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Functional properties of cricket and grasshopper protein isolates☆

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ABSTRACT

This study aimed to assess the extraction of protein isolates from crickets (CPI) and grasshoppers (GPI) and determine their techno-functional, physicochemical, and biofunctional properties. Defatting by solvent remission and supercritical fluid showed the highest efficiency (>90 % in both samples) compared to pressing and gravimetric defatting (<25 % in both samples). Combining alkaline-acid precipitation, sonication, heat treatment, and enzymatic hydrolysis of cricket and grasshopper supercritical fluid defatted flours generated CPI and GPI (95.67 % and 91.60 % protein content, respectively). Significant improvements ($p < 0.05$) were observed in the techno-functional properties of CPI and GPI, including emulsifying capacity (>45 % and 50 %, respectively) and foaming properties (33 % and 225 %, respectively). The physicochemical parameters of CPI and GPI protein isolates were improved, including clarification (brightness 30–33 %, hue intensity 14 %, and saturation 43 %), compressibility (Hausner ratio 5–28 %), and particle surface (74–89 %). CPI and GPI showed higher values ($p < 0.05$) of theoretical protein quality parameters and a higher antioxidant potential (ABTS, DPPH, iron chelation, and ORAC) in a range of 1.0–3.9-fold compared to raw flours. These findings emphasize improvements in the favorable techno-functional, physicochemical, and nutritional characteristics of edible insect protein isolates. CPI and GPI could represent environmentally friendly food ingredients that improve the nutritional value of foods.

1. Introduction

The quest for alternative and sustainable protein sources has gained significant attention in response to growing global concerns regarding food security, environmental sustainability, and the ever-increasing demand for high-quality proteins (Lange & Nakamura, 2023). Edible insects have emerged as an exceptionally promising and ecologically viable source of protein. Crickets and grasshoppers (Orden: Orthoptera) have garnered considerable attention for their protein content and remarkable nutritional profile (Magara et al., 2021; Papastavropoulou et al., 2023).

However, consumers, especially in Western countries, often experience repulsion or aversion towards the idea of consuming whole crickets (*Acheta domesticus*) and grasshoppers (*Sphenarium purpurascens*) due to cultural prejudices or negative perceptions of insects as food (Hartmann & Siegrist, 2017; Kröger et al., 2022). Incorporating edible insects in the form of flour or protein isolates as ingredients can help overcome the barriers to entomophagy, such as neophobia and repulsion (Awobusuyi et al., 2020; Hartmann & Siegrist, 2017). Also, it could offer the benefits of a sustainable food source compared to most animal-based food options (Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019; Sánchez-Velázquez et al., 2024).

Therefore, reducing lipid content in cricket and grasshopper flours or powders eliminates volatile compounds responsible for the sensory characteristics of insects, such as flavor and odor, and enhances protein concentration and solubility (Antunes et al., 2024; Ma et al., 2023; Mintah et al., 2020). One of the primary defatting methods involves using organic solvents such as hexane, acetone, chloroform, and petroleum ether, among others (Rahman et al., 2024). These solvents are highly effective at dissolving lipids from tissues; however, their

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application is limited due to significant environmental concerns and the risk of food contamination from residual solvent traces. Alternative lipid removal techniques, including mechanical pressing and the use of supercritical fluids, could offer a sustainable approach for defatting cricket and grasshopper flours for food applications (Antunes et al., 2024).

Treatments such as alkaline solubilization-acid precipitation, enzymatic hydrolysis, sonication, and heating are effectively applied to insect flours to obtain protein isolates (Damasceno et al., 2023; Jeong et al., 2021). These methods have been used individually or in combination on cricket and grasshopper flours. However, it is important to note that the protein extraction processes may influence the integrity and techno-functionality of proteins as food ingredients (Mishyna et al., 2021; Ribeiro et al., 2019; Rose et al., 2023; Singh et al., 2020; Tengweh Forkwa et al., 2022; Villaseñor et al., 2022; Yang et al., 2023).

The techno-functional and physicochemical properties of cricket (CPI) and grasshopper (GPI) protein isolates are critical determinants of their suitability for incorporation into food products. (Mishyna et al., 2021; Villaseñor et al., 2022). Therefore, assessing these parameters before integrating CPI and GPI into food formulations is crucial.

Moreover, these protein extraction/purification processes may alter the nutritional and biological potential of CPI and GPI proteins (de Castro et al., 2018; Sánchez-Velázquez et al., 2024). It is crucial to maintain a balanced proportion of essential amino acids from the raw material through the protein isolation process. Besides the nutritional benefits of edible insect proteins, their therapeutic potential has been gaining interest, including their antioxidant potential (Fashakin et al., 2023). Several studies have reported the antioxidant potential of peptides and amino acids (histidine, arginine, lysine, and arginine) derived from proteins from Orthoptera species; however, scarce studies for cricket and grasshopper are available (Fashakin et al., 2023; Akande et al., 2023; Chatsuwan et al., 2018; Zielińska, Baraniak, & Karaś, 2017, Zielińska, Karaś, & Jakubczyk, 2017). Humans have consumed these two orthopterans since ancient times. Still, in recent years, their incorporation as functional, healthier, and sustainable food ingredients has been valorized and is gaining interest among researchers, producers, and consumers. In this sense, this work aimed to investigate the effects of defatting and protein purification processes on the techno-functional, physicochemical, nutritional, and antioxidant properties of cricket and grasshopper protein isolates.

2. Materials and methods

2.1. Biological materials and reagents

House cricket (*Acheta domesticus* L.) flour was supplied by Crickex© (Zapopan, Mexico) and kept at −20 °C. Adult milpa grasshoppers (*Sphenarium purpurascens* Charp.) were collected from an alfalfa field located near Españita, Tlaxcala, Mexico (19°27′48.2“ N, 98°25’29.4” W). The field was specifically maintained for the collection of grasshoppers and kept free of any pesticide or chemical treatments. Taxonomic identification was carried out by the entomologist Dr. Jhony Navat Enriquez-Vara, following the same methodology as previously described by Villaseñor et al. (2022). After collection, the insects were blanched for 2 h to empty the gastrointestinal tract, cooled to −20 °C, milled using a Hamilton Beach® Brands blender (Guadalajara, Mexico), and stored at −20 °C until further processing. The reagents 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, A9941), di(phenyl)-(2,4,6-trinitro phenyl) iminoazanio (DPPH, D9132), potassium sulfate ($K_2S_2O_8$, 216224), 2,2′-azobis(2-amidinopropane)-dihydrochloride (AAPH, 440914), Trolox (238813), iron(III) chloride tetrahydrate ($FeCl_2 \bullet 4H_2O$, 380024), pyrocatechol violet (P7884), copper (II) sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$, RES10395-B702X), ethylenediaminetetra acetic acid (EDTA, ED4SS) were purchased from Merk® (St. Louis, USA), unless otherwise indicated.

2.2. Defatting procedures

For the defatting stage, four lipid-removing procedures were performed on cricket (CF) and grasshopper (GF) flours:

Gravimetric: a 5 % solution of cricket or grasshopper flour was diluted in 1.0 L NaOH (0.05 N, pH adjusted to 10.0) and stirred at room temperature for 1 h for fat extraction. Samples were centrifuged at 15,000*g* for 30 min at 4 °C (Centrifuge SL4D, Thermo Fisher Scientific Inc., Osterode am Harz, Germany). Then, pH was adjusted to 3.0 with 6 N HCl and stirred for 1 h at room temperature. Solution was centrifuged at 15,000*g* for 30 min at 4 °C. Fatty layer was recovered from the surface of the solution. Samples were freeze-dried and weighed.

Solvent: 20 g of CF or GF were placed in a Whatman cellulose extraction thimble and exposed to *n*-hexane (1,15, *w/v*) in a Soxhlet system for 3 h at 80 °C. Then, the solvent was recovered, and the residue was evaporated from the flat-bottomed flasks (previously constant-weighed). Finally, the flasks were constant-weighed again;

Pressing: 100 g of CF or GF were placed in a horizontal press (T15 Oil Press Machine, Henan, China) and pressed for 15 min at 100 °C. Then, the samples were recovered and weighed again;

Supercritical fluid: 150–200 g of CF or GF were placed in a supercritical fluid extractor (Thar Technologies-Water®-SFE-500 MR, Thar Designs, Inc., Pittsburgh, USA). Carbon dioxide was pumped with a constant flow rate of 10–20 mg/min for 4–12 h at 60 °C and 400–450 bar. Defatted samples were stored at 4 °C. Defatting efficiency was determined by gravimetric, solvent, pressing, and supercritical fluid methods using differential weight.

2.3. Protein extraction strategies

A combination of physical and enzymatic methods were used to enhance the protein concentration in defatted cricket and grasshopper flours (Fig. 1). The flours defatted by supercritical fluid were used for further experiments.

2.3.1. Solubilization-precipitation

About 100 g of defatted cricket flour (DCF) and defatted grasshopper flour (DGF) were stirred for 1 h at room temperature in 1.0 L NaOH 0.05 N (pH 10.0). Then, the slurries were centrifuged in an SL4DR centrifuge system (ThermoFisher Scientific Inc., Osterode am Harz, Germany) at 4000 rpm, 15 min, 4 °C. The DCF and DGF non-soluble fractions were stored at 4 °C for subsequent extraction. The pH of supernatants was adjusted to pH 3.0 for cricket and pH 3.5 for grasshopper samples, stirred at 250 rpm, 1 h, room temperature, and centrifuged (4000 rpm, 15 min, 4 °C). Precipitates were recovered and stored at 4 °C.

2.3.2. Ultrasonic, heat and enzymatic-hydrolysis treatments

Heat and ultrasonic treatments were used as complementary enhancers of protein release in the DCF and DGF non-soluble fractions after resuspension in 500 mL of 0.05 N NaOH (pH 10.0). Independent and combined treatments were performed as follows: A) 15 min at 80 °C; B) 30 min at 80 °C; C) 15 min sonication (20 kHz); D) 30 min sonication (20 kHz); E) 15 min at 80 °C + 15 min sonication (20 kHz); F) 30 min at 80 °C + 15 min sonication (20 kHz); G) 15 min at 80 °C + 30 min sonication (20 kHz); H) 30 min at 80 °C + 30 min sonication (20 kHz). Samples were centrifuged (4000 rpm, 15 min, 4 °C) using an SL4DR centrifuge (ThermoFisher Scientific Inc., Osterode am Harz, Germany). The supernatants were then adjusted to pH 3.0 for cricket samples and pH 3.5 for grasshopper samples, and stirred for 1 h at 250 rpm and room temperature. Consecutive centrifugation was applied before recovering the precipitate, which was then stored at 4 °C. The combination of ultrasonic-heat treatments with the highest protein concentration was selected for subsequent exposure to a protease to enhance the extracted protein content in DCF and DGF. The following enzymatic hydrolysis was performed on the non-soluble fractions of the treatments B, D, G, and H. Precipitates were suspended in distilled water (1,5, *w/v*), pH

