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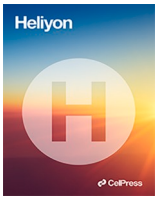

Research article

Physicochemical and techno-functional characterization of soluble proteins extracted by ultrasound from the cricket *Acheta domesticus*

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ABSTRACT

The growing interest in using insects for human consumption is due to their numerous benefits. Insects offer efficient protein generation, rapid growth rates, and high nutritional value. The objective of this work was to evaluate the physicochemical and techno-functional properties of the different soluble protein fractions of the cricket *Acheta domesticus* using various methods: grinding (CF), defatting (DCF), alkalinization (SPA), and ultrasound-assisted extraction (SPS). CF, DCF, SPA, and SPS were used as extenders in food models and compared with a control group prepared with meat and a commercial soy protein (SPI). Defatting increased the protein content (52 %) in CF, improving digestibility, while the SPS extraction method improved solid recovery (40.46 %), protein recovery (41.94 %), total protein content (53.85 %), and digestibility (53.7 %) compared to SPA. Proteins exhibited pH-dependent solubility, with higher solubility at pH 12–13 and an isoelectric point of 4.5. In techno-functional properties, SPS had the highest water/oil retention capacity (2.8 g/g, 3.49 g/g), foam formation (386.66 %), and emulsifying stability (32.96 m^2/g). CF showed no foam formation, although defatting (DCF) improved foam formation (8.33 %) and emulsifying stability (6.23 m^2/g). Heat coagulation was higher for CF and DCF (30.58 % and 30.33 % respectively). All meat models with SPA and SPS showed high elasticity and cohesiveness but low hardness, gumminess, and chewiness. The model prepared with 15 % SPS reduced cooking losses (0.91 %) and total expressible fluid separation (1 %), improved water retention capacity (83.02 %), and increased total soluble protein content (5.32 mg/mL). The ultrasound-assisted extraction method proved to be an efficient way to obtain soluble proteins from *Acheta domesticus* with techno-functional properties suitable for use as a food additive or meat extender.

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1. Introduction

Even as food production keeps pace with population growth, the 2022 United Nations Report on the State of Food Security and Nutrition in the World reveals alarming trends: 828 million people are hungry, 29.3 % of the world's population faces food insecurity, and 22 % of children under five are undersized. Additionally, many people suffer from poor-quality diets and micronutrient deficiencies, contributing to obesity, diabetes, and other diet-related diseases. Furthermore, agri-food systems harm the environment and climate, affecting soil and water resources [1]. Therefore, the focus remains on researching and developing practices that target the nutritional needs of the world's population economically and efficiently, thus helping underdeveloped countries [2].

The notion of using insects for both human consumption and animal feed is gaining attention due to their numerous advantages. Insects are more efficient in protein generation because they possess rapid growth rates and offer substantial nutritional value. Cultivating insects has notable environmental and economic implications, as they demand fewer resources for growing and exhibit lesser ecological impact, particularly concerning greenhouse gas emissions and water footprint, than traditional livestock production. These factors position insect production as a potentially eco-friendly industry [3–5].

Entomophagy, the consumption of insects, has been part of the human diet for centuries and is recognized as an essential nutritional source, providing high-quality proteins, lipids, carbohydrates, and minerals [6]. Today, entomophagy remains a form of nutrition, mainly in Africa, Asia, and Latin America, with more than 2300 species of insects used for consumption [7]. Illa and Yuguero [8] have suggested that edible insects can be considered a complementary protein to the traditional diet based on animal protein. However, the significant obstacle of food neophobia interferes with the widespread acceptance of this alternative food source [9]. Numerous studies propose that edible insects could be integrated as flours, protein concentrates, or isolates in various food products to improve consumer acceptance [10–12].

The Orthoptera order within edible insects represents 13 % of the total [13]. These insects are grasshoppers, locusts, and crickets [14]. Crickets are native to Southwest Asia, although they are currently found in many continents, such as Europe, North Africa, North America, and Australia [15].

Breeding and massive consumption of the common cricket (*Acheta domesticus)* is an alternative for livestock feed and other farm animals. It can be used for human consumption, taking advantage of its high nutritional value, low production cost, and diminished environmental impact [16]. Currently, *Acheta domesticus* is cultivated commercially as a viable option for human feeding. Furthermore, it can be used as bait for sport fishing [17].

Despite the high protein content of edible insects, comparable to conventional protein sources, enrichment of the protein content is desirable. Therefore, the removal of indigestible fractions, e.g., chitin, has to take place. Defatted insect powder is typically the source of insect protein extraction. Methods for extracting protein are commonly divided into conventional techniques (such as aqua-based, salt, solvent, detergent, and alkali methods) and non-conventional or advanced methods (including enzyme, ultrasound, microwave, and pulsed electric field-assisted extraction) [18]. Among them, alkaline extraction is the most common method for isolating protein fractions. The solubility of proteins increases with an increase in the pH of the solvent due to the ionization of acidic and neutral amino acids at high pH. Therefore, protein extraction in an alkaline environment produces higher protein yields [19]. Furthermore, several reports reveal that the protein extraction of insects increases with higher pH [20–23]. Ultrasound-assisted extraction is considered a green alternative technique because it is simple to operate, is time-efficient, and requires less solvent; therefore, it is considered environmentally friendly [24]. Moreover, the proteins extracted by this method have yields from 35 to 94 % due to the cavitation effect caused by the ultrasound [25].

Acheta domesticus is an insect cultivated on farms, providing readily available raw material. In addition, the techno-functional properties of the soluble protein of this insect still need to be documented. Moreover, there is recent interest regarding methods for extracting insect proteins for use as meat extenders or food additives. This study aimed to assess the physicochemical and techno-functional characteristics of various soluble protein fractions derived from the cricket *Acheta domesticus*. These fractions were obtained through grinding, defatting, alkalinization, and ultrasound treatment. The goal was to determine their suitability as extenders or additives in food formulations.

2. Materials and methods

2.1. Defatted flour

The flour was obtained from adult dehydrated crickets (*Acheta domesticus*) purchased from Insect in Nutrition (Apaseo el Grande, Guanajuato, Mexico). The insects were reduced in size in a blender (Oster BLSTTSCB2000, Coahuila, Mexico) to obtain a powder labeled as cricket flour (CF). CF was defatted according to the method described by Choi et al. [25], with some modifications. Hexane (Meyer, Mexico) was used as a solvent with a ratio of 1:5 (insect-solvent). The mixing was shaken at 1050 rpm in a stirring hot plate at 25 °C (CORNING® PC-420D, Massachusetts, USA), and the solvent was replaced every 24 h up to 72 h. The flour was left on an aluminum tray at room temperature for two days until the remaining hexane was eliminated. The flour obtained was labeled as Defatted-Cricket flour (DCF).

2.2. Protein solubility

The solubility determination was carried out according to the method described by Buβler et al. [21]. DCF (7 g) was mixed with 50 mL of distilled water. It was adjusted to various pHs in an interval of 1–13 with augments of one unit, using 1 mol L^{-1} HCl (J.T. Baker,

USA) and 1 mol L^{-1} NaOH (J.T. Baker, USA) and centrifuged (Velab PRO 8M, CDMX, Mexico) at 2450×*g* for 8 min. The supernatants were characterized by determining the soluble protein content using the Bradford method.

