

**ARTICLE**

## Characterization of Cooked Meat Models using Grasshopper (*Sphenarium purpurascens*) Soluble Protein Extracted by Alkalisiation and Ultrasound as Meat-Extender

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**Abstract** The most abundant Orthoptera in Mexico is a small grasshopper (*Sphenarium purpurascens*) which is considered a food source with increased nutritional value due to its high protein content. Insect proteins have gained relevance because of their high potential as gelling, texturing, and extender agents in the food industry. The objective of this study was to evaluate the effect of substituting meat with a soluble protein extract from grasshopper obtained by alkalisiation or alkalisiation-piezoelectric ultrasound, on the techno-functional, physicochemical, and sensory characteristics of cooked meat models (sausages). The soluble protein was extracted in NaHCO<sub>3</sub> pH 8 and a piezoelectric ultrasound 5-mm sonotrode at 20 kHz with 99% amplitude. Different formulations with meat substitution: 0%, 5%, 10%, and 15% were prepared and characterised for their rheological behaviour, emulsion stability, weight loss by cooking, total protein content, colour, and texture. Sensory evaluation was conducted with consumers using a test involving check-all-that-apply and overall liking. The alkalisiation-piezoelectric ultrasound method improved the solubility and the techno-functional properties of the soluble grasshopper protein when applied in sausages at maximum levels of 10% meat substitution. The sensory evaluation indicated that the formulation with 5% meat substitution exhibited the same acceptability as the control sample. Given these results, the soluble protein treated with alkalisiation and piezoelectric ultrasound could be used as an extender in meat products.

**Keywords** edible insect, soluble protein, functional properties, sausages, sensory

## Introduction

Around 2,100 insect species have been identified worldwide as food products (Jongema, 2017). One of the most common are grasshoppers, locusts, and crickets

(13%) (van Huis et al., 2013). The grasshopper *Sphenarium purpurascens* (SP) of the order Orthoptera is endemic of Mexico and it is distributed in the States of Oaxaca, Chiapas, Puebla, Mexico, Hidalgo, Queretaro, and Tlaxcala. It is often known as *saltamontes* or *chapulín de la milpa* due to its abundance in agro-ecosystems where maize is grown (Serrano Limon and Ramos Elorduy, 1989; Torruco-Uco et al., 2019), and is considered a plague by farmers due to the floral and foliar damage it inflicts on crops (van Huis et al., 2013).

SP is used in gourmet dishes and has a high nutritional value comparable to meat (Yi et al., 2013). It has been reported that SP the nutritional composition in 100 g of dried product is given by 52.6 to 75.87 g of protein, with a 26.95 to 30.09 g essential amino acids and 66.48 to 68.93 g non-essential amino acids (NAA). In addition, 11.04 to 24.89 g chitin, 6.02 to 14.86 g crude fat, 15.59 to 30 g carbohydrates and 10 to 31.81 g crude fibre. The micronutrients found in this product are 34.61 to 37.64 mg sodium, 1,007 to 1,028 mg potassium, 201 to 235 mg calcium, 17.84 to 17.98 mg zinc, 13.33 to 18.29 mg iron, 124 to 131 mg magnesium, 0.27 mg thiamine, 0.59 mg riboflavin, 1.56 mg niacin. Adding up to 1.42 to 4.1 g ashes and giving a total energy of 1,736.28 kJ (Ibarra-Herrera et al., 2020; Kosečková et al., 2022; Melo Ruiz et al., 2015; Rodríguez-Miranda et al., 2019; Torruco-Uco et al., 2019).

The Food and Agriculture Organization of the United Nations (FAO) has suggested that insects can be incorporated into the diet to counter hunger; however, this idea has dealt with reluctance from some neophobic consumers due to the visual characteristics of insects (Dobermann et al., 2017; Megido et al., 2016). Some authors indicate that edible insects can be incorporated into food in the form of flour (pulverized whole insects) or as a soluble protein extract (Kim et al., 2019; Mishyna et al., 2019). This could work as a strategy to increase acceptance of insects that are incorporated into foods such as sausages, protein bars, pork pate, bread, and pasta (Mishyna et al., 2021; Smarzyński et al., 2019; van Huis, 2020).

The Orthoptera order has high concentrations of protein; however, its digestibility varies between species due to the high chitin content of the exoskeleton, rendering it indigestible to humans (van Huis, 2016). One way to eliminate the chitin is to extract the protein by pulverising the whole insect. The main methods used are alkaline extraction (ALK), isoelectric precipitation, and ultrasound (Choi et al., 2017a; Kim et al., 2019; Mishyna et al., 2019; Udomsil et al., 2019; Yi et al., 2013). Mishyna et al. (2019) extracted soluble protein from grasshoppers (*Schistocerca gregaria*) and bees (*Apis mellifera*) with a process of defatting and ultrasound-assisted ALK obtaining average yields of 56% from both insects. Choi et al. (2017a) obtained yields from 35% to 94% in protein extraction with sonication from defatted mealworms (*Tenebrio molitor*), adult crickets (*Gryllus bimaculatus*), and silkworm pupae (*Bombyx mori*). The proposed methodologies for protein extraction from each insect are particular to each species and geographic region depending on the structure and functionality of the proteins (Kim et al., 2019).

While there is little bibliographic information on Orthoptera in general, in the case of SP the only information available concerns its nutritional value and provides no scientific evidence on protein extraction methods. On the other hand, the meat industry has a huge interest in reducing production costs by incorporating alternative ingredients, such as high-protein non-meat ingredients as insect proteins whose aim is to substitute the meat content of a product, or to extend the amount of meat used (extenders). Some orthopterans, such as *Gryllidae* sp., *Grylloides sigillatus*, *Locusta migratoris*, *S. gregaria*, *Acheta domesticus*, and *Sphenarium purpurances* have demonstrated to have techno-functional properties. These properties can be grouped in water absorption capacity, oil absorption capacity, water solubility capacity, emulsifying capacity (EC), foaming capacity and foam stability (da Silva Lucas et al., 2020; Mishyna et al., 2019; Purschke et al., 2018; Torruco-Uco et al., 2019). Due to the functionality that these insects have shown, some authors have used those protein extracts to replace a portion of meat in processed meats like sausages. *T. molitor* larvae and silkworm pupae, which produced increased cooking

loss and food hardness (Kim et al., 2016). Whereas results obtained by using yellow mealworm in frankfurters at a similar level to the control sample (50% pork ham) maintained the quality of this type of products (Choi et al., 2017b). Other researchers have used the whole insect in the form of flour as the house cricket *A. domesticus* (Kim et al., 2017), silkworm pupae *B. mori* (Park et al., 2017) and superworm *Zophobas morio* larvae (Scholliers et al., 2020b).