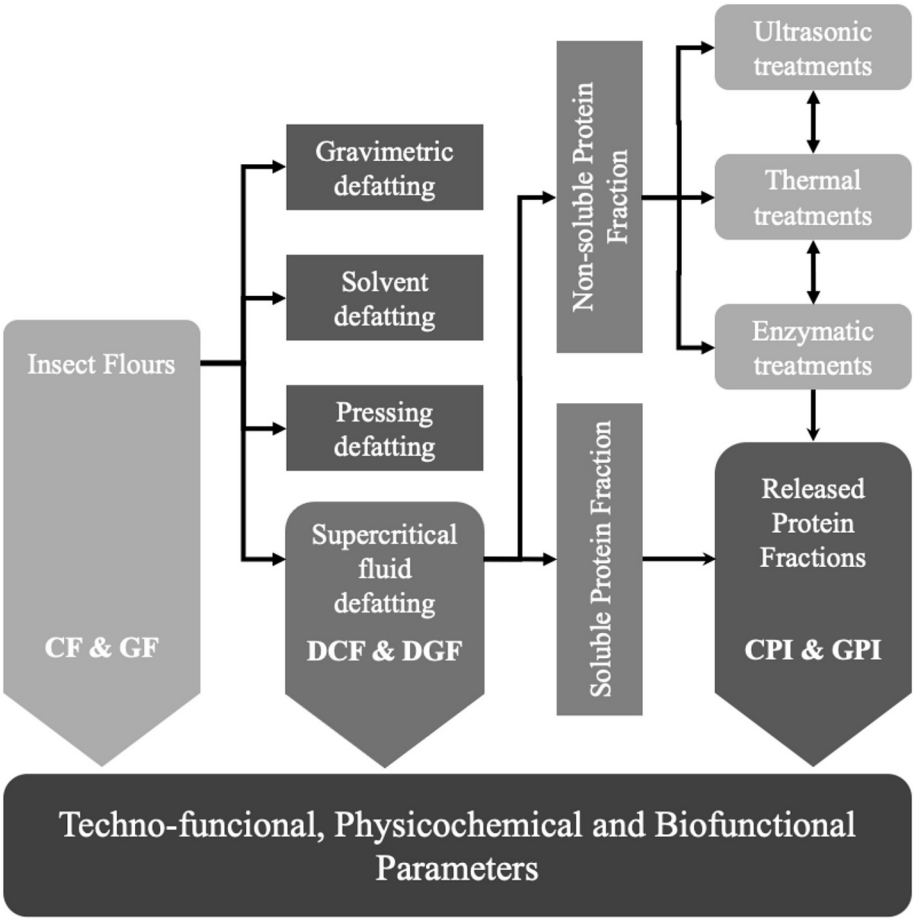


Fig. 1. Schematic procedure for production and evaluation of cricket and grasshopper samples. CF, cricket flour; GF, grasshopper flour; DCF, defatted cricket flour; DGF, defatted grasshopper flour; CPI, cricket protein isolate; GPI, grasshopper protein isolate.

adjusted to 8.0 before adding a ratio of 4 % Alcalase®:protein (*v/w*) (P4860, Sigma Aldrich, St. Louis, USA), and stirred for 15 min at room temperature. The combination of ultrasonic-heat treatments with the highest protein concentration was selected for subsequent exposure to Alcalase® to enhance the extracted protein content in DCF and DGF, using controlled conditions (i.e., <1 h, <50 °C, and < 5 %) to prevent excessive hydrolysis of the residual proteins. In brief, samples were centrifuged (4000 rpm, 15 min, 4 °C), supernatant precipitated to pH 3.0 (250 rpm, 1 h, room temperature), and centrifuged again (4000 rpm, 15 min, 4 °C). The soluble protein fractions were pooled and solubilized in 2 L of distilled water at pH 7.0 and 4 °C.

2.3.3. Spray-drying process

About 2 L of soluble proteins of cricket and grasshopper samples (pH 7.0) were injected into the spray dryer system (MOBILE MINORTM GEA® Model MM standard, Düsseldorf, Germany) using a peristaltic pump (WATSON MARLOW®, model 520S, Düsseldorf, Germany) at a flow rate of 10 mL/min. The blower was maintained with an air pressure of 3.5 Bar (22,000 rpm) to keep the atomizer rotating. A constant flow of hot air at 150 °C was established inside the drying chamber. The resulting powders were labeled cricket (CPI) and grasshopper (GPI) protein isolates and stored at −20 °C.

2.4. Protein content, solubility and digestibility

The efficiency of protein extraction assisted by heat, ultrasonication, and enzymatic treatments was estimated using protein microelemental analysis (Vario MICRO cube Elementar, Frankfurt, Germany). Protein solubility was quantified by the method reported by Silva-Sánchez et al. (2008) using the Quick Smart™ DC-Protein Assay kit (BioRad Laboratories, Hercules, USA), and protein concentration was calculated using a bovine albumin standard curve. The protein digestibility of cricket and grasshopper samples was determined using the method reported by Hsu et al. (1977), with casein (Cas) as a control. 30 mL of sample (6.25 mg/mL) was adjusted to pH 8.0, and 3 mL of a multienzyme solution (1.6 mg/mL trypsin, 3.1 mg/mL chymotrypsin, and 1.3 mg/mL peptidase) was added to the samples or control in a thermal bath with constant agitation at 37 °C for 10 min. After that, the protein digestibility was estimated following this formula:

$$\textit{Protein digestibility}\ (\%) = 210.464 - 18.3x$$

where x is the pH value at 10 min of enzymatic digestion.

2.5. Techno-functional and physicochemical parameters

2.5.1. Oil and water retention

The water absorption capacity was assessed following the method outlined by Alfaro-Diaz et al. (2021) with slight adjustments. Briefly, 1.0 g of sample was dispersed in 10 mL, vortexed for 1 min, and centrifuged at 1238 *g* for 10 min. Then, the supernatant was discarded, and the tubes were re-weighed. The oil absorption capacity was determined using the method described by Stone et al. (2015). 1.0 g of the sample was mixed with 10 mL of sunflower oil, vortexed for 1 min, then centrifuged at 1238 *g* for 10 min. The supernatant was discarded, and the tubes were re-weighed. The water and oil absorption capacities were then calculated using the provided equation and expressed as g of water or oil bound per g of sample:

$$Water \Big/ Oil\ retention\ (g/g) = \frac{W_2 - W_1}{W_0}$$

Where W_2 is the weight of the tube plus the sediment (g), W1 is the weight of the tube plus the dry sample (g), and W_0 is the weight of the dry sample (g).

2.5.2. Emulsion capacity and stability

Emulsion capacity was assessed following the procedure outlined by Ulloa et al. (2017), with some modifications. Solutions containing 2.5 % (*w/v*) of the samples were dissolved in deionized water and adjusted to pH levels of 3.0 and 5.0 using HCl and NaOH, respectively. The solution was centrifuged (D-78564 HERMLE Labortechnik GmbH, Wehingen, Germany) at 3000 *g*, 5 min, and 2 mL of the supernatant was combined with sunflower oil in a 1:1 ratio. Subsequently, the samples were emulsified at 20,000 rpm using a homogenizer (Ultra Turrax® T25, IKA Works, Inc., Wilmington, USA) and centrifuged at 400 *g* for 5 min. Emulsion capacity was quantified as the ratio of the height of the emulsified layer to the volume of the total solution using the following equation:

$$Emulsion\ capacity\ (\%) = \frac{Emulsion\ volume}{Total\ solution\ volume} \times 100$$

The emulsion stability of the samples was achieved through a 30-min heating of the emulsions at 80 °C in a water bath. The tubes containing the samples were centrifuged at 400 *g* for 5 min. Emulsion stability was quantified as the ratio of the height of the emulsified layer to the volume of the total solution using the following equation:

$$Emulsion\ stability\ (\%) = \frac{Foam\ volume_{30\ min}}{Foam\ volume_{0\ min}} \times 100$$

2.5.3. Foaming capacity and stability

Foaming capacity and foaming stability were assessed using the procedure outlined by Alfaro-Diaz et al. (2021). Solutions of 1 % sample (*w/v*) in deionized water at pH 3.0, 5.0, and 7.0 (using 0.5 N HCl and 1 N NaOH) were then subjected to centrifugation (D-78564 HERMLE Labortechnik GmbH, Wehingen, Germany) at 400 *g* for 5 min, and 5 mL of each solution were extracted and homogenized at 13,000 rpm for 1 min. Foaming capacity was quantified as the ratio of the foam volume to the initial volume using the following equation:

$$Foaming\ capacity\ (\%) = \frac{Foam\ volume}{Total\ volume} \times 100$$

The foam was also measured at both time zero and after 30 min. Foaming stability was quantified as the ratio of the volume of the foamed layer to the volume of the total solution with the following equation:

$$Foaming\ stability\ (\%) = \frac{Foam\ volume_{30\ min}}{Foam\ volume_{0\ min}} \times 100$$

2.5.4. Water activity and hygroscopicity

Approximately 2.0 g of sample was placed in a plastic dish, and water activity was measured using a water activity indicator (Rotronic HygroLab, Hauppauge, USA) for 4 min. Also, 1.0 g of cricket or grasshopper samples were placed in a sealed glass container containing saturated NaCl solution at 25 °C for one week (Correia et al., 2017). Hygroscopicity was estimated as the amount of water absorbed per 100 g of sample in dry weight (g/100 g dw).

2.5.5. Flour density

For estimating bulk density (ρ_β), 1.0 g of cricket and grasshopper samples were placed into a 10 mL graduated glass cylinder, and the resulting volume was recorded. Bulk density was estimated following the equation:

$$\rho_\beta = \frac{\text{Sample weight (g)}}{\text{Sample volume (mL)}}$$

The tapped density (ρt) of the samples was estimated by measuring the volume of 1.0 g of powder after tapping 100 times on a soft rubber mat 15 cm above the ground in a glass cylinder. Occupied volume was recorded, and tapped density was calculated using the following formula:

$$\rho_t = \frac{\text{Sample weight (g)}}{\text{Sample volume (mL)}}$$

Carr's index and the Hausner ratio were calculated following the respective equations:

$$Carr's\ index = \frac{\rho_t - \rho_\beta}{\rho_t} \times 100$$

$$Hausner\ ratio = \frac{\rho_t}{\rho_\beta}$$

2.5.6. Color

Approximately 5 g of sample was placed on a quartz plate and read using a Colorimeter (PCE Instrument Reader Americas Inc., Southampton, UK). The L^*, a^* and b^* values were recorded. The obtained data was used for Chroma, ΔE, and Hue angle (h°) estimations with the following equations:

$$Chroma = \sqrt{a^2 + b^2}$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

$$h^\circ = tan^{-1}\left(\frac{b}{a}\right)$$

where "*L*", "*a*" and "*b*" letters were substituted by the respective lightness, redness and yellowness values.

2.6. Amino acid profile and protein quality parameters

For total amino acids, 2 mg of cricket and grasshopper samples were hydrolyzed in 6 N HCl (4 mL) at 110 °C for 24 h in tubes sealed under nitrogen. For tryptophan estimation, a basic hydrolysis was done (Yust et al., 2004). Amino acid profiles were determined after derivatization with diethyl ethoxymethylenemalonate by HPLC (Alaiz et al., 1992), using D,L-α-aminobutyric acid as an internal standard and a 300 × 3.9 mm reversed-phase column (Novapack C_{18}, 4 μm; Waters, Milford, MA, USA). The protein quality parameters assessed were the proportion of essential amino acids (PEAA), amino acid score (AAS), essential amino acid index (EAAI), biological value (BV), and protein efficiency ratios (PER) were estimated according to the amino acid profile of samples using the equations summarized by Sánchez-Velázquez et al. (2021).