2.3. Ultrasonic protein extraction

Protein extraction was, according to Choi et al. [25], with some modifications. Piezoelectric ultrasound (Sonics® Vibra-Cell™ VCX 130 PB, Connecticut, USA) was used at a maximum power of 130 W and 20 kHz with 90 % amplitude. DCF (7 g) was mixed with 50 mL of a 0.5 mol L^{-1} NaOH (J.T. Baker, USA) solution (pH 13) and 1 % food-grade sodium metabisulfite (it is used to avoid enzymatic browning and to obtain a less dark extract) [26]. The mixture was subjected to ultrasound for 20 min (pulse duration, 4 s; pulse interval, 1 s in an ice bath, ensuring that the temperature did not exceed 60 °C, and then centrifuged (Velab PRO 8M, CDMX, Mexico) for 8 min at 2450×*g*. The supernatant was filtered using Whatman No. 1 filter paper to remove flour residues. The supernatant was lyophilized (Labconco FreeZone® 2.5, Houston, USA), and the powder was labeled as soluble protein by sonication (SPS).

2.4. Alkalinization protein extraction

Protein extraction was according to the method described by Cruz-López et al. [23], with some modifications. DCF (7 g) was mixed with 50 mL of a 0.5 mol L^{-1} NaOH (J.T. Baker, USA) pH 13 and 1 % sodium metabisulfite. The suspension was stirred (CORNING® PC-420D, Massachusetts, USA) at 1050 rpm for 30 min. The mix was centrifuged (Velab PRO 8M, CDMX, Mexico) for 8 min at 2450×*g*, and the supernatant was filtered to eliminate flour residues. The supernatant was lyophilized (Labconco FreeZone® 2.5, Houston, USA), and the powder was labeled as soluble protein by alkalinization (SPA).

2.5. Proximal analysis and digestibility

Moisture, fat, ashes, and total protein were performed according to method 23.003 A.O.A.C [27]. The digestibility of CF, DCF, SPA, and SPS was assessed using the modified pepsin digestion method reported by Wang et al. [28]. The procedure involved dissolving 0.04 g of pepsin-simulated gastric fluid (P6887, Sigma-Aldrich) at a pH of 2 in 100 mL of 0.075 eq L^{-1} HCl (J.T. Baker, USA). Samples were combined with the pepsin solution to achieve a protein concentration of 1 %. The solution was equilibrated at 37 °C in a water bath for 90 min, corresponding to the typical food transit time within the human stomach. Following this digestion period, the pepsin activity was stopped by adjusting the pH to 7 using 1 mol L^{-1} $NaHCO_3$. Results were reported based on 100 % crude protein.

2.6. Percentage yield of solids and protein recovery

Solids yield and recovery percentage were calculated using Equations (1) and (2) [29].

$$\%\ yield\ of\ solids = \frac{freeze - dried\ concentrate\ weight}{weight\ DCF + sodium\ metabisulphite} * 100 \quad (1)$$

$$\%\ protein\ recovery = \frac{\%\ solids\ yield * \%\ protein\ concentrate}{\%\ DCF\ protein} * 100 \quad (2)$$

2.7. Techno-functional properties as a food additive

2.7.1. Water holding capacity

The method for determining water-holding capacity (WHC) outlined by Jeong et al. [22] was used with minor adjustments. The 0.5 g sample was mixed with 10 mL of water and shaken for 30 min at 500 rpm using a CORNING® PC-420D (Massachusetts, USA). The supernatant was weighed after centrifugation at 2450×*g* for 30 min using a Velab PRO 8M centrifuge (CDMX, Mexico). As a positive control, soy protein isolate (SPI) (FABPSA, CDMX, Mexico) was tested according to this methodology.

The WHC was determined using the formula shown in Equation (3), which is given in g of water absorbed/g of sample.

$$WHC = \frac{weight\ of\ water\ used - weight\ of\ supernatant}{sample\ weight} \quad (3)$$

2.7.2. Oil holding capacity

The oil retention capacity (OHC) was determined as described in the previous point but using canola oil instead of water and mixing at 550 rpm. A soy protein isolate (SPI) (FABPSA, CDMX, Mexico) was used as a positive control. The OHC was calculated as Equation (4), expressed as g of oil absorbed/g of sample.

$$OHC = \frac{weight\ of\ oil\ used - weight\ of\ supernatant}{sample\ weight} \quad (4)$$

2.7.3. Foaming properties

Foaming ability and foam stability were evaluated according to Antonic et al. [30] and Mishyna et al. [31]. After dispersing 0.2 g of

the samples in 20 mL of phosphate buffer (pH 7, 0.2 mol L^{-1}), the samples were homogenized for 90 s at 21,000 rpm using a frothier (Dahlfoll, EW-071Berlin, Germany). The foam volume was measured in a 100 mL graduated cylinder just after the end of the homogenization. A soy protein isolate (SPI) (FABPSA, CDMX, Mexico) was used as a positive control. The foaming capacity was calculated according to Equation (5), where V_T is the final volume (mL) at different times (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 110 min) and V_0 is the initial volume of the sample (mL). Foam stability was observed every 5 min until 110 min. Foaming stability was determined by Equation (6), where FC_0 is the foaming capacity at cero time.

$$Foaming\ capacity\ (FC\ \%) = \frac{V_T - V_0}{V_0} \times 100 \tag{5}$$

$$Foaming\ stability\ (FS\ \%) = \frac{FC}{FC_0} \times 100 \tag{6}$$

2.7.4. Emulsifying properties

The emulsifying activity index (EAI) was determined with some modifications following Kim et al. [32]. The 0.2 g of the sample was mixed in 20 mL of 0.2 mol L^{-1} phosphate buffer at pH 7 and homogenized with 2 mL of canola oil at 21,000 rpm for 90 s using a frothier (Dallfoll, EW-071, Berlin, Germany). After homogenization, 50 μL of the emulsion was collected and combined with 10 mL of 0.3 % (w/v) sodium dodecyl sulfate (SDS) (Sigma-Aldrich, USA) to evaluate the emulsifying activity index. As a positive control, soy protein isolate (SPI) (FABPSA, CDMX, Mexico) was tested. The absorbance was measured using a spectrophotometer (Velab-V-5000, CDMX, Mexico).

The EAI was calculated using Equation (7) reported by Zhang et al. [33]:

$$EAI\left(\frac{m^2}{g}\right) = 2 \times 2.303 \times \frac{A_0 \times FD}{10000 \times L \times \Theta \times C} \tag{7}$$

where EAI represents the emulsifying activity index, A_0 represents the absorbance at time 0, FD is the dilution factor of the sample, L is the length of the cell (1 cm), Θ the portion of the oil, and C is the concentration of the aqueous phase (g/cm^3).

The determination of emulsion stability (SE) was calculated according to Mishyna et al. [31] with some modifications. The emulsion was prepared according to the previous section, where 50 μL were immediately dispersed in 10 mL of 0.3 % SDS (Sigma-Aldrich, USA). The tubes were inverted several times, and the absorbance at 500 nm was measured at 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min. The stability was calculated according to Equation (8).

$$SE\ (\%) = \frac{A_T}{A_0} \times 100 \tag{8}$$

where SE represents the stability of the emulsion, A_0 is the absorbance of the emulsion at time zero, and A_T is the absorbance of the emulsion at different times.

2.7.5. Coagulation percentage

The percentage of coagulation was determined following the methodology of Mishyna et al. [34], with some modifications. 0.1 g of

Table 1
Meat models (sausages) formulations using different fractions of *Acheta domesticus* as meat-extender.