Therefore, SP protein can be a low-cost alternative to the use of food extenders since it is considered nowadays a plague in maize crops and could be used as an ingredient for value added products. It should be mentioned that there are currently no studies that explain the effect of the addition of SP protein in sausages, making this research of novelty. The aim of this study was to evaluate the effect of substituting meat with soluble SP protein obtained by alkalisation or alkalisation-ultrasound on the techno-functional, physicochemical, and sensory characteristics of sausage-type cooked meat models.

## Materials and Methods

### Grasshopper powder

The ready-to-eat grasshoppers SP are harvested manually during the month of November in the maize fields of some localities of the state of Puebla in their adult stage (body size 10–23 mm) (Rodríguez-Miranda et al., 2019). After it is seasoned, roasted, and refrigerated until sale in different states of southern Mexico. SP used in this study was purchased from an exotic meat market “San Juan” in Mexico City and was refrigerated at 4°C until used. Protein content (method 981.10) were determined according to the Association of Official Agricultural Chemists guidelines (AOAC, 2000). The crude protein content was determined using a conversion factor of  $N_{Kjel} \times 4.5$  (Janssen et al., 2017; Mishyna et al., 2019). The powder was obtained from SP previously cleaned of foreign matter and subsequently dried in an oven (Felisa model FE-291AD, Jalisco, Mexico) at 105°C for 24 h.

### Grasshopper flour degreased with hexane

The dried SP was defatted according to the method described by Choi et al. (2017a) with some modifications. Hexane was used as solvent, in a sample-solvent- ratio of 1:10 (p/v). Samples were stirred for 24 h at room temperature and the hexane was removed by filtering, then replaced every 24 h for a total time of 72 h. Samples were emptied onto aluminium foil and left to dry overnight at room temperature under a fume hood. Once dried, a size reduction was performed to obtain a powder, which was sieved in a No.20 mesh until becoming a fine flour. Three batches were obtained and labelled as defatted grasshopper powder (DGP).

### Protein solubility

Protein solubility was determined based on the method described by Mishyna et al. (2019) with modifications. A DGP solution was prepared with distilled water at 10% (p/v); this was divided into nine fractions in triplicate and the pH of each was adjusted in a range from 1 to 9 using HCl 0.1 M and NaOH 0.1 M. The samples were centrifuged at 3,000×g for 20 min, and decanted. The soluble protein concentration was determined in the supernatants using the Bradford method (Bradford, 1976). The results were reported as mg soluble protein/g DGP.

### Soluble protein extraction

The extraction of soluble protein from DGP was done according to Yi et al. (2013). Briefly, a 10% (p/v) solution of DGP

was prepared in NaHCO<sub>3</sub> 3% (p/v) at pH 8.0 and divided into 2 equal parts to assess the different extraction methods: 1) ALK: the DGP solution was shaken for 30 min and 2 mL aliquots were taken after 0, 5, 15, and 30 min. The samples were labelled as ALK. 2) Alkalisiation-ultrasound extraction (PUP) was performed according to Choi et al. (2017a) with some modifications. The DGP solutions were treated in ultrasound equipment with 5 mm sonotrode (Sonics® Vibra-Cell™ VCX 130P, Connecticut, USA) at 20 kHz with 99% amplitude in an ice bath. Aliquots were taken at different times (0, 5, 15, and 30 min) and labelled as PUP. All the samples were centrifuged, and the soluble protein concentration was determined in the supernatants using the Bradford method. The results were reported as mg soluble protein/g DGP. Finally, the supernatant was freeze-dried and stored at room temperature in vacuum-sealed bags for further characterization.

### Preparation of meat models with the soluble protein as meat extender

Table 1 shows the seven formulations made with the two protein extracts: ALK and PUP, that were used at three different substitution levels (5%, 10%, and 15%) and that were compared to a control sample without meat substitution. All the formulations were made in triplicate. To make the sausages, the meat was manually minced into small pieces of about 7×7 cm, removing the bone. Before the process, the ice was divided into three equal parts approximately and the phosphates into two equal parts. Later, it was mixed with 20 g of frozen lard (pork back fat) in an immersion blender to maximum power (Hamilton Beach, Glen Allen, VA, USA). The powdered ingredients were then added (0.3 g curing salts, 0.25 g phosphates and the grasshopper protein ALK or PUP, according to Table 1), in addition with 10 g of ice. The ingredients were homogenised for 1 min without pausing the blender and another 10 g of ice was added to the mix. The mix was homogenised for an additional 2 min and the remaining phosphate mixture was added (0.25 g). Lastly, approximately 9.2 g of ice was added to the mixture until a homogeneous emulsion or paste was obtained. The emulsion was stuffed into 22 mm cellulose sleeves and cooked at 80°C until the sausages reached an internal temperature of 75°C, at which they were immediately placed in cold water for 30 min, until the internal temperature dropped to 15°C. Finally, the product was vacuum packaged employing a vacuum machine (EVD4 Torrey, México City, Mexico) in oxygen-impermeable bags.

**Table 1. Meat models (sausages) formulations added with SP protein as meat-extenders**

Ingredients (%)	Treatments						
	Control	T1	T2	T3	T4	T5	T6
Pork meat	50	47.5	45	42.5	47.5	45	42.5
Grasshopper protein (ALK) <sup>1)</sup>	-	-	-	-	2.5	5	7.5
Grasshopper protein (PUP) <sup>2)</sup>	-	2.5	5	7.5	-	-	-
Frozen lard	20	20	20	20	20	20	20
Sodium nitrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Phosphate mixture Hamine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ice	29.2	29.2	29.2	29.2	29.2	29.2	29.2

<sup>1)</sup> ALK, alkaline extraction of grasshopper protein.

<sup>2)</sup> PUP, alkalisiation-ultrasound extraction of grasshopper protein.

Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat+7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat+2.5% ALK); T5, sausages with 10% meat substitution (45% pork meat+5% ALK); T6, sausages with 15% meat substitution (42.5% pork meat+7.5% ALK).

SP, *Sphenarium purpurascens*.

### Evaluation of the physicochemical characteristics of meat models

A texture profile analysis (TPA) of the sausages was performed in a Brookfield CT3 Texture Analyzer, using a TA3/100 cylindrical probe 2.5 mm in diameter. The sausages were cut into slices 20 mm wide and 10 mm thick. Data for hardness, adhesiveness, brittleness, cohesiveness, elasticity, and firmness were obtained. Crude protein content was determined by the Kjeldahl method (method 981.10) (AOAC, 2000), using a conversion factor of  $N_{Kjel} \times 6.25$  (Mæhre et al., 2018). The pH was determined according to Choi et al. (2017b) with a previously calibrated electronic potentiometer 120. The colour of each sausage formulation was determined using a previously calibrated colorimeter (ColorFlex-HunterLab, Reston, VA, USA), with a 19.1 mm aperture, Illuminate D65 and 10° standard observer. The determinations were carried out in quadruplicate. The parameters measured were CIE Lab\* (Urbina et al., 2021).

### Evaluation of the techno-functional properties of meat models

The stability of the meat emulsions with and without SP protein extract was determined as reported by Choi et al. (2017b), with some modifications. Screw top tubes of 50 mL that were modified with a mesh at the bottom were filled with 20 g of the meat batter and placed in a hot water bath at 75°C, where they were kept for 30 min. After this time, the samples were cooled to 4°C with ice water. The water and fat content found in the bottom of the tube was quantified and the stability of the emulsion was reported as total expressible fluids in mL/batter. Cooking loss (CL) of the meat batters was evaluated by the weight difference before and after the heat treatment (Park et al., 2017). The viscoelastic properties were evaluated according to Gibis et al. (2017) with some modifications. The meat batters were analysed after heat treatment with an MCR 300 rheometer (Paar Physica Messtechnik GmbH, Stuttgart, Germany) with a striated PP 50/P2 geometry (25 mm diameter), with a 1 mm gap for uncooked samples and 9 mm gap for cooked samples, using approximately 10 g of sample for each determination. Frequency sweeps were performed at 1% deformation (ensuring their measurement within the linear viscoelastic zone) at a frequency range of 0.1–100 Hz at 25°C. Temperature was controlled with a Paar Physica circulation bath and a controlled Peltier system (TEZ 150/MCR) with precision of  $\pm 0.1^\circ\text{C}$ . We obtained graphs of the storage modulus ( $G'$ ), loss modulus ( $G''$ ) and absolute viscosity  $\eta^*$ . The data were analysed with US200/32 Rheometer V2.50 software.

### Sensory evaluation of sausages made with *Sphenarium purpurascens* protein

Sensory evaluation was applied to the formulations that presented the best techno-functional and physicochemical characteristics (T1 and T2) and these were compared to the control (100% meat). The sensory analysis was done by consumers ( $n=100$ ) aged between 19 and 40 years. Sausages were cut with a length of 10 mm and 3 portions of different sausage formulations were served to the panellists randomly. Consumers were instructed to cleanse their palates between samples using crackers and water. The sausages were evaluated according to general liking using a 7-point hedonic horizontal scale, from “Dislike a lot” (1) to “Like a lot” (7). Finally, the check-all-that-apply (CATA) test was applied, in which consumers chose the descriptors that apply to the sample from a list of 34 sensory attributes related to taste, smell, texture and appearance (Ares et al., 2014; Jaeger et al., 2020). All participants agreed to participate in the sensory analysis of this research and signed the Informed Consent Form. This work is part of the divisional project "Techno-Biofunctional and Sensory properties of Biomolecules and their Application in Food" and it has the approval of the Ethics Committee of UAM-Iztapalapa under the number 1913.

## Statistical analysis

All determinations were made in triplicate and the results are presented as the average with SD. Statistical analyses were done with XLSTAT software version 2014.5.03 (Addinsoft, Paris, France) using an alpha limit value of 0.05. The results were analysed using a one-way analysis of variance (ANOVA) and Fisher's means comparison tests between the treatments for each of the methodologies used. For the sensory tests, a factorial correspondence analysis was performed for the CATA data, Friedman's non-parametric test and frequency distribution tests for degree of liking. The preference map was made through principal component analysis (PCA) and hierarchical agglomeration (clustering).