2.7. Particle size and scanning electron microscopy

The droplet size distribution of the cricket and grasshopper samples diluted in distilled water (1100, *w/v*) was evaluated at room temperature using the Malvern Mastersizer 3000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The average droplet size of the DEs was expressed as the volume diameter ($D_{[3,2]}$ and $D_{[4,3]}$). The relative span factor, particle size uniformity, and surface area were also recorded.

The particle structure of cricket and grasshopper samples was examined using a Scanning Electron Microscope (Hitachi SU8230 FESEM, Hitachi, Japan). The samples were placed on specimen stubs and dried with hot air until complete evaporation of water, a process that took approximately 10 min. Subsequently, the dried sample particles were coated with a 4 nm thick layer of platinum using a sputtering

technique in a Leica ACE 600 sputter coater. The coated samples were then analyzed at magnifications ranging from 200× to 5000×, with an accelerating voltage of 20 kV. Micrographs at 1500× magnification were selected as representative images for each sample and then utilized for subsequent analysis.

2.8. In vitro antioxidant activity

Briefly, proteins from cricket and grasshopper samples were diluted in distilled water (1:20, w/v). Soluble protein fractions were prepared at concentrations ranging from 0.05 to 10 mg protein/mL for all samples. These soluble proteins were subsequently used in antioxidant assays to determine the half-inhibitory concentration of oxidative radicals (IC_{50}) in triplicate.

2.8.1. ABTS

The scavenging activity of cricket and grasshopper samples on the radical $ABTS^{\bullet+}$ was assessed following the procedure outlined by Carrasco-Castilla et al. (2012). A solution of 7 mM ABTS reagent was prepared by dissolving 40 mg of ABTS salt in 6.95 mg of 2.45 mM K2S2O8 solution, and then adjusting the volume to 10 mL with distilled water. The solution was kept in the dark for 16 h at room temperature. Subsequently, the ABTS reagent solution was diluted with EtOH to absorb 0.80 ± 0.1 at 734 nm. The ABTS solution (1:10, *v*/v) was mixed with blank standards (Trolox curve of 0–700 μM), and samples were vortexed for 10 s, and the absorbance was measured at 0 and 6 min at 734 nm in a plate reader (Infinite 200 PRO, Tecan Trading AG, Männedorf, Switzerland). The ABTS scavenging activity was calculated following the equation:

$$ABTS\ inhibition\ (\%) = \frac{Abs_{sample\ 0\ min - sample\ 6\ min}}{Abs_{sample\ 0\ min} - (Abs_{B\ 0\min - B\ 6\ min})/Abs_{B\ 0\ min}} \times 100$$

where Abs_{sample} is the absorbance of samples at 0 and 6 min, while Abs_B is the absorbance of the blank at 0 and 6 min.

2.8.2. DPPH

DPPH radical scavenging activity was conducted using a modified version of the method reported by Sánchez-Vioque et al. (2012). The DPPH radical solution (0.1 mM) was prepared by weighing 9.85 mg of the reagent DPPH, adjusting the volume to 25 mL with methanol, then diluting 10 mL of this solution with 10 mL of distilled water. In a 96-well plate, 125 μL of 0.1 mM DPPH were mixed with 125 μL of standard (0–1 μg BHT/mL) or samples. The plate was incubated for 30 min at room temperature with agitation before measuring the absorbance at 517 nm in a plate reader (Infinite 200 PRO, Tecan Trading AG, Männedorf, Switzerland). The following equation was used to estimate the DPPH scavenging activity:

$$DPPH\ inhibition\ (\%) = \left(1 - \frac{Abs_s}{Abs_b}\right) \times 100$$

where Abs_s is the absorbance of the samples, and Abs_b is the absorbance of the blank.

2.8.3. Metal chelation

The Fe^{2+} chelating activity was determined using the procedure described by Carrasco-Castilla et al. (2012) with slight modifications. In a 96-well plate, 25 μL of sample, standard 0–0.0051 μg EDTA/μL), or control (0.0137 μg EDTA/μL) were mixed with 225 μL of 100 mM sodium acetate buffer at pH 4.9, and 30 μL of $FeCl_2 \bullet 4H_2O$ (0.036 mg/mL in sodium acetate buffer). Following a 30-min incubation at room temperature, 12.5 μL of a 40 mM Ferrozine solution, prepared in the same buffer, was added, and the absorbance was measured at 562 nm using a plate reader (Infinite 200 PRO, Tecan Trading AG, Männedorf, Switzerland).

Similarly, the Cu^{2+} chelating activity was evaluated using a modified version of the method outlined by Carrasco-Castilla et al. (2012). Approximately 20.5 μL of sample, standard (0–0.0203 μg EDTA/μL), or control (0.0436 μg EDTA/μL) was introduced into each well of a 96-well transparent plate. This was mixed with 185 μL of sodium acetate buffer, 15 μL of copper solution (0.1968 mg/mL in 50 mM sodium acetate buffer, pH 6.0), and 9 μL of 2 mM pyrocatechol violet prepared in the same buffer. After incubating the plate for 10 min at room temperature with agitation, the absorbance was measured at 632 nm in a plate reader (Infinite 200 PRO, Tecan Trading AG, Männedorf, Switzerland).

$$Iron\Big/copper\ chelating\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.8.4. ORAC

The ORAC assay was conducted following the procedure outlined in a prior study (Huang et al., 2002). A volume of 25 μL of sample or standard (0–100 μM Trolox in PBS, pH 7.4) was placed in a transparent-bottomed black plate with 150 μL of fluorescein (0.08 μM) and then 25 μL of AAPH• (150 mM). The reduction in fluorescence caused by AAPH• (150 mM) at 37 °C was monitored at 5 min intervals over a 60 min period at an excitation/emission wavelength of 485/528 nm in a plate reader (Infinite 200 PRO, Tecan Trading AG, Männedorf, Switzerland). The ORAC values were calculated using the following equation:

$$AOX = \frac{AUC_{Sample} - AUC_{Control}}{AUC_{Trolox} - AUC_{Control}}$$

where AUC_{Sample} is the area under the curve of each sample, $AUC_{Control}$ is the area under the curve of control, and AUC_{Trolox} is the area under the Trolox curve.

2.9. Statistical analysis

Results were expressed as mean ± standard deviation and analyzed using one-way ANOVA using Prism version 9.4.1(458) software (GraphPad Software, LLC, Boston, USA). Dunnett's test was employed to compare protein solubility against the control (CF or GF). The Tukey test was used to detect significant differences in other analyses, with a significance level of $p < 0.05$ in both cases. All experiments were conducted in triplicate.

3. Results and discussion

3.1. Defatting process and protein extraction

Protein concentrates and isolates from sustainable food sources have gained significant relevance in recent years. In this sense, the first concern for increasing the protein concentration in insect powders is removing other major macromolecules, such as lipids and chitin. Four different defatting techniques were used on CF and GF. Solvent extraction was the most effective method for removing lipids in both samples (>96 %), followed by supercritical fluid (removed 95.54 % and 93.36 % of the total fat, respectively) (Table 1).

Gravimetric fat extraction showed defatting efficiency of 16.85 % and 20.18 % in CF and GF, respectively; meanwhile, pressing extraction on CF showed an efficiency of <1 %, compared to 10.03 % in GF. In contrast to solvent and supercritical fluid extraction, the gravimetric and pressing methods for lipid separation in the studied samples demonstrated significantly lower fat removal yields. The lipids found in orthopteran tissues, such as triglycerides and phospholipids, are often tightly bound to proteins, peptides, and structural carbohydrates such as chitin (Antunes et al., 2024). These bonds are more effectively broken by chemical or physicochemical methods compared to physical processes. Moreover, the lipid composition of these insects, particularly the presence of saturated fatty acids and polar lipids such as phospholipids, plays a crucial role in the effectiveness of extraction. As described by

Table 1
Efficiency of defatting processes on cricket and grasshopper flours.

Sample	Initial Lipid Content (g/100 g dw)	Final Lipid Content (g/100 g dw)	Efficiency of Defatting (%)
Cricket flour			
Gravimetric	22.78 ± 0.01	18.95 ± 0.34	16.85 ± 2.09^{e}
Solvent extraction		0.35 ± 0.02	98.47 ± 0.97^{a}
Pressing		22.76 ± 0.06	0.07 ± 0.00^{g}
Supercritical fluid		1.02 ± 0.04	95.54 ± 2.33^{c}
Grasshopper flour			
Gravimetric	7.68 ± 0.02	1.55 ± 0.06	20.18 ± 0.22^{d}
Solvent extraction		0.28 ± 0.03	96.33 ± 0.81^{b}
Pressing		6.91 ± 0.03	10.03 ± 0.04^{f}
Supercritical fluid		0.51 ± 0.00	93.36 ± 0.00^{c}

Different letters indicate statistical differences (Tukey's test, $p < 0.05$) in efficiency of defatting treatments. Data are the mean and SD of three replicates.

Pellerin and Doyen (2024), phospholipids exhibit low solubility under acidic or alkaline aqueous conditions and tend to remain embedded within protein–lipid complexes, limiting the efficacy of gravimetric defatting. These conditions can also promote protein unfolding and aggregation, potentially entrapping lipids and further hindering their removal. In contrast, non-polar solvents such as hexane or supercritical CO_2 exhibit a greater affinity for lipids and can efficiently disrupt these complexes, resulting in significantly higher fat extraction yields.It is essential to note that solvent extraction is not considered eco-friendly and has limitations when applied to food formulations (Cravotto et al., 2022). Still, supercritical fluid extraction for fat is a sustainable technology with low or no risk to foods that can maintain the food matrix free of external chemicals (Ziero et al., 2020). However, this technology currently faces limitations in scaling up to industrial levels, particularly due to the high costs of equipment.

In another study, Sipponen et al. (2018) reported 21 % of remaining lipids in defatted cricket flour by supercritical fluid, which is 3.2- and 5.9-fold higher than the retained fat in DCF and DGF, respectively. The residual fats in defatted flours can be related to phospholipids, tri- or diglycerides, and free fatty acids in the flours (Sipponen et al., 2018). Due to these results, the following protein extraction and isolation steps, as well as the subsequent evaluations, were conducted using the supercritical fluids DCF and DGF.

3.2. Protein content, solubility and digestibility

Defatting and protein extraction (using sonication combined with thermal and enzymatic treatments) significantly increased ($p < 0.05$) the protein content from 61.34 % to 95.67 % in cricket and from 60.93 % to 91.60 % in grasshopper samples (Table 2). This increase may be attributed to the removal of lipids by supercritical fluid extraction and the elimination of other impurities, such as residual phospholipids, insoluble carbohydrates, minerals, and non-soluble proteins, during the protein extraction process.

Table 2
Protein content, soluble protein and protein digestibility of cricket and grasshopper samples.