Treatments	Ingredients (%)[a]									
	Pork meat	Frozen lard	CF	DCF	SPA	SPS	SPI	Sodium chloride	Nutrifos 088	Sodium nitrate
Control	50	20	–	–	–	–	–	3.0	0.3	0.3
CF 5 %	47.5	20	2.5	–	–	–	–	3.0	0.3	0.3
CF 10 %	45	20	5.0	–	–	–	–	3.0	0.3	0.3
CF 15 %	42.5	20	7.5	–	–	–	–	3.0	0.3	0.3
DCF 5 %	47.5	20	–	2.5	–	–	–	3.0	0.3	0.3
DCF 10 %	45	20	–	5.0	–	–	–	3.0	0.3	0.3
DCF 15 %	42.5	20	–	7.5	–	–	–	3.0	0.3	0.3
SPA 5 %	47.5	20	–	–	2.5	–	–	3.0	0.3	0.3
SPA 10 %	45	20	–	–	5.0	–	–	3.0	0.3	0.3
SPA 15 %	42.5	20	–	–	7.5	–	–	3.0	0.3	0.3
SPS 5 %	47.5	20	–	–	–	2.5	–	3.0	0.3	0.3
SPS 10 %	45	20	–	–	–	5	–	3.0	0.3	0.3
SPS 15 %	42.5	20	–	–	–	7.5	–	3.0	0.3	0.3
SPI 5 %	47.5	20	–	–	–	–	2.5	3.0	0.3	0.3
SPI 10 %	45	20	–	–	–	–	5.0	3.0	0.3	0.3
SPI 15 %	42.5	20	–	–	–	–	7.5	3.0	0.3	0.3

Cricket-flour (CF), Defatted-cricket-flour (DCF), Soluble-protein by alkalinization (SPA), Soluble-protein by sonication (SPS), and Soy protein isolate (SPI).

[a] Ice (frozen water) was employed to complete 100 %.

the samples were dispersed in 10 mL of 0.2 mol L^{-1} phosphate buffer, pH 7; the suspension was stirred (CORNING® PC-420D, Massachusetts, USA) at 650 rpm for 30 min and centrifuged (Velab PRO 8M, CDMX, Mexico) for 25 min at 2450×*g*. The supernatant was divided into two fractions. One fraction was heated to 85 ± 2 °C for 30 min and cooled to room temperature. The second fraction did not receive any heating treatment. 0.5 mL for each fraction was mixed with biuret reagent (2 mL) (Meyer, Mexico) and incubated for 30 min in the dark. The absorbance was measured at 540 nm using a spectrophotometer (Velab, V-5000, CDMX, Mexico). As a positive control, soy protein isolate (SPI) (FABPSA, CDMX, Mexico) was tested.

The percentage of heat coagulation (%HC) was calculated with Equation (9).

$$\%\ HC = \frac{Abs\ before\ warm\ up - Abs\ after\ warm\ up}{Abs\ before\ warm\ up} \times 100 \tag{9}$$

2.8. Techno-functional evaluation as meat-extender

2.8.1. Elaboration of the cooked meat batter (sausages)

A control group consisting of 100 % meat and various formulations in which meat was replaced with CF, DCF, SPA, SPS, and SPI (soy protein isolate) (FABPSA, CDMX, Mexico) were prepared (n = 15; see Table 1). Each formulation was evaluated at three different substitution levels: 5 %, 10 %, and 15 %. Lean pork was purchased at a local market in Mexico, and its connective tissue was removed. The meat was ground in a food processor (Moulinex DPA2, Ecully, France) and mixed with sodium chloride, salt Fabpsa (FABPSA, CDMX, Mexico) (curing salt), and nutrifos-088 (phosphates) (FABPSA, CDMX, Mexico), incorporating half the ice used in this formulation for 3 min. Lard (pork loin fat) was added and emulsified for an additional minute. The rest of the ice was added to make it 100 % complete and emulsified for 2–3 min, maintaining the temperature of the meat batter (12 ± 2 °C). CF, DCF, SPA, SPS, and SPI were added with the different salts. The mixtures were stuffed into a 20-mm cellulose casing and cooked in a water bath until an internal temperature of 70 ± 2 °C for 30 min. Samples were cooled in an ice bath until 15 °C [10]. The sausages were vacuum-packed and stored in refrigerated conditions until use.

2.8.2. Emulsion stability

Samples of meat batter were evaluated before cooking according to Kim et al. [35], with some modifications. For which, 20 g of emulsified paste was placed in 50 mL tubes. The samples were heated for 30 min in a water bath at 75 °C and then cooled to 4 °C. The water and fat content were quantified in the liquid phase expelled after cooking. Emulsion stability was reported as total expressible fluid separation (TEFS) (*mL/g sample*) according to Equation (10) [36].

$$TEFS\ (mL\ /\ g\ sample) = \frac{WL(mL) + LF\ (mL)}{RMW(g)} * 100 \tag{10}$$

where TEFS represents the total expressible fluid separation (mL/g sample), WL represents the water layer (mL), LF is the layer of fat (mL), and RMW is the raw meat weight (g).

2.8.3. Cooking losses

Samples were weighed before and after the heat treatment. The yield was calculated by determining the weight loss by cooking the sausage according to Equation (11)[37].

$$\%\ weight\ cooking\ loss = \frac{(initial\ weight - final\ weight\ after\ cooking)}{initial\ \ weight} * 100 \tag{11}$$

2.8.4. Water holding capacity

The measurement of water holding capacity was carried out following the compression technique of Orozco et al. [38], with some modifications. A sample of cooked meat batter (sausages) of approximately 0.6 ± 0.05 g was placed between two coffee filters, and a 1 kg weight was applied for 60 min. WHC was determined according to Equation (12).

$$\%\ water\ holding\ capacity = \frac{weight\ after\ compression}{Initial\ sample\ weight} * 100 \tag{12}$$

2.8.5. Protein solubility

The soluble protein content in the meat batter samples was determined following Lee et al. [39] adjusted with some modifications. 10 mL of buffer (1.1 mol L^{-1} potassium iodide in 0.1 mol L^{-1} potassium phosphate buffer pH 7) was added to 1 g of paste. For sarcoplasmic proteins, 1 g of paste was added with 10 mL of buffer (0.025 mol L^{-1} potassium phosphate at pH 7). Each sample was homogenized using a frothier (Dallfoll, EW-071, Berlin, Germany) for 2 min at 23,000 rpm and kept for 16 h at 4 °C. Later, it was centrifuged at 2450×*g* for 15 min, and the supernatant of both samples was filtered. The soluble protein content was measured using the Bradford method.

2.8.6. Texture and color analysis

Texture profile analysis (TPA) was evaluated using a texture analyzer (Brookfield model CT3, AMETEK, Berwyn, PA, USA)

equipped with a cylindrical probe (TA-AACC36) (40 % deformation, 0 s of waiting, 0.0068 kg of load and speed of 5 mm/s). The sausages were cut into 20 mm slices. The data on hardness, adhesiveness, tractability, cohesiveness, elasticity, firmness, and chewiness were obtained.

Sausage samples were cut transversely with a thickness of 25 mm, and color was determined using a colorimeter (ColorFlex EZ 45/0 spectrophotometer; Hunter Lab, Reston, VA, USA) previously calibrated. The CIE LAB color coordinates L* (lightness), a* (redness), and b* (yellowness) were established with an angle of 10° and a D65 light source. The average color per sample was determined from 4 readings rotating the sample 90° [40].

2.9. Statistical analysis

All determinations were performed in triplicate, and the results are presented as the average and its standard deviation. Statistical analyses were performed with the XLSTAT software version 2014.5.03 (Addinsoft, Paris, France) using a limit alpha value of 0.05. The results were analyzed using a one-way analysis of variance (ANOVA) and mean comparison tests with the Tukey method between the treatments for each of the methodologies used. Principal Component Analysis (PCA) was obtained for color and texture tests.