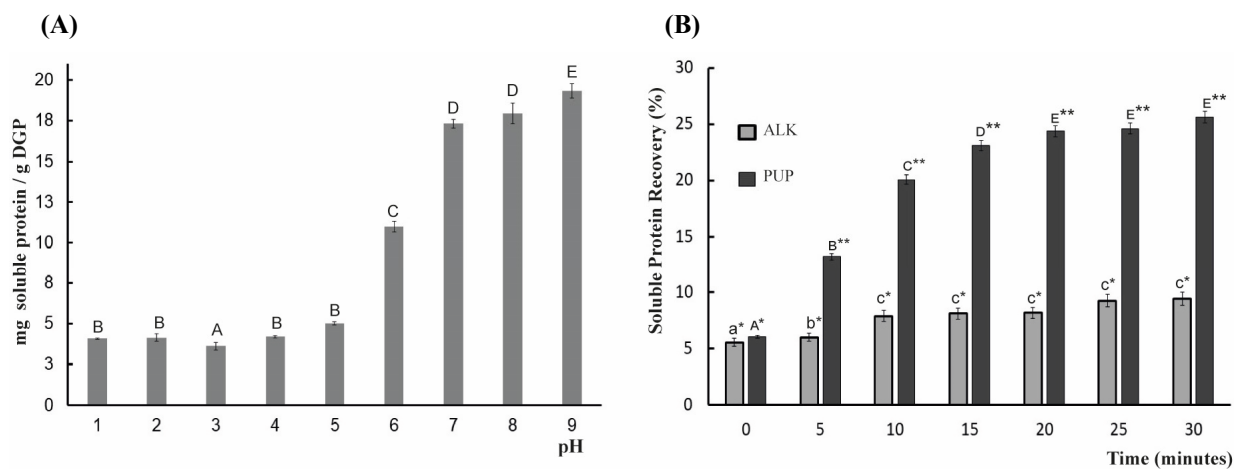
## Results and Discussion

### Protein quantification of grasshopper

SP showed a total protein content using the  $N_{K_{jel}}$  factor 4.5 of  $39.39 \pm 0.84\%$ . This value is lower when compared to other type of grasshopper which is not *ready to be consumed* (seasoned and roasted), making them eligible to be considered as fresh insects such as the case of *Schistocerca* spp., *Melanoplus femrrubrum*, *S. histrio* (Melo Ruiz et al., 2015) and SP (Ibarra-Herrera et al., 2020; Rodríguez-Miranda et al., 2019; Torruco-Uco et al., 2019). However, the protein content reported is within the range for insects from 13% to 77%, these differences depending on the species, habitat, age, diet, season, age, gender, processing, and method of determination (de Carvalho et al., 2020; Kouřimská and Adámková, 2016). The most described method in the literature is that of Kjeldahl, which uses a protein conversion factor depending on the protein source. Nevertheless, for insects, a  $N_{K_{jel}}$  factor ranging from 4.67 to 5.62 has been reported (Janssen et al., 2017), while in the case of Orthoptera such as grasshoppers, the  $N_{K_{jel}}$  factor has been established at 4.5 based on amino acid analysis (Mishyna et al., 2019). However, some authors point out that this varies because these insects have non-protein nitrogen in their structure, as is the case of excretion products in the intestinal tract (ammonia) and chitin which forms part of the exoskeleton in ratios of 5.3% to 6.6% (Janssen et al., 2017). The protein concentration found in SP is higher when compared with the protein of beef (18.4%), chicken (22%) and fish (18.3%) (Yi et al., 2017). Although there is little information in the literature on total protein determination in Orthoptera using the  $N_{K_{jel}}$  factor 4.5, among the data reported and with similar results is the desert locust *S. gregaria* that presents 30.1% protein (Mishyna et al., 2019). Likewise, the orthopter are considered a good source of protein such as a desert locust *S. gregaria*, nymphs of the migratory locust *L. migratoris*, crickets *G. bimaculatus*, *Schistocerca* spp., *M. femrrubrum* and *Shpenarium histrio* (Melo Ruiz et al., 2015; Mishyna et al., 2019; Udomsil et al., 2019). But because of its structure it produces neophobia to some people (Sogari et al., 2019), some authors have indicated that concentrates or isolates of protein can be obtained from insects, promoting the acceptance of these novel foods with added value (Shelomi, 2016). Currently, there is no research reported in literature that mentions how to obtain protein concentrates from SP, the information that is published corresponds mainly on the way of using the complete insect for edible purposes (Cruz-López et al., 2022; Cuj-Laines et al., 2018).

### Protein solubility

Some techno-functional properties of proteins such as foaming properties, EC and gel formation are dependent on the degree of protein solubility (Jeong et al., 2021; Torruco-Uco et al., 2019). The solubility of proteins is also influenced by the structure of their molecules and the ratio of polar to non-polar groups, making pH an important parameter to change the solubility of the proteins (Jeong et al., 2021). Fig. 1A shows the solubility profile of proteins present in the DGP. The



**Fig. 1. Soluble protein extraction.** (A) Protein solubility of defatted grasshopper powder (DGP) as function of pH. Different capital letters indicate significant differences ( $p < 0.05$ ) with respect to pH. (B) Recovery yield for ALK and PUP soluble protein. Each value is expressed as the mean ( $n=3$ ) $\pm$ SD. <sup>A-E</sup> Means treatments with different capital letters are significantly different ( $p < 0.05$ ) with respect to time using the PUP extraction method. <sup>a-c</sup> Means treatments with different lowercase letters are significantly different ( $p < 0.05$ ) with respect to time using the ALK extraction method. <sup>\*\*\*</sup> Means treatments with asterisk are significantly different ( $p < 0.05$ ) between ALK and PUP extraction method evaluated at the same time. ALK, alkaline extraction; PUP, alkalisation-ultrasound extraction.

solubility of the proteins is observed to increase significantly at pH values ranging 7.0 and 9.0, reaching a maximum solubility at pH 9.0 ( $19.33 \pm 0.45$  mg soluble protein/g DGP). Meanwhile, at a pH between 7.0 and 8.0 no significant difference ( $p > 0.05$ ) was found in the soluble protein content. These results are similar to those reported by using alkaline pH values between 10.0 and 12.0 to solubilise insect proteins with high yields (Bußler et al., 2016; Mishyna et al., 2019; Purschke et al., 2018; Udomsil et al., 2019; Yi et al., 2016; Zhao et al., 2016). In acidic conditions (pH 1.0–5.0), lower soluble protein concentrations were achieved, being pH 3.0 the one that presented the lowest value ( $3.61 \pm 0.23$  mg soluble protein/g DGP), suggesting that the isoelectric point (pI) of these proteins is between pH 2.0 to 4.0. These results are similar to those found for other insect protein sources, which in acid conditions (pH 4.0–5.0) decrease the solubility of their proteins, as reported for silkworm pupae *B. mori* (Kim et al., 2016); crickets such as *G. bimaculatus* and *A. domesticus* (Udomsil et al., 2019); grasshoppers *S. gregaria*; western honeybees *A. mellifera* (Mishyna et al., 2019), migratory locust *Locusta migratoria* (Purschke et al., 2018); mealworm larvae *T. molitor*, and black soldier fly *Hermetia illucens* (Bußler et al., 2016). It is surmised that SP grasshopper proteins are more soluble in alkaline media since pH values above the isoelectric point favour the dissociation of the carboxyl group and negatively charged amino acids present in the proteins. This gives as a result, an increase in the surface charge leading to a greater electrostatic repulsion, which in turn increases the solubility of the proteins in the supernatant phase (Yi et al., 2016). Until now, the characterization of proteins in SP have not been reported; however, some authors have noted the presence of structural and globular proteins in Orthoptera such as *A. domesticus*, which are soluble in saline or low alkaline solutions like actin and myosin (Montowska et al., 2019).

### Extraction of *Sphenarium purpurascens* soluble protein

Proteins play an important role in food technology, and the extraction method is different according to the protein characteristics and their extraction source. In the case of grasshoppers some authors mentioned that the insect protein extract has a high particle size with a granular texture, which is not pleasant to the palate when incorporated into food products (Cruz-López et al., 2022), also for some consumers the appearance of grasshoppers causes neophobia (Sogari et al., 2019).