Sample	Protein Content (g/100 g dw)	Soluble Protein Proportion (%)	Protein Digestibility (%)
CF	61.34 ± 1.76^{e}	38.41 ± 6.06^{c}	85.49 ± 1.00^{b}
DCF	81.80 ± 2.37^{d}	51.20 ± 6.53^{b}	86.47 ± 0.37^{b}
CPI	95.67 ± 0.35^{a}	74.28 ± 3.29^{a}	91.22 ± 1.74^{a}
GF	60.93 ± 2.71^{e}	41.15 ± 5.13^{c}	80.76 ± 0.95^{c}
DGF	86.62 ± 2.76^{c}	56.28 ± 4.80^{b}	86.11 ± 0.88^{b}
GPI	91.60 ± 0.68^{b}	71.11 ± 2.14^{a}	89.25 ± 0.11^{a}

CF, cricket flour; DCF, defatted cricket flour; CPI, cricket protein isolate; GF, grasshopper flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate. Data are the mean and SD of three replicates. Same letter indicates non-statistical differences (Tukey's test, $p < 0.05$) between protein characteristics.

Applying single ultrasonication or single thermal treatment, the efficiency of protein extraction in DCF increased significantly ($p < 0.05$) from 16.85 mg/mL to 17.95–18.84 mg/mL (Fig. 2A), while in DGF, the protein extraction from followed the same tendency with values of 18.23 mg/mL to 19.50–22.11 mg/mL, except for sonication for 15 min with no significant changes ($p > 0.05$) (Fig. 2B). Following the application of proteases to the ultrasonic-heating treatments, protein extraction values increased to 38.92 and 42.05 mg/mL, for cricket and grasshopper samples, respectively. Therefore, the combination of 30 min heating+15 min sonication+enzymatic hydrolysis was selected to produce cricket (CPI) and grasshopper (GPI) protein isolates.

Defatting and protein isolation processes increased the proportion of soluble protein concentration statistically ($p < 0.05$) by 1.93-fold in crickets and 1.72-fold in grasshoppers (Table 2). Protein digestibility exceeded 80 % in all samples. Moreover, defatting by supercritical fluid followed by protein extraction increased digestibility significantly ($p < 0.05$) to 91 % in CPI and 89 % in GPI.

Protein solubility at different pH values showed typical behavior of high protein food sources (Fig. 2). However, in cricket samples (Fig. 2C), DCF showed higher ($p < 0.05$) protein solubility at pH 7.0–10.0 (until 34.01 % higher) compared to CF, while CPI demonstrated significantly higher ($p < 0.05$) protein solubility (19.61–73.54 %) compared to CF (8.01–51.87 %) at above pH 3. Meanwhile, in grasshopper samples (Fig. 2D), DGF exhibited superior protein solubility ($p > 0.05$) at pH 9.0 (57.81 %) compared to GF. In contrast, GPI protein solubility was significantly higher ($p < 0.05$) across the pH evaluated (43.51–77.57 %) compared to GF (28.41–54.07 %). Defatting by supercritical fluid negatively affected protein solubility in most evaluated pH levels in both samples, but the effect was more pronounced in the grasshopper results. Changes in protein solubility could be influenced by factors such as protein aggregation, conformational structure, chemical nature, amino acid sequence, interactions with chelating compounds, and matrix physicochemical characteristics (Sęczyk et al., 2019; Grossmann and McClements, 2023), which they can occur mainly during the defatting step by supercritical fluid.

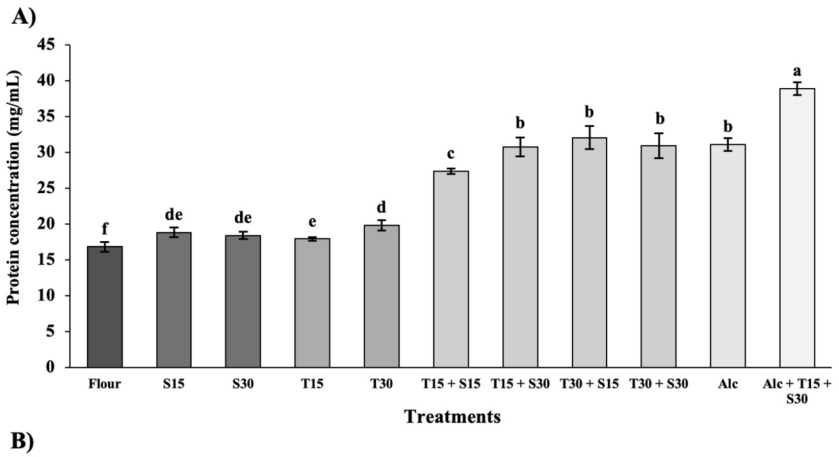
Meanwhile, the protein digestibility of all samples was higher than 80 % (Table 2). Defatting and protein extraction statistically improved ($p < 0.05$) protein digestibility by 1.07-fold in cricket and 1.11-fold in grasshopper samples. Ibarra-Herrera et al. (2020) reported an in vitro protein digestibility of 87.92–90.01 % in grasshoppers fed with alfalfa or maize green fodder. Based on this information, differences in protein digestibility depend not only on dietary modulation and genotypic influences on protein composition but also on the technological processes applied.

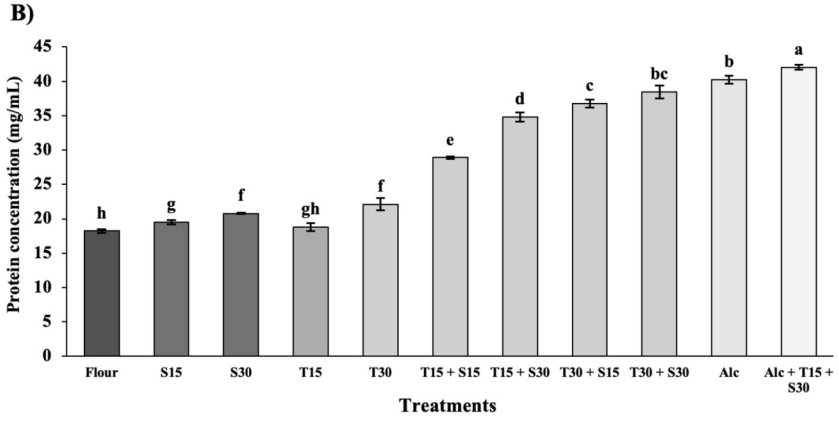
3.3. Techno-functional characterization

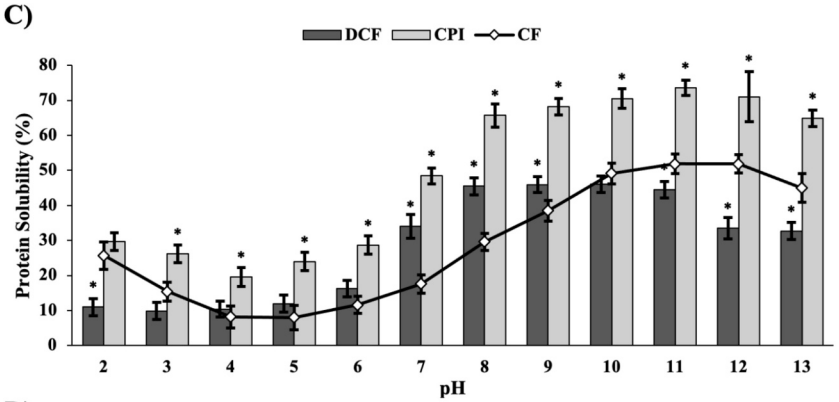
Fig. 3 shows the changes in techno-functional parameters of CF and GF following protein purification processes. These evaluations provide information needed during food formulation, guiding decisions on preparation, processing methods, and potential applications of the new ingredient. Additionally, they facilitate compliance with regulatory standards, ensuring that the ingredient meets safety and quality requirements.

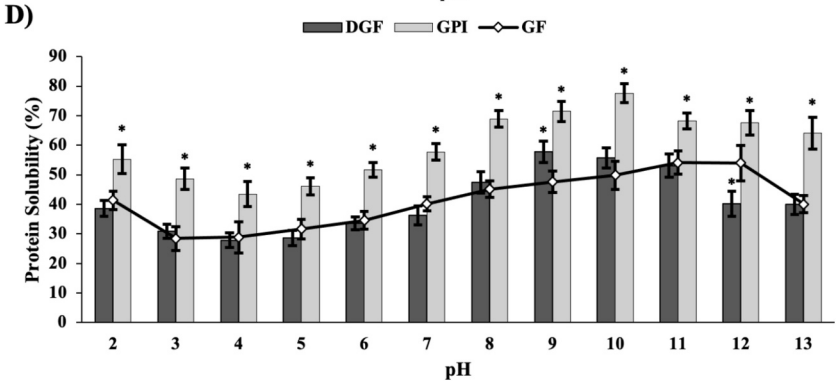
3.3.1. Water and oil holding capacity

Water holding capacity decreased statistically ($p < 0.05$) by 47.7 % in CPI (2.19 g water/g flour) in contrast to CF (3.98 g water/g flour), while GPI (1.79 g water/g flour) showed no statistical changes ($p > 0.05$) compared to to GF (1.76 g water/g flour) (Fig. 3). Similar values to GPI were observed in *Gryllodes sigillatus* (17.6 g water/g flour) *Tenebrio molitor* (16.2 g water/g flour), and *Gryllus bimaculatus* (1.75 g water/g flour) flours (Damasceno et al., 2023; Stone et al., 2019). However, CPI









(caption on next page)

Fig. 2. Cricket and grasshopper protein extraction and solubility. Efficiency in the extraction of proteins from cricket (**A**) and grasshopper (**B**) flours. Protein solubility of cricket (**C**) and grasshopper (**D**) samples. Treatments: S15, sonication 15 min; S30, sonication 30 min; T15, heat 15 min; T30, heat 30 min; T15 + S15, heat 15 min + sonication 15 min; T15 + S30, heat 15 min + sonication 30 min; T30 + S15, heat 30 min + sonication 15 min; T30 + S30, heat 30 min + sonication 30 min; Alc, Alcalase 4 % 5 min; Alc + T15 + S30, Alcalase 4 % 5 min + heat 15 min + sonication 30 min. DCF, defatted cricket flour; CPI, cricket protein isolate; CF, cricket flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate; GF, grasshopper flour. Data are the mean and SD of three replicates. Same letter above bars indicates non-statistical differences (Tukey's test, $p < 0.05$) between treatments. Asterisks (*) indicate statistical differences (Dunnett's test, $p < 0.05$) against controls (CF or GF) at the same pH.