3. Results and discussion

3.1. Proximal analysis and digestibility

The proximal analysis parameters of each sample obtained from *Acheta domesticus* are presented in Table 2. Degreasing with hexane removed 98.9 % of the total fat present in CF. The treatments DCF, SPA, and SPS showed a residual fat content of 1.17 %, 1.2 %, and 1.8 % w/w, respectively. The results are similar to other authors that mention that the residual fat content in *Gryllus bimaculatus* after defatting with hexane was 1.07–1.83 % [22,25]. An increase in protein, ash, and carbohydrate content is observed due to extracting the fat content for DCF. The carbohydrate content reported by Montowska et al. [41] presented a range of 19.6–21.8 %, and Ayeiko et al. [42] reported 25.8 % for *Acheta domesticus* in western Kenya. Both values are comparable with the carbohydrate content obtained in this work (23.68 %). On the other hand, SPS showed the lowest carbohydrate content, and it was significantly different ($p < 0.05$) from other treatments, which can be attributed to the presence of remaining chitin, which is gradually lost at each extraction stage. The total protein content for CF was 45.75 % using the conversion factor $N_{kjel4.5}$, according to Mishyna et al. [31], for Orthoptera. Nevertheless, considering the content of chitin, phospholipids, and excretion products present in insects, there is an overestimation when using the common factor of $N_{kjel6.25}$ since not all nitrogen is of protein origin [43]. Montowska et al. [41] reported similar results in powders from *Acheta domesticus* (roasted and finely milled) from different origins (Thailand and Canada) with a conversion factor of $N_{kjel4.5}$, in which the protein content is between 42.0 and 45.8 %. SPA was significantly different ($p < 0.05$) from CF, DCF, and SPS and showed the lowest protein content because it is an extract derived from CF and DCF. According to the results, SPA extraction had lower protein recovery (11.29 %) than SPS (41.94 %), indicating that the alkalinization method is not efficient for soluble protein extraction from flour (DCF). On the other hand, the flours (CF and DCF) could be an overestimation of total protein by the presence of non-protein nitrogen [43]. Similar results were observed by Mishyna et al. [31], where total protein increased from 39.6 to 55.2 % after an ultrasound process in *Apis melífera*. The increase in ash is due to the extraction method, as sodium hydroxide and sodium metabisulfite were concentrated, showing evidence that the sodium in the samples was not removed. Salts have various effects on proteins and, in high concentrations, affect their functional properties due to the change of charges and the reduction of electrostatic repulsion between proteins [44,45]. However, treatments with higher ash content (SPS and SPS) presented the best techno-functional properties.

Digestibility measures the degree of net absorption of nutrients in the digestive tract; therefore, the higher the digestibility, the better the protein quality [46,47]. CF and SPA samples showed the lowest digestibility compared to DCF and SPS (Table 2). DCF

Table 2
Proximate composition of the fractions from the cricket *Acheta domesticus*.

Component (%)	CF	DCF	SPA	SPS
Moisture	6.94 ± 0.070^{D}	6.34 ± 0.072^{C}	2.3 ± 0.141^{A}	3.46 ± 0.032^{B}
Ashes	3.1 ± 0.000^{A}	8.83 ± 0.183^{B}	46.21 ± 0.513^{D}	30.73 ± 0.024^{C}
Total protein	$45.75 \pm 0.640^{B+}$	$51.94 \pm 0.301^{C+}$	$35.65 \pm 0.591^{A++}$	$53.85 \pm 0.253^{C++}$
Fat	20.53 ± 0.182^{C}	1.17 ± 0.032^{A}	1.2 ± 0.002^{A}	1.89 ± 0.014^{B}
Carbohydrates[a]	23.68 ± 0.031^{C}	31.84 ± 0.540^{D}	14.49 ± 0.224^{B}	9.9 ± 0.254^{A}
Digestibility[b]	40.95 ± 0.362^{A}	57.65 ± 0.631^{D}	42.58 ± 0.341^{B}	53.67 ± 0.533^{C}
Yield of Solid	NA	NA	16.45 ± 0.123^{A}	40.46 ± 0.180^{B}
Protein recover	NA	NA	11.29 ± 0.082^{A}	41.94 ± 0.181^{B}

All values are expressed on a percentage.
NA = does not apply In total protein, the symbol (+) means that the conversion factor was used $N_{Kjel4.5}$, and the symbol (++) means that the conversion factor was used $N_{Kjel6.25}$ Cricket flour (CF), Defatted cricket flour (DCF), Soluble protein by alkalinization (SPA), and Soluble protein by sonication (SPS). All values are mean ± SD of three replicates (n = 3).
[A–D] Different letters in the same row mean significant differences between samples at $p < 0.05$.
[a] Carbohydrates = 100 – Protein – Fat – Ashes - Moisture.
[b] Digestibility with pepsin (based on 100 % crude protein).

showed increased protein digestibility (from 40.95 to 57.65 %) after the fat was removed in CF. Bryan & Classen [48] mention that this method can be misleading if the analyzed samples contain an increased content of fat or carbohydrates.

For SPA, the low digestibility may be attributed to the alkaline pH in the extraction process. Studies have shown that alkaline protein extraction severely affects protein digestibility and causes adverse chemical reactions, such as converting serine and cysteine residues to lysinoalanine compounds, which reduces protein bioavailability. Additionally, it can lead to the racemization of amino acids, introduce a bitter taste, and cause protein aggregation [49–51]. Proteins extracted from other sources have the best digestibility; for example, combining deep eutectic solvent (DES) with microwave technology improved the extraction and digestibility of wheat germ proteins, compared to alkaline extraction [52]. On the other hand, ultrasound extraction leads to structural changes to the proteins, increasing crude protein and increased digestibility [29].

Marin-Morales et al. [53] analyzed in vitro protein digestion using a multi-enzymatic method of an insect native to Mexico, early and adult *Sphenarium purpurancens*, obtaining 81.59 % and 85.21 %, respectively, higher values than those obtained in this study for *Acheta domesticus*, which belongs to the same order (Orthoptera). This could be possible because the multi-enzymatic method used in that research leads to a more complete digestion than the one used in the present work. DCF and SPS digestibility is higher than raw pork, fish, beef, and chicken (47.22 %, 46.98 %, 42.75 %, and 44.67 %, respectively), making the insect protein a high quality one when compared to raw meat proteins [54,55].

Parameters such as the yield of solids and percentage of protein recovery are important for the industry since they demonstrate the extraction process's efficiency. In addition, it is shown that SPS is significantly higher than SPA when recovering solids. Similar results are reported by Kingwascharapong et al. [29] after an ultrasound process used for extracting protein from *Patanga succinta* L. Furthermore, the same behavior was observed when using ultrasound-assisted extractions for the recovered protein from chicken eggshells and skin collagen from *Chitala ornata* [56,57]. Ultrasound allows the penetration of extraction solvents into the internal structure of the food product, enhancing mass transfer and resulting in increased extraction yield [58].