On other hand, there are no reports regarding the extraction method of SP protein or their techno functional properties. Ultrasound has been widely used in protein extraction or in changing the structural characteristics of proteins, decreasing particle size, improving rheological properties, solubility, and emulsifying activity (Wang et al., 2021).

The establishment of the best pH extraction condition for soluble proteins was based on the suitability of the SP proteins to perform as a good meat extender and contribute to the protein content of the product from an unconventional source of protein. Preliminary assays showed that soluble protein extracted at pH 9 did not produced meat emulsions with adequate stability during sausage stuffing (data not shown), so it was decided to try different pH conditions for extracting the soluble protein in SP. In agreement to the protein solubility described in previous section, at pH 7 and 8 the solubility of SP protein did not display significant differences, nevertheless, in accordance with the normative regulations on the addition of acidity regulators in food products (Codex Alimentarius, 1995), the use of NaHCO<sub>3</sub> (pH 8 at 3% w/v) presents a higher acceptability and compatibility in meat-like products, preferably than NaOH commonly used for reaching more alkaline conditions (pH>9), allowing to extract high yields of soluble protein from SP. Therefore, the soluble protein recovery was done at pH 8 by using two extraction methods as shown in Fig. 1B. The protein extraction recovery in alkaline medium without ultrasound (ALK) did not show significant differences after 10 min of extraction ( $p>0.05$ ) and it was lower than 10%. The maximum recovery was 25% and it was obtained for the PUP method after 20 min, without significant difference ( $p>0.05$ ) for longer times. The results indicate that the application of ultrasound by sonotrode increased the yield 2.5-fold compared to the alkalisation method. The result of PUP method is in accordance with crude protein recovery percentages of 17% to 23% for insects such as mealworms *T. molitor*; crickets *A. domesticus* and *Acheta diaperinus*; beetles *Z. morio* and cockroaches *Blattella germanica* (Yi et al., 2013). These results are similar to those reported by Mishyna et al. (2019) for the protein extracted using an ultrasound-assisted alkaline method on grasshoppers *S. gregaria* yielding for 19.4%. Nevertheless, the protein percentages with PUP recovery using the sonication method were relatively lower than those reported in *T. molitor* larvae (94%), crickets *G. bimaculatus* (34%) and silkworm pupae *B. mori* (28%) (Choi et al., 2017a). These differences in the protein yielding when using PUP may be due to the extraction conditions, the type of ultrasound device and configuration (bath or piezoelectric sonotrode), surface area of the pre-treated samples, residual fat percentage of the powder, and the presence of chitin in the case of Orthoptera (Choi et al., 2017a). Moreover, because of the high energy addition to the protein molecules due to ultrasound application, variations on the protein recovery are also attributed to changes in the surface hydrophobicity of proteins, due to splitting and fractionation of the protein structure due to the cavitation phenomenon, which leads to changes in the conformation of the secondary, tertiary, and quaternary structures of the protein, affecting the functional properties such as the solubility (Kingwascharapong et al., 2021). In this sense, proteins in their native state usually perform as aggregates with low dispersibility in aqueous media, but when ultrasound is applied, a large number of cavitation bubbles are produced, which cause a rapid increase in local temperature and pressure at the neighborhood of the collapsing bubbles. This cavitation causes the disruption of hydrogen bonds, hydrophobic interactions and peptide bonds by hydrolysis mechanisms, provoking the unfolding of protein structure (Jambrak et al., 2009), dissociating the former protein aggregates, reducing the particle size, and the exposing greater number of inner sulfhydryl (SH) groups, and therefore increasing the surface area and particle charge (Jeong et al., 2021; Téllez-Morales et al., 2020) which contributes to stronger protein-water interactions and improving the protein solubility (Zhang et al., 2017). Some works state that during ultrasound application on protein samples, covalent bonds are not broken, but instead small changes in the secondary structure of the protein are occurred; inducing a decrease in  $\alpha$ -helix content and increasing the  $\beta$ -laminar structure, besides the increase in free SH groups causes changes in the tertiary structure with significant effect on the protein solubility (Jeong et al., 2021;



Télliez-Morales et al., 2020). Additional factors that contribute to modifying the protein solubility include the amino acid composition, three-dimensional structure in native proteins, pH, temperature, and ionic strength (Su et al., 2021; Télliez-Morales et al., 2020).

### Physicochemical characteristics of meat models

The results for pH, colour, and total protein of the sausage like meat products are given in Table 2. The pH values among treatments presented significant differences when compared to the control. The pH value increased accordingly to the percentage of meat substitution when compared to the control. These results may be attributed to the pH of the meat ranging 5.5–6.0 and the SP protein extracts having a pH of 8.0 due to the extraction method. Urbina et al. (2021) observed that the final pH of the cooked meat emulsions incorporated with extract from the cricket *A. domesticus* depends on the type of extraction used, in acid conditions, it presented an acidic final pH of 5.0 to 6.5 and the extracts obtained under basic conditions had a final pH of 8.0 to 9.0.

The characteristics colour of the cooked meat models decreased in terms of the CIE L\* compared to the control, while the CIE a\* and CIE b\* parameters increased across all treatments prepared with SP protein extract. It is important to mention that no colourants were used in any of the formulations. Significant differences ( $p < 0.05$ ) were observed in all the colour parameters compared to the control, although there was no significant difference among treatments. Based on the results, the cooked meat models with SP extract tend towards red (CIE a\*) and yellow (CIE b\*) in darker tones (CIE L\*). These results may be due to the protein extraction method with no significant differences between them: ALK CIE Lab\* 57.70±0.30, 5.74±0.01, 20.99±0.07 and PUP CIE Lab\* 60.36±0.04, 6.01±0.37, 22.2±0.41. The colour values obtained in the a\* parameters may be due to the roasting of the SP for consumption, which may promote Maillard reactions due to the presence of amino acids, sugars and proteins causing darkening of the grasshoppers (Kinyuru et al., 2009). Another factor, which may enhance red and yellow tones in insect extracts, is the oxidation of pigments such as melanin and primarily pheomelanin