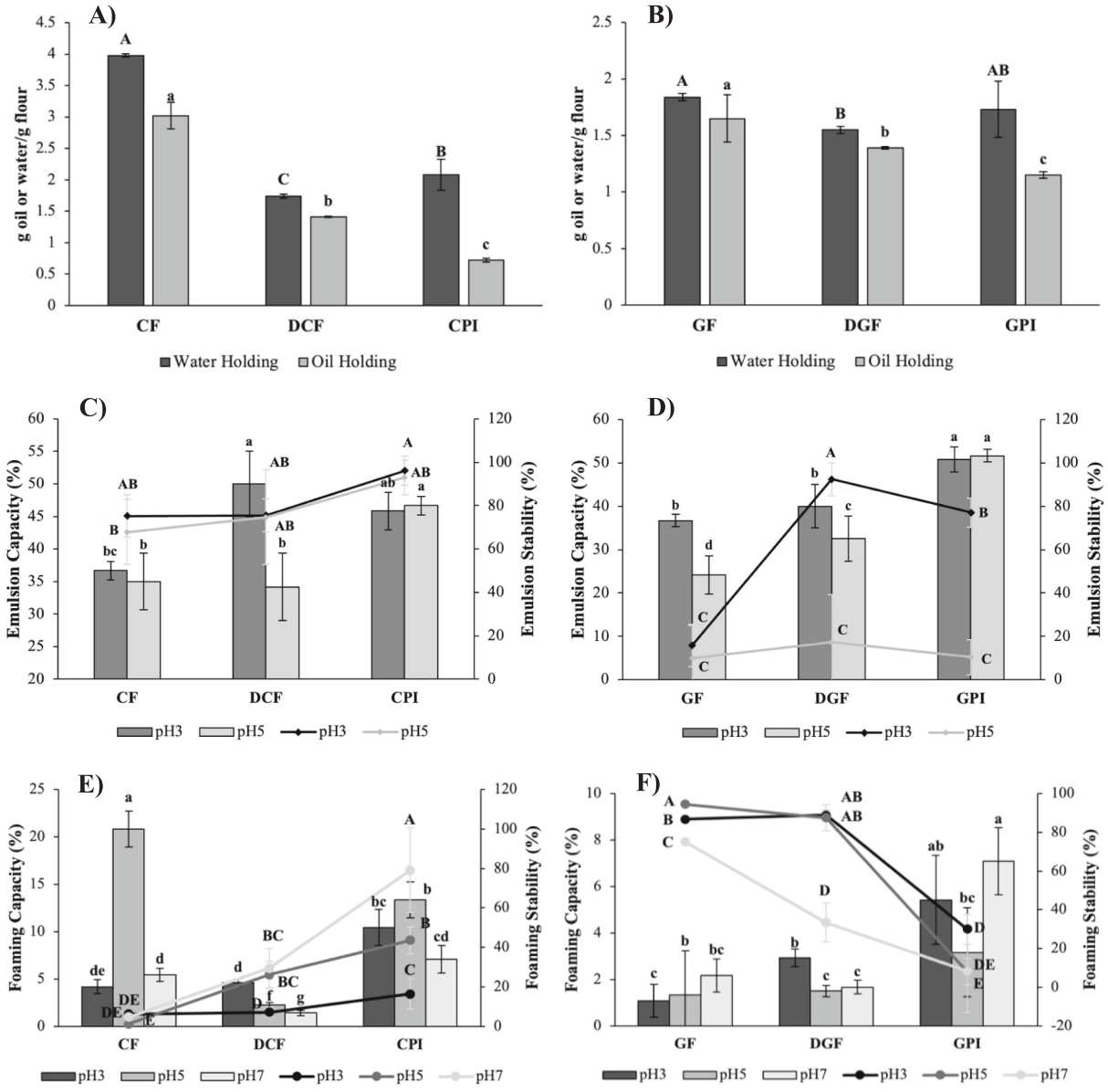


Fig. 3. Techno-functional properties of cricket and grasshopper samples. Water and oil retention of cricket (**A**) and grasshopper (**B**) samples; emulsion activity and capacity of cricket (**C**) and grasshopper (**D**) samples; and foaming activity and capacity of cricket (**E**) and grasshopper (**F**) samples. CF, cricket flour; DCF, defatted cricket flour; CPI, cricket protein isolate; GF, grasshopper flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate. Data are the mean and SD of three replicates. Same super-index letter in the line indicates non-statistical differences (Tukey's test, $p < 0.05$) between physicochemical or technofunctional parameters.

and GPI had lower values than protein preparations from *Hermetia illucens* (3.95 g water/g powder), *Tenebrio molitor* (3.44 g water/g powder) and *Schistocerca gregaria* (2.31 g water/g powder) (Mintah et al., 2020; Zielińska, Karaś and Baraniak, 2018)). Zielińska, Karaś and Baraniak (2018) attributed variations in water-holding capacity to the protein purification process. However, in this study, protein isolation reduced this capacity, possibly due to the removal of chitin or polysaccharides that contribute to water retention by trapping water within their structure (Zhang et al., 2022). While protein denaturation can increase solubility, it can also reduce water retention. Denatured proteins may lose their native structure and ability to form a gel-like matrix that holds water effectively, as they may have a lower capacity to entrap water within their three-dimensional structure in their native state (Yousefi & Abbasi, 2022). Furthermore, during the protein isolation process, some hydrophilic groups on the proteins might become involved in intra- or intermolecular interactions, such as hydrogen bonding or hydrophobic interactions, reducing the number of sites available to bind water (Alrosan et al., 2022).

After processing, oil holding capacity decreased significantly ($p < 0.05$) by 76 % in cricket and 30 % in grasshopper. In *Gryllodes sigillatus*, *Tenebrio molitor* and *Schistocerca gregaria* protein preparations, these values were higher (2.74–3.22 g oil/g powder) (Zielińska, Karaś and Baraniak, 2018). Like water-holding capacity, lipid removal and protein purification negatively affected oil-holding capacity.

Each step in the protein isolation process contributes to reducing the oil retention capacity by either removing lipid components or disrupting the protein structure, thereby reducing its ability to interact with and trap oil. For example, since lipids play a crucial role in oil retention, their removal decreases the overall ability of the protein matrix to retain oil. Thus, the presence of lipids creates a hydrophobic environment within the protein matrix, which traps and holds oils (Galves et al., 2019). Moreover, the denaturation of proteins caused by alkaline-acid extraction reduces the number of hydrophobic pockets within the protein structure that are capable of interacting with and retaining oil (Ma et al., 2023). Additionally, the drastic pH changes during this step can further disrupt the protein's tertiary structure, limiting its capacity to entrap oil molecules (Momen et al., 2021). The combined effects of defatting, protein denaturation, structural disruption, and hydrolysis ultimately result in a protein isolate with significantly lower oil retention capacity compared to the original material.

3.3.2. Emulsion capacity and stability

Emulsion capacity increased significantly ($p < 0.05$) by 1.2–1.3-fold in CPI and 1.4–2.1-fold in GPI (Fig. 3). Contrary to these results, Bußler et al. (2016) reported a 51 % decrease in emulsion capacity for *Tenebrio molitor* at pH 5.0 when protein concentration increased. However, a similar behavior to ours was observed at pH 7.0. In CPI samples, emulsion stability (after 30 min) remained >92 %, while in GPI, it was recorded at 77.06 % and 10.35 % at pH 3.0 and 7.0, respectively. Chatsuwan et al. (2018) reported emulsion stability equivalent to 0 % at 15.67–33.34 min. This indicates that flours and protein isolates studied in this work maintain the emulsion phase longer than water-soluble proteins from other grasshopper species.

3.3.3. Foaming capacity and stability

At pH levels of 3.0 and 7.0, CPI samples exhibited statistically higher foaming capacities ($p > 0.05$) than CF (2.5-fold and 1.3-fold, respectively). In comparison, GPI exhibited higher foaming capacity than GF (2.4–5.0-fold) at the evaluated pH levels (Fig. 3). Other authors reported foaming capacities of 1.1–2.4-fold after protein purification from several insect raw flours (Zielińska, Karaś and Baraniak, 2018). Approximately 79.05 % of the foam in CPI remained stable at pH 7.0, and > 75 % in GF was stable at pH 3.0, 5.0, and 7.0. GPI at pH 3.0 presented a stability value of 30 %, while foaming capacity was reduced by 65–91 % with purification. Zielińska, Karaś and Baraniak (2018) also reported a decrease in foaming stability in some insect protein preparations, but these were not as pronounced as those obtained in this study. Chatsuwan et al. (2018) found a maximum foaming capacity of 25.71 % in grasshopper samples, at least 20 % higher than any assayed samples. The authors also reported foaming stability of 84.41–98.72 %, similar to GF and DGF at pH levels of 3.0 and 5.0.

3.4. Physicochemical parameters

In 2023, the European Commission approved the introduction of partially defatted powder derived from whole *Acheta domesticus* (cricket) as a novel food (European Comission, 2023). Consequently, an in-depth examination of the physical and chemical characteristics of various insect presentations for food purposes and their derivatives is crucial for facilitating the introduction of pioneering food products by the food industry while ensuring their safety (European Comission, 2023). Attributes such as color, density index, hygroscopicity, water activity (a_w), moisture content, and particle size distribution of cricket and grasshopper samples (Fig. 3) play essential roles in shaping consumer acceptance of insects as food ingredients.

3.4.1. Color

Measuring L^*, a^*, and b^* parameters in insect flours ensures color consistency, quality control, and consumer satisfaction. This enables manufacturers to produce visually appealing, high-quality food products that meet industry standards and expectations (Pathare, Opara, & Al-Said, 2013). Generally, a high L^* value ($L^* \geq 55$) indicates lighter or brighter colors, while lower values represent darker or more intense colors. In contrast, the values of a^* (redness) and b^* (yellowness) range from −60 to +60. The highest lightness (L^*) values for each insect sample were observed in both protein isolates (CPI = 80.07 and GPI = 56.77), while the lowest were in raw flours (CF = 53.34 and GF = 39.91). Both samples are darker than powders made with *Acheta domesticus* in Kenya, Spain, and Belgium ($L^* = 65.50, 64.58, 66.29$) and *Gryllus assimilis* ($L^* = 64.15$) (Ndiritu et al., 2017; Lucas-González, Fernández-López, Pérez-Álvarez and Viuda-Martos, 2019; Khatun et al., 2021), but GF was similar to the value reported by González, Garzón and Rosell (2019) for cricket ($L^* = 39.32$). These results are consistent with those reported by (Téllez-Morales et al., 2022), who used cricket flour in various proportions with nixtamalized corn flour to alter the color of extruded snacks, attributing the dark hue of crickets, similar to grasshoppers, to melanin pigments in their integuments (Mishyna et al., 2019).

DCF showed substantially higher lightness (74.84) compared to DGF (52.46), indicating a lighter color after the defatting process in cricket flour. However, both samples exhibited similar redness (a^*) values, with DCF at 4.62 and DGF at 10.84. The yellowness (b^*) in DCF (14.60) was slightly lower than in DGF (14.61), suggesting comparable yellowness between the two defatted flours (Table 3). Compared to CF and GF, the L^* value of this color loss could be attributed to the defatting process, which dissolves fat and some dyes from the insect flour (Bußler et al., 2016), thereby increasing the lightness of the flours.

With defatting and protein isolation, redness values statistically decreased ($p < 0.05$) in cricket from 10.58 to 5.0, but increased ($p < 0.05$) in grasshopper samples from 10.24 to 12.02. Moreover, yellowness decreased ($p < 0.05$) from 22.96 to 15.37 in cricket samples but increased ($p < 0.05$) from 14.08 to 24.90 in grasshopper samples. Chroma value decreased from 25.28 to 16.16 in cricket powders, while grasshopper samples increased from 17.41 to 27.65. CPI became clearer with processing, while GPI maintained similar color parameters (L^*, a^*, b^*, and Chroma) to those of unprocessed CF. In *Gryllus bimaculatus* powder, Damasceno et al. (2023) reported the same behavior in color parameter changes as the cricket samples in this study. Still, these were observed in grasshopper powders from this research or others (Chatsuwan et al., 2018). However, in wheat flour, $L^* > 60$ (Siddiq et al., 2009) indicates that the brightness of DCF and CPI is higher than that of common flour used in food formulations. It is important to note that the differences in the L^*, a^*, and b^* parameters compared to CF and GF are proportional. The dominance of dark colors in grasshopper flour persists through downstream processes for defatting and protein isolate production. This suggests that the initial unprocessed flour is relevant for producing more acceptable products. Awobusuyi et al. (2020) demonstrated that products made with dark insect flour had lower acceptability, suggesting that defatted flours and protein isolates could have achieved greater acceptance and market penetration in the food industry.