3.2. Protein solubility

The solubility of DCF at different pH's is observed in Fig. 1. The Tukey statistical analysis indicates no significant differences ($p > 0.05$) between the values from 1 to 8. At these pHs, the lowest solubilization values were obtained. Significant differences exist in the basic pH's (10–13), and the solubility increases when observing the highest solubility at pH 13 (0.194 ± 0.002 mg soluble protein/mg DCF). These results were similar to Kim et al. [59] on the solubility of cricket flour. The lowest solubilization is obtained at pH 4, referring to its possible isoelectric point. Similar results were reported from the family *Gryllidae*, belonging to the order (Orthoptera) like *Acheta domesticus.* Jeong et al. [22] studied the solubility of *Gryllus bimaculatus,* and their research indicated that the solubility is greater at basic pH, and the lowest solubility occurs at pH 5. Moreover, *Gryllodes sigillatus* presented its lowest solubility at pH 3 [60].

3.3. Techno-functional properties as a food additive

3.3.1. Water holding capacity and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) are important parameters for the food industry, especially for texture development and yield. These properties are significantly affected by the composition of the protein extracts, as well as the native state of the protein, protein composition, pH value, and ionic strength [61]. Table 3 presents the techno-functional properties of fractions derived from the cricket *Acheta domesticus* when used as food additives. Degreasing does not significantly affect ($p > 0.05$) the water retention capacity when comparing CF (2.43) and DCF (2.33). Similar results were observed with *Gryllodes sigillatus* (2.34 g/g) and *Schistocerca gregaria* (2.18 g/g) [62]. Furthermore, *G. bimaculatus* showed no significant difference before and after a hexane defatting [22]. SPA could not hold water; this may be because the proteins obtained in this fraction do not have exposed hydrophilic groups that allow them to interact with water molecules. Moreover, SPS presented the best water retention capacity (2.8 g/g), which can be attributed to the structural change of the proteins due to ultrasound's cavitation and its solubility increase. Mshiyisa et al. [63] mention a significant increase in WHC when using protein isolates from *Hermetia illucens* compared to flours. All samples show a lower

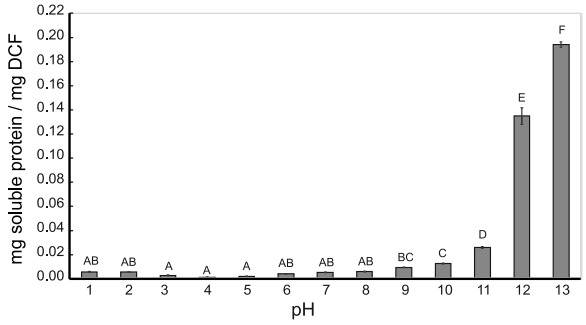

Fig. 1. Effect of pH on defatted-cricket-flour protein solubility. All values are mean ± SD of three replicates (n = 3) $^{A-F}$ Different letters in the same column mean significant differences between samples at $p < 0.05$.

Table 3
Techno-functional properties as food-additive of the fractions from the cricket *Acheta domesticus*.

Samples	WHC (g/g)[a]	OHC (g/g)[b]	FC (%)	EAI (m^2/g)	HC (%)
CF	2.43 ± 0.051^{A}	2.54 ± 0.032^{C}	0	5.5 ± 0^{B}	30.58 ± 0.823^{C}
DCF	2.33 ± 0.062^{A}	2.04 ± 0.051^{B}	8.33 ± 0.572^{A}	6.23 ± 0.052^{C}	30.33 ± 1.122^{C}
SPA	0	2.56 ± 0.042^{C}	363.33 ± 7.632^{C}	16.20 ± 0.003^{D}	4.25 ± 0.370^{A}
SPS	2.8 ± 0.191^{B}	3.49 ± 0.113^{D}	386.66 ± 10.401^{D}	32.96 ± 0.164^{E}	9.64 ± 0.752^{B}
SPI	7.31 ± 0.062^{C}	1.57 ± 0.002^{A}	135 ± 5.000^{B}	3.7 ± 0.052^{A}	41.80 ± 1.681^{D}

Cricket flour (CF), Defatted Cricket Flour (DCF), Soluble Protein by alkalinization (SPA), Soluble Protein by sonication (SPS), and Soy protein isolate (SPI). Water holding capacity (WHC), oil holding capacity (OHC), Foaming capacity (FC), Emulsifying activity index (EAI), and Heat coagulation (HC). All values are the mean ± SD of three replicates (n = 3).
$^{A-D}$ Different letters in the same column mean significant differences between samples at $p < 0.05$.
[a] g of water absorbed/g of sample.
[b] g of oil absorbed/g of sample.

WHC than the commercial control, possibly due to the high protein content present in SPI (according to the supplier, 84.43 %), unlike the total protein content obtained by *Acheta domesticus* in the different fractions (35.65 %–53.85 %). Peng et al. [64] reported similar results, where commercial SPI presented higher WHC than proteins extracted from soybeans by alkalization and demonstrated that the WHC is directly proportional to the protein content. Furthermore, SPI presented the minimum oil retention capacity (1.57 g/g), followed by the DCF sample (2.04 g/g). SPA and CF did not present oil holding capacity (OHC). However, CF is significantly different from DCF ($p < 0.05$). Some authors have reported no significant decrease in OHC after defatting flour from *Hermetia illucens* and *T. molitor* [21,63]. Finally, SPS presented the best oil retention capacity, significantly different from the other treatments ($p < 0.05$). The increase in OHC can be attributed to the unfolding of polypeptide chains and conformational modifications in the macromolecular structure. These changes are induced during ultrasonication, where the transmission of sound waves oscillates through the food, leading to pressure fluctuations and resulting in the formation and collapse of bubbles, a phenomenon called *cavitation* [65]. This cavitation causes the breaking of hydrogen bonds. It exposes the nonpolar hydrophobic side chains of the amino acids within the polymer, thereby enhancing the binding affinity of oil molecules to the protein molecules [29,66].

3.3.2. Foaming properties

The foam can be defined as air bubbles trapped within a liquid and stabilized by a protein network at the air-liquid interface [63]. Table 3 shows the foaming capacity of the fractions obtained from *Acheta domesticus*. Foam stability is highly influenced by protein structure, protein concentration, ionic strength, particle size, carbohydrates, and fats [67–69]. Specifically, it has been observed that globular proteins and carbohydrates have a reduced capacity to unfold at the air-water interface, limiting their ability to encapsulate air bubbles, which results in a low foaming capacity [70]. *Acheta domesticus* has been reported to have globular proteins [67]. Consequently, CF did not exhibit foaming capacity due to its high fat and carbohydrate content, as shown in Table 2. Some insects have low or null percentages regarding their foaming ability (0–4.1 %). Examples of these insects are *Locusta migratoria* (3.33 %), *Hermetia illucens* (5.69 %), and *Macrotermes subhylanus* (4.1 %) [71–73]. DCF increased its foaming capacity (8.33 %) with respect to CF. Similar results were reported after defatting with hexane for flours from *Gryllus bimaculatus*, *S. gregaria,* and *A. mellifera* [22,31]. SPA (386.66 %), SPS (363.33 %), and SPI (135 %) presented higher foaming capacities, with significant differences ($p < 0.05$). SPS increased 23 % the foaming capacity in comparison with SPA. This behavior is attributed to the cavitation effect, which causes the breaking of hydrogen bonds, hydrophobic interactions, and peptide bonds through hydrolysis mechanisms, leading to the unfolding of the protein structure and the breakdown of proteins into peptides [74,75]. Additionally, the unfolding of the polypeptide chain exposes the hydrophobic regions essential for the molecules adsorption at the air-water interface, thus forming a stable film surrounding the air bubble in the foam system [76,77]. Kingwascharapong et al. [29] reported a similar behavior in protein extracted from *Bombay locust*, where foaming capacity increased 36 % compared to the control after applying ultrasound for 30 min.

