondary structure because of HPAE (Tables 3 and 4) could be linked to the reduced emulsifying activity (Fig. 4).

Pressure treatments favoured the destruction α-helix and β-turns and favoured the formation of random coils, especially at 200 MPa for *A. diaperinus* and 250 and 300 MPa for *T. molitor*. In general, β-sheet structures remained mainly unchanged. The deconvolution results showed a pattern similar to what was observed for protein solubility (with HPAE appeared to have decreased hydrogen-bond stability, which may contribute to decrease α-helix content in some HPAE samples (Lv et al., 2020). Random coils and α-helices are typically negatively correlated; increased changes in secondary structure may lead to higher proportions of random coils and lower proportions of α-helices (Carbonaro et al., 2012). The loss of α-helix, coupled with an increase in random coils, suggests that moderate unfolding is the dominant mechanism driving the observed protein structural changes (Peng et al., 2016).

## Emulsifying Activity

Figure 4 presents the emulsifying activity, EA, of *T. molitor* and *A. diaperinus* proteins obtained after HPAE. The emulsifying activity measurements were all performed at the same protein concentration (0.1% w/v) and compared to the control, untreated sample (Fig. 4). The capacity of the extracted proteins to stabilize the oil/water interphase decreases with the applied pressure for both insect species. In the case of *T. molitor*, the emulsifying activity is significantly reduced at pressure values above 100 MPa (Fig. 4a). As for the values of emulsifying activity at 200 MPa, when the highest protein extraction was obtained for both insect species, the reduction is very similar, with a 41% decrease compared to the control for *T. molitor* and a 44% decrease for *A. diaperinus*. In terms of the most suitable HPAE conditions for *T. molitor*, our results suggest that 100 MPa increases protein extraction without compromising emulsifying activity (Fig. 4a). In case of *A. diaperinus*, the HPAE treatment significantly reduces emulsifying activity even at the lowest pressures (Fig. 4b). The results obtained for *T. molitor* align with those of Kim et al. (2021). The authors reported that both control and 100 MPa treated samples of *P. brevitarsis seulensis* had the highest emulsion capacity, which decreased with the increasing high pressure. The fact that *A. diaperinus* did not follow the same trend regarding the EA is a clear indication that the impact on emulsifying activity of the HPAE has to be evaluated individually for each insect species. Previous studies on plant proteins—such as soy protein isolate (Wang et al., 2008), peanut protein (Dong et al., 2019), hazelnut protein (Sarıcaoğlu et al., 2018), and quinoa protein (Luo et al., 2022)—have demonstrated that high-pressure-assisted extraction (HPAE) enhances their emulsifying activity at pressures ranging from 0.1 to 150 MPa. This effect has been attributed to structural modifications in the proteins, leading to the exposure of hydrophobic groups, which may enhance emulsification. Certain components of the protein secondary structure have been linked to interfacial properties. Pokorski

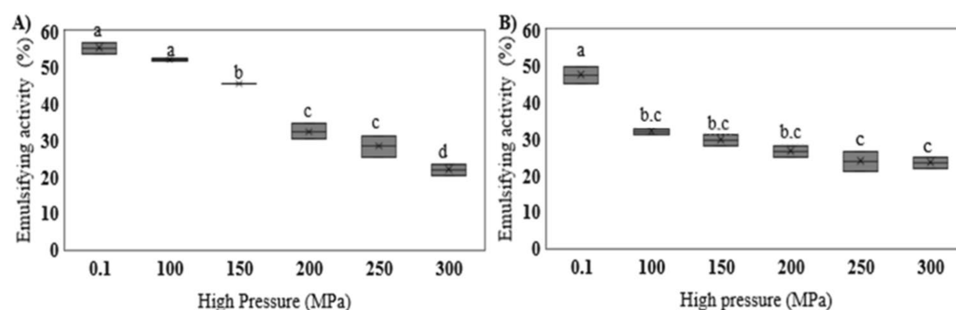


**Fig. 4** Emulsifying activity of protein samples extracted from *T. molitor* (**A**) and *A. diaperinus* (**B**) species, by applying high pressure assisted extraction (HPAE) for various pressures from 0.1 MPa (control) to 300 MPa at 25 °C for 5 min. The protein concentration of samples treated at HPAE were 1% w/v. Means that do not share a letter are significant different ($p < 0.05$)

et al., (2025) used the alfa-helix/beta-sheet ratio as an indication of the balance between flexibility and stiffness of *T. molitor*, *A. domesticus*, and *L. migratoria* proteins extracted with different salt-assisted methods. In our study, the alfa-helix/beta-sheet ratio for *T. molitor* is similar for the control sample and the samples extracted at 100 and 150 MPa. This value decreases for the samples at 200 and 300 MPa that could be correlated with the loss of flexibility and the lower emulsifying activity. However, the ratio of alfa-helix/beta-sheet for *A. diaperinus* decreases for the sample at 100 MPa compared to the control and keeps dropping for samples at 200–300 MPa, which correlate with lower emulsifying activity values. In certain plant proteins, secondary and tertiary structures begin to break down at pressures exceeding 400 MPa and 100–200 MPa, respectively (Barbhuiya et al., 2021). On the other hand, in this current study of HPAE of insect proteins indicates that the alterations in secondary protein structure (Tables 3 and 4) did not enhance protein adsorption at the interface. Regarding the potential use of the extracted proteins in food formulations, emulsifying stability should also be considered; however, this was not measured in the present study.

## Conclusions

Overall, the findings underscore the potential of HPAE for improving protein extraction from insect powders, while also emphasizing the necessity for careful optimization to retain desirable functional characteristics. HPAE at 200 MPa effectively enhanced protein solubility with increases of 22% for *T. molitor* and 18% for *A. diaperinus*. Regardless of the insect species, HPAE at 200, 250, and 300 MPa led to higher protein solubility than the control sample, with the 200 MPa treatment yielding the highest protein extraction. However, emulsifying activity decreased with increasing pressure, particularly for *T. molitor*, suggesting a need to balance protein yield with emulsifying properties during extraction. SDS-PAGE analysis revealed structural changes in proteins after HPAE, particularly the absence of specific bands in treated *T. molitor* samples, indicating potential denaturation. Additionally, FT-MIR analysis showed that HPAE treatments altered protein secondary structures by reducing α-helices and β-turns while increasing random coils, which may impact their functional properties.. Future studies are needed before incorporation of the proteins in food products for human consumption.

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**Data Availability** Data will be available from authors upon request.

## Declarations

**Competing interests** The authors declare no competing interests.

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