On the other hand, the Chroma value was higher than in wheat flour in all samples (Chroma ≥ 15.32), indicating that redness and yellowness are considerably higher in all insect samples. ΔE of DCF and CPI showed values (ΔE = 11.70 and 11.04, respectively) closer to wheat flour (standard, $L^* = 80.59$, $a^* = 2.17$, $b^* = 4.71$). The dark brownish color in powders could be associated with melanin accumulation in tissue, a lipophilic compound that can be removed along with lipids during defatting (Damasceno et al., 2023). However, this does not explain the dark brown color of grasshopper samples since their fat content (and

Table 3
Physicochemical and techno-functional parameters of cricket and grasshopper samples.

Parameters		CF	DCF	CPI	GF	DGF	GPI
Color	L^*	53.34 ± 0.14^{c}	74.84 ± 1.74^{b}	80.07 ± 2.20^{a}	39.91 ± 1.32^{d}	52.46 ± 0.87^{c}	56.77 ± 0.49^{c}
	a^*	10.58 ± 0.28^{b}	4.62 ± 0.39^{c}	5.00 ± 0.20^{c}	10.24 ± 0.10^{b}	10.84 ± 0.14^{b}	12.02 ± 0.07^{a}
	b^*	22.96 ± 0.35^{b}	14.60 ± 0.75^{cd}	15.37 ± 0.34^{c}	14.08 ± 0.51^{d}	14.61 ± 0.16^{d}	24.90 ± 0.12^{a}
	Chroma	25.28 ± 2.23^{a}	15.32 ± 1.54^{c}	16.16 ± 0.29^{bc}	17.41 ± 1.01^{bc}	18.19 ± 1.78^{b}	27.65 ± 2.04^{a}
	ΔE	33.85 ± 5.15^{b}	11.70 ± 1.33^{c}	11.04 ± 1.05^{c}	42.51 ± 3.05^{a}	31.05 ± 2.81^{b}	32.74 ± 0.82^{b}
	Hue	65.25 ± 9.53^{b}	72.43 ± 2.34^{a}	71.99 ± 7.89^{a}	53.97 ± 2.50^{c}	53.43 ± 4.46^{c}	64.24 ± 1.03^{b}
Density index	ρ_{β} (g/mL)	0.38 ± 0.02^{d}	0.49 ± 0.02^{b}	0.34 ± 0.01^{e}	0.42 ± 0.02^{c}	0.54 ± 0.02^{a}	0.28 ± 0.02^{f}
	ρ_{t} (g/mL)	0.49 ± 0.01^{b}	0.55 ± 0.04^{a}	0.45 ± 0.03^{bc}	0.44 ± 0.02^{c}	0.57 ± 0.02^{a}	0.40 ± 0.01^{d}
	Carr's index (CI)	21.41 ± 3.13^{b}	11.35 ± 3.16^{c}	24.46 ± 3.97^{ab}	4.17 ± 0.17^{e}	5.36 ± 0.17^{d}	30.82 ± 2.39^{a}
	Haunser ratio (HR)	1.27 ± 0.05^{b}	1.13 ± 1.04^{c}	1.33 ± 0.07^{a}	1.04 ± 0.00^{e}	1.06 ± 0.00^{d}	1.45 ± 0.05^{a}
Hygroscopicity (g/100 g dw)		5.07 ± 0.28^{a}	1.25 ± 0.03^{d}	1.56 ± 0.02^{c}	−1.30 ± 0.18^{f}	0.94 ± 0.00^{e}	1.85 ± 0.06^{b}
a_w		0.38 ± 0.01^{a}	0.23 ± 0.01^{c}	0.25 ± 0.00^{c}	0.31 ± 0.00^{b}	0.36 ± 0.00^{a}	0.25 ± 0.00^{c}
Total solids (g/100 g)		96.06 ± 1.46^{ab}	96.16 ± 1.00^{ab}	96.58 ± 0.85^{ab}	97.18 ± 0.34^{a}	95.99 ± 0.24^{b}	96.16 ± 0.25^{b}
Particle Size Distribution	$D_{[3,2]}$ (μm)	43.32 ± 1.03^{b}	33.70 ± 0.21^{c}	11.44 ± 1.05^{d}	52.79 ± 2.11^{a}	46.33 ± 2.12^{b}	5.81 ± 0.67^{e}
	$D_{[4,3]}$ (μm)	220.10 ± 15.32^{c}	210.02 ± 10.59^{c}	18.34 ± 0.99^{d}	517.49 ± 23.43^{a}	363.37 ± 22.23^{b}	14.11 ± 0.38^{e}
	Uniformity	0.81 ± 0.07^{c}	1.07 ± 0.01^{c}	0.59 ± 0.04^{d}	1.24 ± 0.03^{b}	1.19 ± 0.21^{bc}	1.42 ± 0.07^{a}
	Specific Surface Area (m^2/kg)	125.81 ± 9.08^{c}	162.04 ± 21.31^{c}	476.50 ± 32.90^{b}	103.33 ± 9.78^{d}	117.78 ± 8.98^{cd}	938.32 ± 65.77^{a}
	$Dx_{(10)}$ (μm)	21.51 ± 2.01^{a}	14.42 ± 2.12^{b}	5.96 ± 0.68^{c}	26.70 ± 3.19^{a}	21.49 ± 1.95^{a}	3.19 ± 0.75^{d}
	$Dx_{(50)}$ (μm)	181.00 ± 17.03^{b}	143.53 ± 12.98^{c}	15.21 ± 3.23^{d}	319.97 ± 27.98^{a}	230.02 ± 31.65^{b}	6.77 ± 0.61^{e}
	$Dx_{(90)}$ (μm)	485.43 ± 49.01^{c}	492.07 ± 50.08^{c}	34.12 ± 0.31^{d}	1289.09 ± 89.05^{a}	854.33 ± 54.23^{b}	14.62 ± 1.02^{e}

CF, cricket flour; DCF, defatted cricket flour; CPI, cricket protein isolate; GF, grasshopper flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate. Data are the mean and SD of three replicates. Same super-index letter in the line indicates non-statistical differences (Tukey's test, $p < 0.05$) between physicochemical or technofunctional parameters.

their related lipophilic compounds, about 7.5 % dw), suggesting it could be related to the presence of residual phytochemicals (i.e., carotenoids) or transformed endogenous pigments (e.g., biliverdin) solubilized into the hemolymph or fixed in the body (Varma et al., 2023). These powders can be used as raw materials for formulating food ingredients, providing valuable color values that can be used to generate other food ingredients and commercial foods.

3.4.2. Density indicators

Bulk density (BD) is a crucial property in powder products, as it impacts packaging costs and transportation efficiency. This characteristic is contingent upon the collective impact of various factors, including particle size and interparticle forces (Sharma, Jana and Chavan, 2012). Cricket and grasshopper flours, variations in bulk density are evident based on processing methods: DCF exhibits a higher ($p < 0.05$) bulk density (0.49 g/mL) than CF (0.38 g/mL). In comparison, DGF displays a higher ($p < 0.05$) bulk density (0.54 g/mL) than GF (0.42 g/mL). Comparing samples of different origins but with the same treatment, GPI showed the lowest bulk density (0.28 g/mL), followed by CPI (0.34 g/mL), indicating a correlation between insect type and bulk density. According to Flores-Jiménez et al. (2022), the extraction methods significantly influenced the density of protein materials, as shown in this study. Erdem and Kaya (2021) also noted that defatting impacts the modification of size, shape, spatial distribution, and morphology of whey protein isolates. The bulk densities found for the flour were consistent with expected trends due to particle shape (Fig. 4). The smaller, more spherical particles in CPI and GPI (18.34 μm and 14.11 μm, respectively) facilitate easier and more spontaneous accommodation, filling more inter-particle spaces than the larger, amorphous particles in DGF and DCF, likely retaining insect body parts from CF and GF.

Flowability, commonly determined by Carr's index (% compressibility, CI), is a key characteristic of powders, ranging from "good" to "bad" on a sliding scale (0–40 %) (Moravkar et al., 2022). This attribute significantly impacts the quality of processes such as packaging, filling, mixing, handling, and dosing (Ezzat et al., 2020). GF and DGF can be considered as "free flowing powder" (HR < 1.1), DCF is a "medium flowing powder" (HR < 1.25), CF and CPI are "difficult flowing powder" (HR < 1.4), and GPI is a "very difficult flowing powder" (HR > 1.4) (da Silva et al., 2024). These results are consistent with the CI classification, where GPI is considered "poor flowability" (CI > 25), and other samples exhibit "excellent flowability". The spherical shape and small particle size of the CPI and GPI (**Fig. 4G** and Fig. 4**H**, respectively) result in reduced flowability, as the particle surface area per unit mass increases (476.52 m^2/kg and 938.30 m^2/kg, respectively) with decreasing particle size. This increase in surface area provides a greater opportunity for surface cohesive forces to interact, resulting in a more cohesive flow behavior (Stavrou et al., 2020).

Spray-drying can influence the properties of the produced particles through van der Waals and electrostatic forces. Adsorbed moisture strengthens van der Waals forces by reducing inter-particle distances by adding a thicker layer. In contrast, higher moisture content diminishes electrostatic forces due to water's conductive nature (Tze et al., 2012). The Hausner ratio observed in samples followed the same behavior as CI. Excellent values were found in GF and DGF; good values in DCF; and from passable to poor in the remaining samples. The Hausner ratio provides a valuable measure of a powder's flow character. This ratio, calculated by comparing the bulk density to the tapped (loose) bulk density, indicates the powder cohesiveness and ability to flow. During food manufacturing, this value helps optimize handling, packaging, and transportation processes, ensuring efficient and consistent powder flow (Fitzpatrick, 2013).

3.4.3. Hygroscopicity and water activity

The ability of a powder to absorb moisture from the air, known as powder hygroscopicity, is a critical factor that affects the handling and storage of food powders. According to Ng and Sulaiman (2018), powders with hygroscopicity values above 15–20 % (determined at 75 % relative humidity) are considered hygroscopic. Samples exhibiting lower hygroscopicity (but not negative) are more manageable and packable (Wang et al., 2020).