Fig. 2A shows foaming stability. SPI showed a behavior similar to DCF, with the lowest stability. It lost more than 50 % after 15 min;

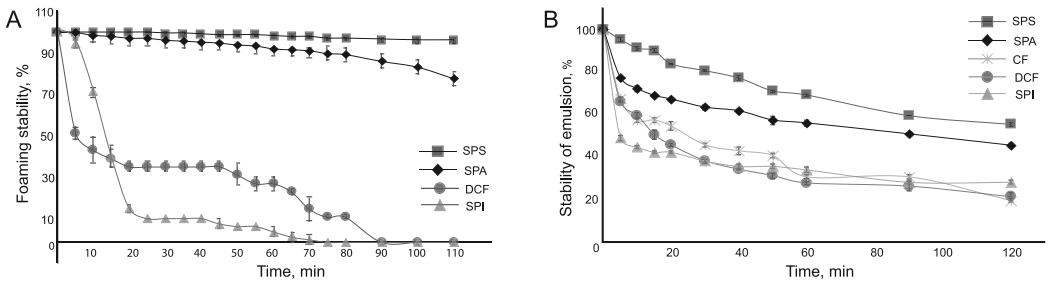

Fig. 2. Foaming stability (A) y Stability of emulsion (B) of different fractions of *Acheta domesticus*: Cricket flour (CF), Defatted Cricket Flour (DCF), Soluble Protein by alkalinization (SPA), Soluble Protein by sonication (SPS), and Soy protein isolate (SPI). All values are mean ± SD of three replicates (n = 3).

a similar result was reported by Martin et al. [78]. DCF is the least stable of the four treatments, with 51.85 % at 5 min, losing all its foaming capacity at 90 min. Similar results have been reported from *Gryllodes sigillatus*, where its stability is quickly lost after 30 min [62]. Regarding SPA, a good foaming capacity is observed. Furthermore, the stability was above 90 % during the first 70 min, and SPS had a stable foaming capacity above 95 % during 110 min. The results are similar to protein concentrates of larvae of *Hermetia illucens*, extracted by alkalization with foam stability (93 %) after 30 min [63].

3.3.3. Emulsifying properties

The Emulsifying activity index (EAI) is related to the amphipathic property of the protein that allows it to form and stabilize the emulsion, creating an electrostatic repulsion on the surface of the oil droplets [79]. The EAI is shown in Table 3. SPI had the lowest EAI (3.7 m^2/g), which is similar to L'hocine et al. [80], who reported low values for EAI, which is due to high WHC and very low OHC. On the other hand, DCF was significantly different and higher ($p < 0.05$) than CF. The same behavior is observed in *Gryllus bimaculatus* and *Hermetia illucens* after defatting [22,63]. An increase in EAI is observed when using SPA and SPS, possibly related to alkaline extraction; consequently, as mentioned above, alkaline extraction improves the solubility of proteins. Abd Rahim et *al.* [81] establish that the EAI of soluble proteins depends on the hydrophilic-lipophilic balance, affected by pH, with a pronounced trend in basic pH. They also demonstrated that alkaline extraction increases the surface hydrophobicity of proteins extracted from rice bran. SPS had the higher EAI (29.96 m^2/g); this may be due to the cavitation effect produced by ultrasound, which exposes the hydrophobic groups and, helps stabilize the protein network at the interface and improve the stabilization of the emulsion [82].

Fig. 2B showed similar results for CF and DCF for the stability of the emulsion. Both treatments present the lowest stability, losing approximately 30 % in the first 5 min. After 30 min, both exhibited a stability of 45.55 % and 38.11 %, respectively. Similar percentages are reported for flours from *Gryllodes sigillatus* (38.3 %) and *S. gregaria* (48.11 %) [62]. SPA showed a medium stability with a loss of 25 % in the first 5 min and 63.28 % at 30 min. The highest stability was demonstrated by SPS, with a loss of 5 % within the first 5 min and maintenance of above 50 % after 120 min. The results are similar to the stability emulsion for black soldier fly larvae protein concentrates (49.83 %) [63].

3.3.4. Gelling properties

The heat coagulation (HC) percentage is an indirect measure of the gelling properties, with high coagulation percentages indicating strong protein-protein interactions after heating, as shown in Table 3. The commercial control, SPI, exhibited the highest HC at 41.80 %. CF and DCF showed HC values of 30.58 % and 30.33 %, respectively, with no significant difference ($p > 0.05$), indicating that degreasing did not have a significant effect. SPA and SPS presented a lower HC (4.25 %); therefore, treatment with ultrasound does not improve HC. Similar results were shown with *Apis mellifera* flour, which reported a higher HC (73.3 ± 5.5 %) than protein concentrates extracted by alkalization and ultrasound (30.2 ± 4.2 and 31.6 ± 2.7 %, respectively) [31]. However, an increase in HC is observed with ultrasound treatment compared to alkalinization. These results can be attributed to changes in protein conformation during sonication and an increase in sulfhydryl groups [83]. On the other hand, Gao et al. [84] observed that alkaline extraction promoted the formation of protein aggregates, thus decreasing protein-protein interaction. Therefore, the soluble protein extract of *Acheta domesticus* does not exhibit gelling properties.

Table 4
Techno-functional properties as meat-extender of the fractions from the cricket *Acheta domesticus* to different substitution levels.

Samples	Cooking loss (%)	TEFS (mL g sample^{-1})	WHC (%)
Control	3.56 ± 0.041^{H}	2.87 ± 0.172^{E}	64.44 ± 0.452^{A}
CF 5 %	3.73 ± 0.072^{I}	3.51 ± 0.123^{F}	72.06 ± 1.091^{BC}
CF 10 %	3.17 ± 0.003^{G}	2.70 ± 0.073^{DE}	75.85 ± 0.262^{DE}
CF 15 %	2.26 ± 0.014^{E}	1.67 ± 0.033^{B}	76.23 ± 1.173^{DE}
DCF 5 %	3.96 ± 0.032^{J}	3.59 ± 0.072^{F}	75.87 ± 0.752^{DE}
DCF 10 %	3.63 ± 0.020^{I}	3.40 ± 0.071^{F}	76.72 ± 1.060^{D}
DCF 15 %	3.57 ± 0.005^{H}	3.40 ± 0.004^{F}	79.52 ± 1.242^{GF}
SPA 5 %	1.19 ± 0.032^{B}	1.67 ± 0.113^{B}	74.08 ± 1.452^{CD}
SPA 10 %	2.46 ± 0.041^{F}	2.35 ± 0.002^{C}	70.37 ± 0.991^{B}
SPA 15 %	3.08 ± 0.052^{G}	2.75 ± 0.003^{DE}	69.83 ± 0.971^{B}
SPS 5 %	2.56 ± 0.030^{F}	2.55 ± 0.071^{CD}	75.49 ± 0.940^{DE}
SPS 10 %	1.58 ± 0.013^{D}	1.50 ± 0.072^{B}	77.09 ± 0.691^{EF}
SPS 15 %	0.91 ± 0.032^{A}	1.00 ± 0.073^{A}	83.02 ± 0.713^{H}
SPI 5 %	1.55 ± 0.053^{D}	1.75 ± 0.005^{B}	75.92 ± 0.582^{DE}
SPI 10 %	1.50 ± 0.032^{CD}	1.55 ± 0.073^{B}	80.38 ± 0.392^{G}
SPI 15 %	1.35 ± 0.013^{C}	1.45 ± 0.071^{B}	84.46 ± 0.421^{H}

Cricket flour (CF), Defatted Cricket Flour (DCF), Soluble Protein by alkalinization (SPA), Soluble Protein by sonication (SPS), and Soy protein isolate (SPI).
Water holding capacity (WHC), Total expressible fluid separation (TEFS).
All values are mean ± SD of three replicates (n = 3).
$^{A-I}$ Different letters in the same column mean significant differences between samples at $p < 0.05$.