**Table 2.** Physicochemical parameters of meat models formulated with SP protein

Treatments	Parameters				
	Total protein <sup>1)</sup> (%)	pH	Colour		
			L*	a*	b*
Control	13.13±0.41 <sup>A</sup>	6.35±0.03 <sup>A</sup>	74.25±3.50 <sup>B</sup>	1.76±1.03 <sup>A</sup>	13.18±0.11 <sup>A</sup>
T1	12.54±1.24 <sup>A</sup>	7.21±0.03 <sup>B</sup>	59.48±4.54 <sup>A</sup>	4.83±1.06 <sup>B</sup>	18.40±0.20 <sup>B</sup>
T2	13.13±0.41 <sup>A</sup>	7.13±0.07 <sup>B</sup>	56.56±3.97 <sup>A</sup>	5.26±0.13 <sup>B</sup>	20.47±0.10 <sup>B</sup>
T3	14.88±0.41 <sup>B</sup>	8.71±0.05 <sup>E</sup>	50.63±1.71 <sup>A</sup>	5.36±0.22 <sup>B</sup>	20.49±0.13 <sup>B</sup>
T4	11.96±0.41 <sup>A</sup>	7.50±0.04 <sup>C</sup>	58.18±7.77 <sup>A</sup>	5.11±0.75 <sup>B</sup>	20.92±1.41 <sup>B</sup>
T5	13.13±0.41 <sup>A</sup>	8.42±0.06 <sup>D</sup>	56.38±1.13 <sup>A</sup>	5.22±0.31 <sup>B</sup>	20.75±1.34 <sup>B</sup>
T6	15.17±0.83 <sup>B</sup>	8.68±0.19 <sup>E</sup>	56.60±8.18 <sup>A</sup>	5.36±1.14 <sup>B</sup>	19.83± 2.44 <sup>B</sup>

All values are mean±SD of three replicates (n=9).

<sup>1)</sup> Kjeldahl N×6.25.

<sup>A-E</sup> Different letters in the same column mean significant differences between samples at  $p < 0.05$ .

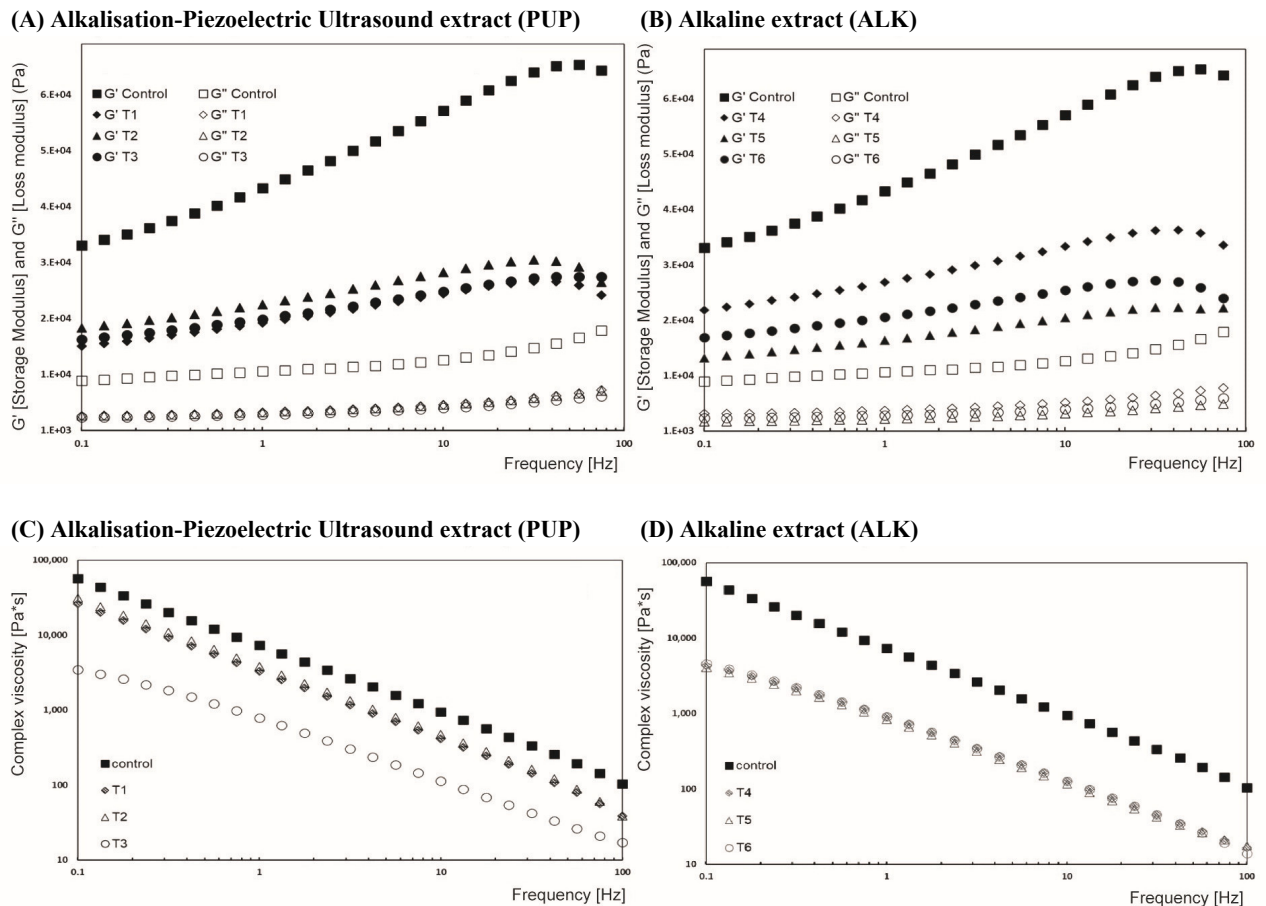
Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat+7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat+2.5% ALK); T5, sausages with 10% meat substitution (45% pork meat+5% ALK); T6, sausages with 15% meat substitution (42.5% pork meat+7.5% ALK).

SP, *Sphenarium purpurascens*.