The hygroscopicity of CPI increased statistically ($p < 0.05$) by 3.25-fold compared to CF, while GPI hygroscopicity decreased ($p < 0.05$) by 49 % compared DGF. However, the hygroscopicity of the studied spray-

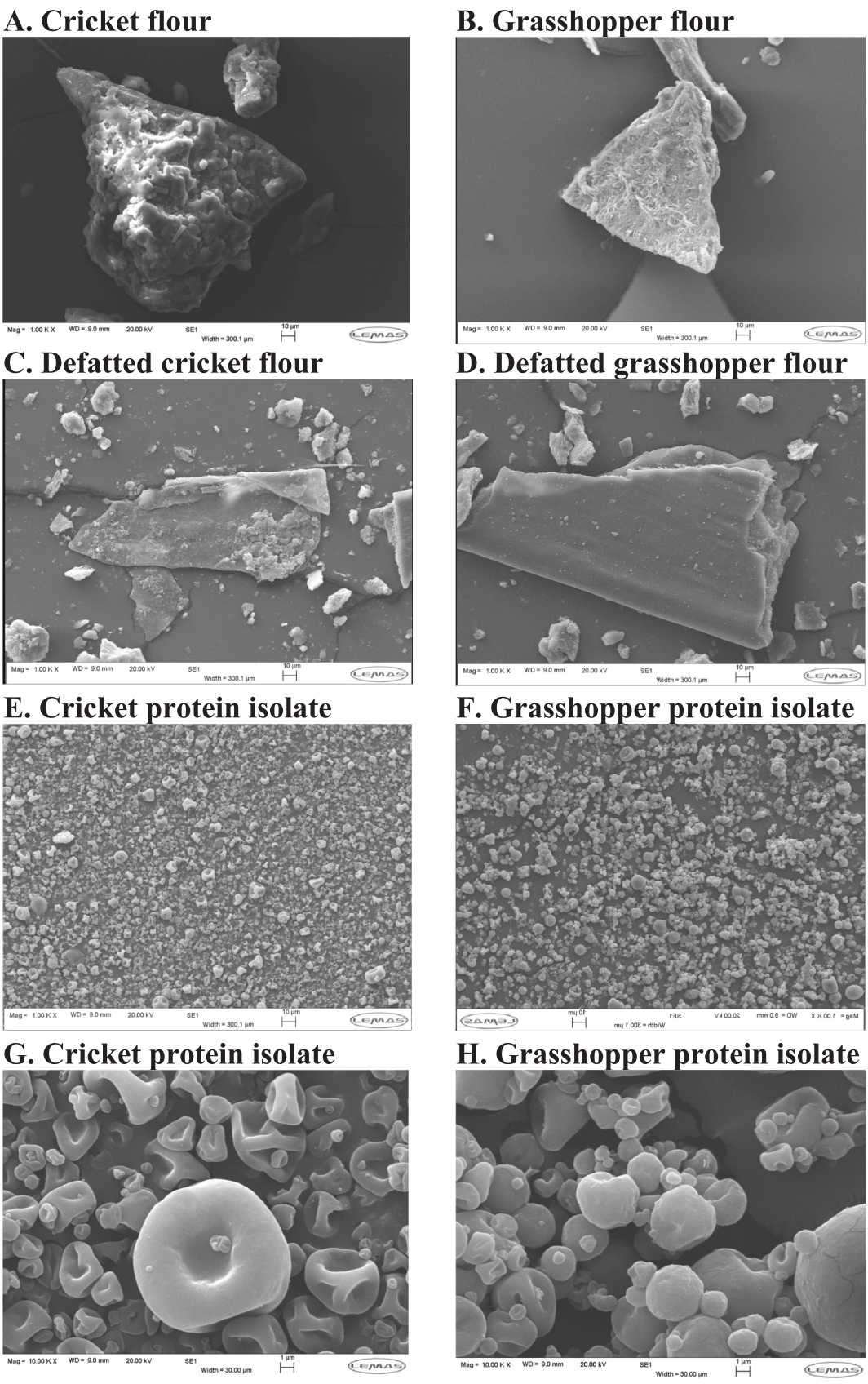


Fig. 4. Scanning electron microscopy (SEM) micrographs of cricket and grasshopper flours and protein isolates at 1000× (A–F) and 20,000× (G and H).

dried protein isolates from edible insects is superior to that of other powders, such as soy protein hydrolysates (18–39 g/100 g dry weight) and maltodextrin-gum Arabic (10.93–16.43 g/100 g) (Laureanti et al., 2023; Wang et al., 2020). Hygroscopicity refers to a material capacity to absorb moisture from the surroundings, impacting the storage and stability of powdered foods, which is also related to water activity. The obtained insect flours can be considered non-hygroscopic, according to the proposed limit range (<15 %). As indicated by Bhandari (2012), hygroscopicity is associated with amorphous structures characterized by open and porous molecules, enabling increased external interaction, as observed in CPI and GPI samples (Fig. 4).

Water activity (a_w) measures water availability for chemical or physical reactions or microbial growth within a food item. Typically, food degradation triggered by microbial proliferation, from yeast and molds to pathogens, occurs below a water activity of 0.6 (Gomes and Kurozawa, 2021). The cricket and grasshopper samples are not susceptible to microbial growth, as all a_w values ranged between 0.23 and 0.38. Both CPI and GPI isolates showed statistically significant ($p < 0.05$) lower aw values (aw $\leq$ 0.25) than insect flours and defatted flours (aw $\geq$ 0.38), indicating a reduction in susceptibility to microbial growth by over 30 % with protein purification processes. Other authors reported $a_w \leq 0.18$ (Lucas-González, Fernández-López, Pérez-Álvarez and Viuda-Martos, 2019).

However, certain enzymatic reactions, such as browning, occur within the range of 0.3–1.0, with a notable acceleration between 0.6 and 0.8 (Vanqa et al., 2022). The a_w values suggest potential susceptibility to rapid enzymatic degradation, which could affect the product stability. The moisture content of all samples was below 5 %, which is considered desirable to prevent powder agglomeration and microbial contamination (Vanqa et al., 2022). The a_w and moisture values suggest that the samples could have an extended shelf-life (Table 3).

3.4.4. Particle size distribution and specific surface area

The defatting and spray-drying processes in both insect samples influenced the average surface diameter (D[3,2]) of the particles. In cricket, the $D_{[3,2]}$ decreased statistically ($p < 0.05$) by 73.6 %, while in grasshoppers it decreased ($p < 0.05$) by 90 %. Meanwhile, after processing, the volume-average diameter ($D_{[4,3]}$) was reduced by 91.7 % and 97.3 % in cricket and grasshopper samples, respectively. The uniformity of the cricket particles decreased to 0.59 after spray-drying, whereas in grasshopper samples, it increased to 1.42, both of which were statistically different ($p < 0.05$) from the previous treatments. However, the specific surface area increased 3.8-fold and 9.1-fold in cricket and grasshopper samples. Particle size distributions ($Dx_{(10,50,90)}$) in spray-dried cricket samples decreased 3.6–14.2-fold and 8.4–88.2-fold in grasshopper samples.

Diameters of 17–35 μm are the most common in wheat flour particles (Tian et al., 2022), suggesting that the spray-dried samples can be easily rehydrated and integrated into other particles in the matrix. Moreover, the particle size of powdered ingredients has a significant influence on physicochemical properties, product quality, nutritional attributes, dough rheology, and processing efficiency. Therefore, achieving particle size characteristics superior to those of traditional food ingredients presents a valuable opportunity for spray-dried insect protein isolates to establish themselves as alternative ingredients with numerous advantages over conventional powders (Zhang Jian et al., 2018; Ma et al., 2022). Sensory properties of insect spray-dried proteins can also be influenced by particle size. Thus, the reduction of $Dx_{(10,50,90)}$ may have positive effects on the integration of other ingredients in various food formulations (Ruggeri et al., 2023).

3.4. Scanning electron microscopy.

Fig. 4 illustrates the morphometric characteristics of the insect samples. Important physicochemical and techno-functional attributes can be directly related to the microscopic morphometry of samples, such as density and hygroscopicity (Fu et al., 2020). For example, CF's high apparent surface area and particle size can be related to higher hygroscopicity (or water absorption capacity) compared to other samples (**Fig. 4A** and Fig. 4**B**). Therefore, the reduced fat content of the DCF and DGF samples, obtained by supercritical fluids (Fig. 4C and Fig. 4D, respectively), appears to increase both the quantity of coarse and fine particles, thereby decreasing uniformity, which the fat could facilitate on the surface of the particles. Typically, achieving a specific and narrow particle size distribution with an average particle size is preferred. These morphometric characteristics could be adjusted using milling technologies and size classification techniques, such as sifting and fine grinding (Fu et al., 2020). If the irregular distribution persists, it could alter physicochemical variables, as described previously, thereby affecting product formulation and final quality.

On the other hand, the protein-isolated powder displays a morphology similar among the particles but in a wide range of sizes (Fig. 4E-H). As observed, the particle size of the protein isolates was drastically reduced after spray drying, resulting in a more uniform particle shape. During spray-drying, a droplet containing abundant protein isolate undergoes rapid surface skin formation, though the thin layer may not dry uniformly. As moisture is removed, the droplet shrinks, settling wet spots on the thin layer, ultimately creating large concavities on dried particles (Fu et al., 2020).

Small and relatively uniform particles of CPI and GPI offer several key advantages for food ingredient formulation. It could improve flowability, making handling and processing more efficient while ensuring consistent mixing and homogeneity with other ingredients, as well as better solubility and dispersibility in liquids, a smoother texture, and a better mouthfeel, thereby enhancing the sensory qualities of the final product (Fu et al., 2020; Li et al., 2019).

3.5. Theoretical nutritional quality of proteins

The theoretical nutritional attributes of proteins from cricket and grasshopper samples are summarized in Table 4. The proportion of essential amino acids (PEAA) ranged from 42.17 to 44.80 % and 42.96–44.17 % in cricket and grasshopper, respectively, being the protein isolates from both samples statistically ($p < 0.05$) higher than their

Table 4
Theoretical parameters of nutritional quality of proteins from cricket and grasshopper samples.

	CF	DCF	CPI	GF	DGF	GPI
PEAA (%)	42.17 ± 0.15^{d}	42.15 ± 0.11^{d}	44.80 ± 0.53^{a}	42.96 ± 0.15^{c}	44.17 ± 0.22^{ab}	44.15 ± 0.14^{b}
AAS	162.00 ± 0.07^{b}	163.40 ± 0.05^{b}	177.83 ± 3.05^{a}	164.71 ± 0.54^{b}	174.72 ± 0.43^{a}	177.89 ± 1.30^{a}
EAAI (%)	905.33 ± 31.65^{c}	959.38 ± 26.81^{c}	1280.64 ± 138.08^{b}	951.46 ± 15.19^{c}	740.38 ± 26.63^{d}	1705.37 ± 54.00^{a}
BV	975.11 ± 34.50^{d}	1034.03 ± 29.22^{cd}	1384.19 ± 150.51^{b}	1025.39 ± 16.56^{c}	795.32 ± 29.02^{e}	1847.15 ± 58.86^{a}
PER_1	3.20 ± 0.04^{bc}	3.19 ± 0.01^{c}	3.44 ± 0.09^{a}	3.39 ± 0.00^{a}	3.25 ± 0.03^{b}	2.99 ± 0.03^{d}
PER_2	2.99 ± 0.02^{c}	3.01 ± 0.01^{c}	3.32 ± 0.05^{a}	3.23 ± 0.00^{b}	3.28 ± 0.04^{a}	3.02 ± 0.02^{c}
PER_3	2.24 ± 0.04^{d}	2.40 ± 0.02^{c}	3.08 ± 0.07^{b}	2.43 ± 0.02^{c}	3.48 ± 0.09^{a}	3.09 ± 0.04^{b}
PER_4	2.93 ± 0.01^{e}	2.93 ± 0.01^{e}	3.16 ± 0.04^{a}	2.98 ± 0.01^{c}	3.03 ± 0.03^{bc}	3.04 ± 0.01^{b}
PER_5	3.10 ± 0.00^{b}	3.11 ± 0.00^{b}	3.23 ± 0.04^{a}	3.06 ± 0.01^{c}	2.96 ± 0.01^{d}	2.98 ± 0.01^{d}

PEAA, proportion of essential amino acids; AAS, amino acid score (chemical score); EAAI, essential amino acid index; BV, biological value; PER, protein efficiency ratio. CF, Cricket flour; DCF, defatted cricket flour; CPI, cricket protein isolate; GF, grasshopper flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate. Same super-index letter in the line indicates non-statistical differences (Tukey's test, $p < 0.05$) between physicochemical or technofunctional parameters.

respective original flours. The amino acid score (AAS) indicates the PEAA relative to the international standard (whole egg) (Oser, 1959), ranging from 162.00 to177.83 % in cricket and 164.71 to177.89 % in grasshopper samples, with the highest AAS values observed in both protein isolates.