3.4. Techno-functional evaluation as a meat extender

3.4.1. Cooking losses

Cooking losses are important since they provide insight into the product's performance. Higher yields result in lower losses and improved technological functionality of the protein. Table 4 shows that cooking losses are significantly higher ($p < 0.05$) at 5 % and 10 % of DCF compared to the control, highlighting those high levels of CF substitution reduced cooking loss. Similar results for increased yield have been reported by Park et al. [37], who use silkworm meal (*Bombyx mori*) with levels from 5 to 15 %. Choi et al. [85] reported reduced cooking losses in sausages when incorporating flour *Tenebrio molitor* at levels of 5 and 10 %, with higher levels causing a high cooking loss. This phenomenon can be attributed to the denaturation of myofibrillar proteins in the drying process, showing no differences among the different levels of SPI substitution. The reduction in cooking losses in the SPI and SPS formulations could be due to the high WHC capacity of both proteins. Results could indicate that SPS may have changes in its structure, exhibiting the presence of free amino acids and exposure to hydrophilic or hydrophobic groups present in the protein. These modifications can enhance the techno-functional properties of the SPS extract, making it superior to the control and comparable to the commercial control [31,86,87].

3.4.2. Emulsion stability

The stability of the emulsion is expressed as total expressible fluid separation (TEFS), which is the sum of fat with water that was not trapped within the matrix after the cooking process due to lower TEFS representing high emulsion stability. Table 4 shows that DCF in all the concentrations presents the highest values of TEFS and the highest cooking losses. The meat model has good emulsion stability, and the TEFS did not exhibit significant differences among treatments of CF 15 %, SPA 5 %, SPS 10 %, and the commercial control SPI (5, 10, 15 %). All of them showed better results than when compared to the control (without substitution of meat). The results obtained for TEFS (1–3.59 %) from all treatments are lower than those reported by *Choi* et al. [85], who replaced pork meat with *Tenebrio molitor* flour in Frankfurter formulations at substitution levels of 5 % (6.71), 10 % (6.01), and 15 % (6.25), so the different fractions from *Acheta domesticus* have better properties of emulsion reducing TEFS (high stability), in comparison with *Tenebrio molitor*. The results obtained in this study are interesting because other authors observed an increase in TEFS (low stability) in the meat model with substitution levels of 5–10 % of superworm (Zophobas morio larvae) [88]. SPS had the lowest TEFS (1.00 ± 0.07 %), attributed to the ultrasonic process and their high values on tecno-functional properties, such as AEI, WHC, OHC, and cooking loss. In general, SPS significantly improved emulsion stability compared to commercial control (SPI). These results are similar to those from Wang et al. [87], who observed that the ultrasound-assisted extraction in flour from *Clanis Bilineata* improves emulsifying activity and rheological properties such as viscosity at different ultrasonic power.

3.4.3. Water holding capacity

The water holding capacity (WHC) is an essential parameter in the quality of the sausages. As shown in Table 4, all formulations have a higher percentage of water retention than the control (64.44 %, without substitution). These results indicate that incorporating some fraction of *Acheta domesticus* improved the WHC of meat models. Kim et al. [89] reported that porcine myofibrillar protein gels formulated with flours (1 %) from *T. molitor* and *G. bimaculatus* improved WHC. SPA with 10 and 15 % substitution have lower WHC (70.37 and 69.83 % respectively) than SPA 5 % (74.08 %). However, there is no significant difference ($p > 0.05$) with the treatments CF and DCF at different levels of concentration tested, although defatting was shown to improve WHC. Scholliers et al. [88] mentioned that this effect could be due to a poor interaction of the insect protein with the water and other proteins, such as porcine myofibrillar proteins. On the other hand, SPS 15 % (83.02 %) had the best WHC, and there is no significant difference ($p > 0.05$) when compared with SPI 15 % (84.46 %). Furthermore, it has been shown that gels with 8, 10, and 13 % SPI isolates present high WHCs (74–84 %)

Table 5
Protein solubility of meat models using the different fractions of *Acheta domesticus* as meat-extender.

Formulations		Total protein solubility (mg/mL)	Myofibrillar protein (mg/mL)	Sarcoplasmic protein (mg/mL)
Control		4.75 ± 0.071^{B}	0.87 ± 0.001^{A}	3.88 ± 0.072^{C}
CF	**5 %**	4.53 ± 0.042^{A}	0.88 ± 0.001^{A}	3.66 ± 0.043^{AB}
	10 %	5.15 ± 0.090^{D}	0.96 ± 0.003^{B}	4.18 ± 0.091^{EF}
	15 %	5.31 ± 0.072^{DE}	0.98 ± 0.002^{BC}	4.33 ± 0.062^{FG}
DCF	**5 %**	4.72 ± 0.041^{B}	0.89 ± 0.003^{A}	3.84 ± 0.043^{C}
	10 %	5.29 ± 0.052^{DE}	0.99 ± 0.023^{CD}	4.3 ± 0.060^{FG}
	15 %	5.45 ± 0.053^{E}	1.02 ± 0.062^{FG}	4.43 ± 0.042^{G}
SPA	**5 %**	4.53 ± 0.091^{A}	1.01 ± 0.002^{EF}	3.52 ± 0.092^{A}
	10 %	4.63 ± 0.030^{AB}	1.01 ± 0.001^{DEF}	3.63 ± 0.032^{AB}
	15 %	4.78 ± 0.603^{B}	1 ± 0.004^{CDE}	3.78 ± 0.061^{BC}
SPS	**5 %**	4.96 ± 0.012^{C}	1.02 ± 0.013^{FG}	3.93 ± 0.090^{CD}
	10 %	5.14 ± 0.053^{D}	1.04 ± 0.003^{GH}	4.09 ± 0.052^{DE}
	15 %	5.32 ± 0.062^{E}	1.03 ± 0.010^{H}	4.29 ± 0.063^{FG}

Cricket-flour (CF), Defatted-cricket-flour (DCF), Soluble-protein by alkalinization (SPA), Soluble-Protein by Sonication (SPS).
All values are mean ± SD of three replicates (n = 3).
$^{A-H}$ Different letters in the same column mean significant differences between samples at $p < 0.05$.

[90].

3.4.4. Protein solubility

Meat protein solubility is an important indicator related to WHC, emulsion stability, and gel formation because of weight loss by cooking and the textural properties in meat emulsion products [91]. The protein solubility of meat models is shown in Table 5. The solubility of SPA (10 and 15 %) and DCF 5 % treatments did not show significant differences ($p > 0.05$) when compared to the control (without any substitution). The formulations CF 15 %, DCF (10 and 15 %), and SPS 15 % presented higher solubility, and they did not show significant differences among treatments ($p > 0.05$). These results could be due to the percentage of total protein present in the different formulations and the meat substitution level. The results are similar to those of Kim et al. [92], who observed an increase in solubility when using protein concentrates, in contrast with a decrease in solubility when using flour and defatted flour. On the other hand, Choi et al. [85] showed an opposite trend: the increase in yellow worm meal substitutes significantly decreased solubility, attributing these results to the denaturation of yellow worm proteins during the drying process. Therefore, for the present study, *Acheta domesticus* was dried at low temperatures to avoid protein denaturation and not lose solubility. The myofibrillar protein concentration increases in all formulations (0.89–1.04 mg/mL), and it shows significant differences ($p < 0.05$) with the control (0.87 mg/mL). The difference between the control and the formulations added with the cricket fractions may be due to the insoluble myofibrillar proteins present in insects. *Acheta domesticus* has reported some myofibrillar proteins, for example, actin and myosin, with short molecular chains [62]. The ultrasound could extract this type of protein due to the cavitation change in the hydrophobicity of the proteins since the highest values were obtained in SPS treatments, which explains the increased solubility in SPS formulations. The sarcoplasmic proteins are soluble proteins, increasing their concentration in all formulations with the highest substitution levels.