(Kim et al., 2020; Urbina et al., 2021). The results of pH and colour coincide to those reported by other authors who incorporated insect protein extracts such as *T. molitor* larvae or silkworm *B. mori* pupae in cooked emulsified products (Park et al., 2017; Kim et al., 2020). The total protein content in the sausages in the treatments T1, T2, T4, and T5 present no significant difference ( $p > 0.05$ ) compared to the control. Treatments T3 and T6 with 15% meat substitution present the highest percentage of total protein. The results obtained agree to previous observations where an increase in the percentage of protein of *B. mori* (Park et al., 2017) and *T. molitor* (Choi et al., 2017b) in meat batters, and *A. domesticus* crickets in pork pate (Smarzyński et al., 2019). In addition, according to Ibarra-Herrera et al. (2020) the SP protein is considered highly digestible (85% to 90%) and comparable to meat (89.6%), as well as having concentrations of essential and NAAs comparable to egg.

### Viscoelastic properties

The dynamic oscillatory rheology for the meat batters with ALK and PUP as meat substitutes are shown in Fig 2. The moduli  $G'$  and  $G''$  for the different treatments present a frequency-dependent behaviour, where the elastic component ( $G'$ ) is above the viscous component ( $G''$ ) throughout the frequency interval (Fig. 2A, B), indicating the formation of ordered and

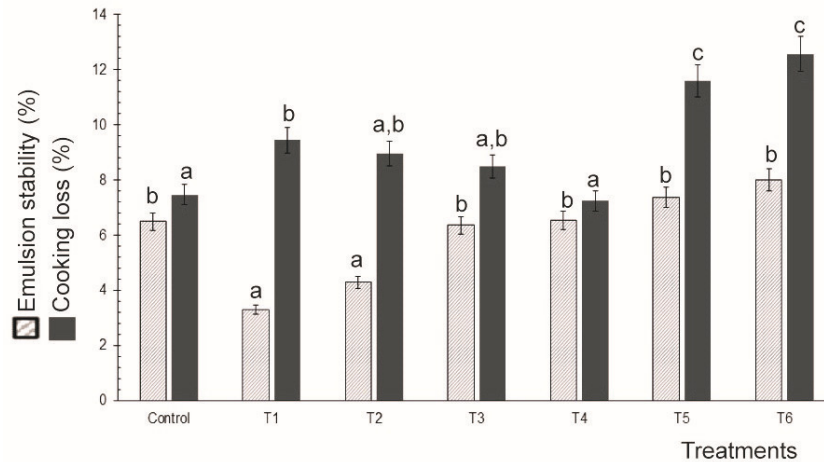


**Fig. 2.** Dynamic oscillatory rheology of meat models with grasshopper protein extracted by PUP (A, C) or ALK (B, D). Storage modulus  $G'$  and loss modulus  $G''$  (Pa) and complex viscosity  $\eta^*$  (Pa s). Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat+7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat+2.5% ALK); T5, sausages with 10% meat substitution (45% pork meat+5% ALK); T6, sausages with 15% meat substitution (42.5% pork meat+7.5% ALK). ALK, alkaline extraction; PUP, alkalisation-ultrasound extraction.

elastic gel structures (Li et al., 2020). This behaviour is characteristic of weak viscoelastic materials, which tend to exhibit a solid-like behaviour where elasticity predominates over viscosity (Gibis et al., 2017; Kim et al., 2022; Scholliers et al., 2020b). There are no studies in the literature regarding the viscoelasticity of meat sausages using grasshopper *Shepenarium purpurances* protein as meat extenders. However, the rheological behaviour obtained for the different treatments of this research is in accordance with some other authors, which used other insects in their studies. Scholliers et al. (2020a) and Scholliers et al. (2020b) evaluated the effect of heating temperature (70°C to 90°C) on the gelation of different ratio solutions of *Z. morio* larvae protein and pork proteins in a hybrid model system and as partial replacement of meat in cooked sausages. Their results showed gels with elastic characteristics where  $G'$  was predominant over  $G''$  showing a slight frequency-dependence. Kim et al. (2022) compared rheological properties among thermal-induced gels using porcine myofibrillar protein and five different edible insect species: *T. molitor* L., *Protaetia brevitarsis*, *Allomyrina dichotoma*, *G. bimaculatus* and *Oxyachinensis sinuosa*, where samples exhibited solid-like behaviour, and  $G'$  was greater than  $G''$  approximately at 50°C due to the formation of a rigid structure. In contrast, some authors obtained different results in emulsified systems using *T. molitor* larvae as partial substitutes for myofibrillar protein (MP) since the  $G'$  and  $G''$  moduli are not grouped between treatments (Kim et al., 2020). The differences could be attributed because a meat matrix is more complex in comparison to controlled systems in terms of pH, temperature, and protein concentration. The control sample profiles were higher among treatments, indicating that SP protein does not have the same capacity to form gels as meat protein. The replacement of meat with SP protein affects the apparent viscosity ( $\eta^*$ ) of the cooked sausages, the  $\eta^*$  of the control was higher in comparison with all treatments with SP protein. This could be explained, because edible insect protein has the capacity of reducing water and fat binding capacities (Choi et al., 2017b; Kim et al., 2016; Kim et al., 2020). The control and all the treatments with SP protein presented a thixotropic behaviour, with  $\eta^*$  values that decreased with increasing rotation time (Fig. 2C, D) (Choi et al., 2017b; Wang et al., 2021). The results are in accordance with some authors that used the protein of *T. molitor* larvae, that presented a lower  $\eta^*$  than the control when comparing sausages with 5% and 10% meat substitute (Choi et al., 2017b; Kim et al., 2020). On the other hand, the  $\eta^*$  of the formulation with meat substitution of 5% and 10% PUP were similar to that displayed by the control treatment (Fig. 2C), whereas all treatments with the ALK method have approximately a viscosity 10-fold lower when compared to the control (Fig. 2D). Therefore, it can be inferred, that the ultrasound treatment favours the development of viscoelastic properties and creates a stronger gel structure in the sausages. The increased viscosity of the sausages added with PUP protein extract may be associated with the formation of more cross-links between protein strands or proteins-coated oil droplets through hydrophobic interactions, sulfhydryl-disulphide interchange, also taking into consideration the high-intensity ultrasound could modify the structure of SP protein and improve the rheological properties (Li et al., 2020; Téllez-Morales et al., 2020).