On the other hand, the essential amino acid index (EAAI) in cricket and grasshopper samples exceeded 7-fold, which is directly related to the high amounts of specific essential amino acids (i.e., His, Val, Phe, Ile, Leu, and Lys) in these samples compared to the international standard (whole egg) (Oser, 1959). The highest EAAI ($p < 0.05$) was observed in CPI and GPI. The biological value (BV) increased proportionally with the EAAI, following the same trend. The theoretical estimation of protein efficiency ratios (PER) also ranged from 2.24 to 3.48. All PER values, except PER1 and PER5 in grasshopper samples, were higher ($p < 0.05$) between original flours and protein isolates. The relatively high values of essential amino acids, like Leu, Lys, and Thr, positively influenced the PER values.

In a study, Oibiokpa, Akanya, Jigam, Saidu, & Egwim, 2018 reported similar content of essential amino acids in unprocessed Nigerian cricket powders. Thus, the AAS values were similar to CF, DCF and GF (<170 %), but inferior to protein isolates. Consequently, the quality parameters of cricket and grasshopper samples are higher than those of some plant-based high-protein products, which are typically limited in these essential amino acids (Sánchez-Velázquez et al., 2021). However, comparisons against other nutritional quality parameters from concentrated or isolated proteins from edible insects remain scarce.

3.6. Antioxidant activity

Antioxidant-rich diets help protect cells from damage caused by harmful molecules, such as free radicals, thereby reducing the risk of chronic diseases, including cancer, cardiovascular diseases, and aging-related conditions. Incorporating ingredients with high antioxidant activity, such as raw and defatted flours, as well as protein concentrates and isolates from edible insects, into food formulations can enhance the functional properties of foods and promote overall well-being when consumed as part of a balanced diet. Table 5 summarizes the antioxidant evaluation of the different edible insect samples.

3.6.1. ABTS

The Trolox equivalent antioxidant capacity showed higher ABTS scavenging activity in grasshopper samples (IC_{50} = 95.77–205.80 μg/mL), with statistical differences among samples ($p < 0.05$). In contrast, DCF and CPI did not show differences ($p > 0.05$), but were higher than CF. In black cricket protein concentrate, the ABTS radical was also scavenged (1.62-fold) with enzymatic hydrolysis, which increases the protein/peptide content and may impact the ingredients' functionality (de Matos et al., 2021). Foods with a fair content of Tyr, Trp, Cys, and Met (>1 %), such as the cricket and grasshopper samples analyzed (data not included), are related to donating electrons or hydrogen atoms to neutralize free radicals in assays like ABTS (Damgaard et al., 2015). Fashakin et al. (2023) identified antioxidant peptides released from fractioned proteins found in *Gryllus bimaculatus*, reporting that small peptides (<3 kDa) exhibited the highest ABTS scavenging activity. In water-soluble proteins from grasshopper, Chatsuwan et al. (2018) reported ABTS IC_{50} = 69.12–81.97 μg/mL, which is 2.98-fold higher than the grasshopper results obtained in this study. The ABTS was even higher in locust samples, with IC_{50} = 16.6–25.9 μg/mL (Zielińska, Baraniak, & Karaś, 2017, Zielińska, Karaś, & Jakubczyk, 2017).

Table 5
In vitro antioxidant activity of cricket and grasshopper samples.

Samples	Antioxidant Activity (IC_{50})				
	ABTS (μg/mL)	DPPH (mg/mL)	Fe chelation (mg/mL)	Cu chelation (μg/mL)	ORAC (μg/mL)
CF	498.80 ± 32.94^{a}	9.69 ± 2.93^{ab}	0.95 ± 0.09^{b}	422.74 ± 32.30^{a}	458.35 ± 4.63^{a}
DCF	358.47 ± 10.98^{c}	14.89 ± 3.77^{a}	1.50 ± 0.14^{a}	194.73 ± 72.33^{b}	342.20 ± 5.57^{c}
CPI	391.12 ± 22.51^{b}	10.93 ± 2.09^{a}	0.61 ± 0.09^{c}	60.32 ± 8.76^{d}	230.74 ± 3.54^{d}
GF	139.90 ± 8.26^{e}	6.19 ± 0.95^{b}	0.68 ± 0.09^{c}	230.64 ± 66.64^{b}	412.61 ± 2.56^{b}
DGF	205.80 ± 17.24^{d}	6.08 ± 0.45^{b}	1.41 ± 0.08^{a}	240.21 ± 15.36^{b}	251.84 ± 17.81^{d}
GPI	95.77 ± 2.35^{f}	3.11 ± 0.35^{c}	0.63 ± 0.07^{c}	80.38 ± 11.22^{c}	103.91 ± 6.15^{e}

CF, cricket flour; DCF, defatted cricket flour; CPI, cricket protein isolate; GF, grasshopper flour; DGF, defatted grasshopper flour; GPI, grasshopper protein isolate. Data are the mean and SD of three replicates. Same super-index letter in each column indicates non-statistical differences (Tukey's test, $p < 0.05$) among antioxidant evaluations.

3.6.2. DPPH

The DPPH scavenging activity in cricket samples did not show changes ($p > 0.05$) with the applied processes (IC_{50} = 9.69–14.89 μg/μL). After defatting and protein isolation, the grasshopper showed the highest DPPH scavenging activity (IC_{50} = 3.11 mg/mL, $p < 0.05$). Similar to ABTS, de Matos et al. (2021) also reported an increased DPPH scavenging activity after applying enzymatic hydrolysis (1.68-fold). Other locusts and grasshoppers showed DPPH scavenging activity of IC_{50} = 0.07–0.20 μg/mL (Chatsuwan et al., 2018; Zielińska, Baraniak, & Karaś, 2017, Zielińska, Karaś, & Jakubczyk, 2017), which it several-fold higher than the results obtained in this research.

3.6.3. Metal chelation

Iron chelating activity was negatively affected by the defatting step (IC_{50} = DCF 1.50 mg/mL, DGF = 1.41 mg/mL); however, it was recovered in grasshopper (IC_{50} = 0.61 mg/mL, $p < 0.05$) but improved in cricket (IC_{50} = 0.63 mg/mL, $p < 0.05$) samples. On the other hand, copper chelating activity improved from IC_{50} = 422.74 to 60.32 μg/mL in cricket samples. No statistical changes were found between GF and DGF values ($p > 0.05$), but the highest chelation for copper was reported in GPI (IC_{50} = 80.38 μg/mL, $p < 0.05$). The metal (iron) chelating activity of *Gryllus bimaculatus* protein hydrolysates showed the highest values in >10 kDa peptides, opposite to what was observed in ABTS, where the best values were observed in smaller peptides (Fashakin et al., 2023). Particularly, defatting removes lipids, which can interfere with metal ion binding by proteins. By reducing lipid content, the chelating sites on proteins, especially those involving amino acids such as His, Cys, and carboxyl groups, become more accessible for binding with iron and copper ions, potentially enhancing chelating activity (Gulcin & Alwasel, 2022).

3.6.4. ORAC

Cricket samples' oxygen radical absorbance capacity improved statistically ($p < 0.05$) with processing from IC_{50} = 458.35 to 230.74 μg/mL. Similarly, the grasshopper displayed the same behavior, with values from IC_{50} = 412.61 to 103.91 μg/mL. David-Birman et al. (2018) found that thermal treatment (cooking and baking) positively impacted the ORAC values of cricket flour. In a cricket protein hydrolysate, the ORAC values (IC_{50} = 34 μg/mL) (Mudd et al., 2022) were higher, at least 3-fold, than the cricket or grasshopper samples analyzed.

Defatting, coupled with protein extraction, concentration, and isolation processes, enhances the antioxidant capacity of edible insect flours through various mechanisms. Defatting removes lipids containing oxidative compounds, reducing potential sources of oxidative stress. The antioxidant capacity, evaluated by ABTS, ORAC, and metal chelation, increases in both insect protein isolates after the protein purification process. However, it is important to note that proteins from these insects may also carry other compounds with redox potential, such as phenolics and other phytochemicals bioaccumulated from their diet. Thus, they can exhibit a synergetic antioxidant activity with insect proteins.

Each step in the isolation process contributes to enhancing the antioxidant activity of the protein isolates by either increasing the concentration of bioactive peptides, unmasking functional groups, forming new antioxidant compounds, or breaking down proteins into smaller, more active peptides (Rahman & Lamsal, 2021). These changes, including enhanced performance in assays such as ABTS, DPPH, iron chelating activity, and ORAC, making the isolated proteins more effective at neutralizing free radicals and chelating metal ions. In summary, these processes collectively enhance the antioxidant capacity of edible insect flours, making them a nutritious and potentially beneficial option for reducing oxidative damage and promoting overall health.

4. Conclusions

Supercritical fluid is an effective, eco-friendly technology for removing lipids from insect flours, contrasting with solvent extraction, pressing, and gravimetric procedures. Combined processing with heating, ultrasound, and partial enzymatic hydrolysis, it was possible to obtain protein isolates with more than 90 % purity. Protein solubility and digestibility were improved on generated protein isolates. The physicochemical parameters of CPI and GPI were modified in most cases with the treatments, improving properties such as color, density, and water-associated indicators compared to whole flours. Particle characterization and SEM revealed significant changes in surface parameters and particle size in protein isolates, which could be favorable for incorporating these powders into food formulations.

The nutritional parameters related to the presence of essential amino acids in the flours and protein isolates remark the high potential of these ingredients as sources of proteins with high nutritional quality, especially the protein isolates. Furthermore, these ingredients could help protect against oxidative stress due to their antioxidant potential. Incorporating cricket and grasshopper protein isolates into functional foods can improve their nutritional and health benefits. This first approach provides a novel green protein extraction process for these potential food sources, contributing to the search for sustainable, innovative, and healthy diets.

Founding statement

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CRediT authorship contribution statement

Oscar Abel Sánchez-Velázquez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alan Javier Hernández-Álvarez:** Writing – review & editing, Supervision, Investigation, Data curation. **Alejandro Davalos-Vazquez:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Stephania Aragón-Rojas:** Writing – review & editing, Visualization, Data curation. **Lorena Moreno-Vilet:** Writing – review & editing, Supervision, Methodology, Data curation. **Gustavo Adolfo Castillo-Herrera:** Writing – review & editing, Supervision, Conceptualization. **Diego Armando Luna-Vital:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Luis Mojica:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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