3.5. Texture and color

Fig. 3A shows the principal component analysis, which explains 94.08 % of the data for texture. Four clusters were formed; 10 % and 5 % CF present the lowest elasticity and cohesiveness. These results are consistent with those reported by Choi et al. [85], who showed that hardness, gumminess, and chewiness decreased with the addition of yellow worm powder. On the other hand, elasticity only decreased at high substitution levels, and it could be attributed to moisture loss throughout the cooking process [93]. Results show that the defatting process benefits the textural properties. Therefore, the cluster with the control, DCF, and CF 15 % formulations present high values of hardness, gumminess, chewiness, cohesiveness, and medium elasticity. The SPA and SPS samples show high elasticity and cohesiveness but low values for hardness, gumminess, and chewiness. Therefore, the PCA suggests that the extraction method affects the texture. As previously mentioned, SPA presented a low coagulation percentage, which impacts the physicochemical properties, including the gel-like structure, through covalent and non-covalent interactions blocking exposed hydrophobic sites, which leads to a surface area reduction, decreasing the concentration of myofibrillar protein available to interact in the formation of the gel structure, which influences the textural properties [94]. The samples with commercial protein (SPI) showed high values of hardness, gumminess, and chewiness, as well as medium values for cohesiveness and elasticity.

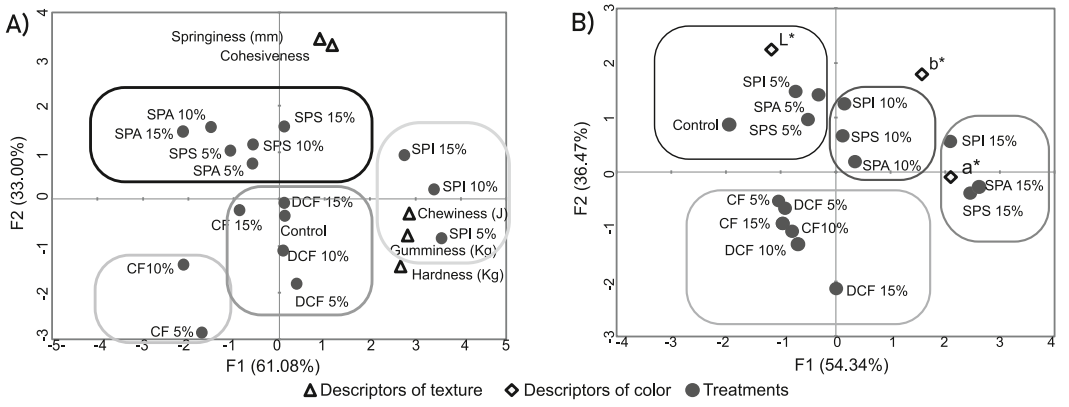

Fig. 3. Principal Component Analysis of the sausages. (A) Texture and (B) color. Control, sausages without meat substitution; CF 5 %, sausages with 5 % meat substitution (47.5 % pork meat+2.5 % CF); CF10 %, sausages with 10 % meat substitution (45 % pork meat+5 % CF); CF15 %, sausages with 15 % meat substitution (42.5 % pork meat+7.5 % CF); DCF 5 %, sausages with 5 % meat substitution (47.5 % pork meat+2.5 % DCF); DCF10 %, sausages with 10 % meat substitution (45 % pork meat+5 % DCF); DCF15 %, sausages with 15 % meat substitution (42.5 % pork meat+7.5 % DCF); SPA 5 %, sausages with 5 % meat substitution (47.5 % pork meat+2.5 % SPA); SPA10 %, sausages with 10 % meat substitution (45 % pork meat+5 % SPA); SPA15 %, sausages with 15 % meat substitution (42.5 % pork meat+7.5 % SPA); SPS 5 %, sausages with 5 % meat substitution (47.5 % pork meat+2.5 % SPS); SPS 10 %, sausages with 10 % meat substitution (45 % pork meat+5 % SPS); SPS 15 %, sausages with 15 % meat substitution (42.5 % pork meat+7.5 % SPS); SPI 5 %, sausages with 5 % meat substitution (47.5 % pork meat+2.5 % SPI); SPI 10 %, sausages with 10 % meat substitution (45 % pork meat+5 % SPI); SPI 15 %, sausages with 15 % meat substitution (42.5 % pork meat+7.5 % SPI). Cricket-flour (CF), Defatted-cricket-flour (DCF), Soluble-protein by alkalinization (SPA), Soluble-protein by sonication (SPS), and Soy protein isolate (SPI).

Fig. 3B shows the color values in PCA, which explains 90.81 % of the data. Of the different formulations, it is observed that CF and DCF present the lowest values of luminosity (L*), yellow (b*), and reddish (a*) colors. Reddish colors dominate the formulations of SPA and SPS 15 % (a*) and medium lightness (L*). SPA, SPS (10 %), and SPI (10–15 %) present average values for the parameters a* and L*, with yellow colors (b*) predominating. Finally, the control, SPA, and SPS 5 % formulations present greater luminosity (L*). The samples generally differ from the control at levels greater than 5 %, decreasing L* and increasing the values of a* and b*. Commercial control (SPI) behaves similarly to SPA and SPS. All the reports reported similar results, with different myofibrillar protein substitutions, pork pastries added with flour from *T. molitor* as an extender, and biscuits enriched with flour from *A. domesticus*. In contrast, the level of insect protein increased, L* decreased, and a* and b* increased [32,95,96].

4. Conclusions

The ultrasound-assisted extraction method improved total protein content, digestibility, solid yield, and protein recovery compared to the traditional alkalinization method. Furthermore, proteins extracted by ultrasound exhibited the best techno-functional characteristics as a food additive (oil holding capacity, foaming capacity, and emulsifying activity) and meat extender (cooking loss, water holding capacity, and emulsion stability) at 15 % substitution level, when compared to the control and the soy protein isolate formulation. These results suggest that soluble proteins extracted with ultrasound from *Acheta domesticus* can be used as food additives and meat extenders. Further studies are crucial due to the high ash content, which may affect techno-functionality, for which an isoelectric precipitation extraction is recommended. Additionally, sensory tests will be performed on sausages at 15 % SPS substitution level to determine consumer acceptance and product shelf life.

CRediT authorship contribution statement

Salvador O. Cruz-López: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Héctor B. Escalona-Buendía:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Isadora Martinez-Arellano:** Writing – review & editing, Visualization, Data curation. **Julieta Domínguez-Soberanes:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology. **Yenizey M. Alvarez-Cisneros:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability statement

Data will be made available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Chat GPT to translate from Spanish to English to improve the quality of the research paper. After using this tool/service Later, the authors used Grammarly to check the document's style. After using these tools/services, the author(s) reviewed and edited the content as needed. Therefore, we take(s) full responsibility for the publication's content.

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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.

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