### Cooking loss and emulsion stability

CL and emulsion stability (ES) of the different meat models are shown in Fig. 3. The results for cooking loss show that the treatments with PUP extracts at 10% and 15% (T2 and T3), and ALK at 5% (T4) present no significant differences ( $p>0.05$ ) compared to the control. The treatments with PUP extract (T1–T3) and the control showed significant differences ( $p<0.05$ ) with the formulations with ALK extract T5 and T6. These treatments with ALK extract showed that when increasing protein concentration in the formulations, the cooking loss and pH of meat models (T3–T6) increased when compared to the control, but the viscoelastic properties decreased with respect to control. Different results were reported by Park et al. (2017) showed that the decrease of cooking loss for meat batter added with silkworm powder has an inverse relation with pH and viscosity.



**Fig. 3. Emulsion stability and cooking loss of meat batters formulated with various levels of soluble protein extracts from SP extracted by ALK or PUP.** Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat+7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat+2.5% ALK); T5, sausages with 10% meat substitution (45% pork meat+5% ALK); T6, sausages with 15% meat substitution (42.5% pork meat+7.5% ALK). All values are represented as the mean value and the vertical bars show the SD of three replicates (n=9). <sup>a-c</sup> Different letters mean significant differences between treatments for each variable at  $p < 0.05$ . SP, *Sphenarium purpurascens*; ALK, alkaline extraction; PUP, alkalisation-ultrasound extraction.

Therefore, when CL decreases the viscosity and pH of the meat batter are increased when compared to the control. Results are similar to those reported by Choi et al. (2017b) that when substituting meat with at least 15% protein from *T. molitor*, the pH and cooking loss increased. This behaviour was explained due to the denaturation of the insect's built-in protein due to the drying process of the insect, which could clarify that CL is not a factor dependent on the increase in pH. On the other hand, the increased CL in sausages with ALK extract could be attributed to the loss stability of the emulsion due to the decrease of MP or possibly because the grasshopper protein has a higher proportion of hydrophobic groups that do not allow a good water absorption causing an increase in the cooking loss. The results indicated that the ALK extract does not have a high-water holding capacity, although some authors have reported that the cooking loss is improved with the insect protein (Pintado and Delgado-Pando, 2020). Torruco-Uco et al. (2019) reported that SP have a WHC of 1.75 g/g that is lower than other insects such as *A. domesticus* (2.03 g/g) and *Gryllidae* sp. (2.38 g/g). The difference among the ALK extract and other insects or their extracts would be due to different protein contents and/or its different extraction methods (Kim et al., 2017). On the other hand, PUP treatments showed a different behaviour in comparison to ALK treatments, where an increasing meat substitution and high pH in the meat model resulted in a decrease in cooking loss (T2 y T3) and these do not present significant differences with the control ( $p > 0.05$ ). These results coincide with those reported by Kim et al. (2016), Kim et al. (2017), Park et al. (2017) and Scholliers et al. (2020b); that established that there is an inverse relation between CL with respect to a higher concentration of insect and the pH, which is observed in mealworm larvae *T. molitor*, silkworm pupae *Bomboxy mori* and *A. domesticus*. The reduction of CL in the treatments with PUP extract could be due to the decrease of moisture, which could be explained by the increased solid content which took place by replacing pork meat portion with grasshopper protein (Kim et al., 2017; Park et al., 2017). Also, the results obtained could indicate that the grasshopper protein obtained by sonication method may have changes in its structure, such as surface charge and exposure of hydrophilic or hydrophobicity groups present in the protein. These changes contributed to improve the solubility and CL in comparison with

ALK extract, making their behaviour similar to the control (without substitution) (Mishyna et al., 2019; Su and Cavaco-Paulo et al., 2021; Wang et al., 2021).

The percentage of total separation of fluids such as fat and water in the meat batter was determined with lower values of expressible fluids representing good ES (Choi et al., 2017b). As shown in Fig. 3, the meat emulsion has better stability at concentrations of 5% (T1) and 10% (T2) of PUP which are significantly different ( $p < 0.05$ ) to the values from ALK treatments and the control. These results indicate that the SP protein extract has functional properties that help to stabilise the emulsion formed in the meat models, even at 15% meat substitution. The results obtained match those reported by Choi et al. (2017b) when incorporating *Tenebrio molitor* L. as a meat substitute at levels of 5%, 10%, and 15%. Kim et al. (2016) observed no difference in the EC of the control with meat batters that incorporated 10% of *T. molitor* and *B. mori* as a meat substitute. Finally, the SP protein that was obtained by ultrasound method presented cooking loss and ES properties like the control. The results obtained can be attributed to the PUP method of extraction that improved functional properties with ultrasound treatment, which can bind to water and avoid cooking loss. Furthermore, some studies have shown that the ultrasound method applied during pre-treatment of insect proteins such as *S. gregaria*, *A. mellifera* (Mishyna et al., 2019), *Bilineata tingtauca* Mell (Wang et al., 2021), and *H. illucens* (Mintah et al., 2019) has different effects. For example, it modifies particle size, solubility, increases sulfhydryl content, increases surface hydrophobicity and rheological properties in proteins extracted due to its physical effects such as capillary surface waves and acoustic cavitation. Also, Majzoubi et al. (2012) reported that higher protein solubility would increase protein adsorption and protein migration rate when considering the water–oil interface, thereby increasing the emulsion properties of proteins, which can cause a low loss due to cooking.

## Texture

The results of the TPA for the meat models prepared with different levels of SP protein extracted by ALK or PUP are shown in Table 3. The parameters brittleness, adhesiveness, elasticity, and cohesiveness presented no significant differences ( $p > 0.05$ ) among the samples and the control. The results for elasticity and cohesiveness matched those of other authors who noted no difference in these parameters (Kim et al., 2016; Kim et al., 2020; Park et al., 2017). The extraction method does not

**Table 3. Texture parameters of meat models formulated with SP protein**

Treatments	Texture parameters					
	Hardness (kg)	Adhesiveness (mJ)	Brittleness (kg)	Elasticity (mm)	Cohesiveness	Firmness (kg)
Control	1.07±0.20 <sup>B</sup>	0.26±0.05 <sup>AB</sup>	1.19±0.16 <sup>A</sup>	3.60±0.13 <sup>AB</sup>	0.80±0.05 <sup>A</sup>	0.95±0.07 <sup>C</sup>
T1	0.84±0.03 <sup>AB</sup>	0.37±0.08 <sup>B</sup>	1.01±0.08 <sup>A</sup>	3.66±0.02 <sup>B</sup>	0.77±0.01 <sup>A</sup>	0.78±0.05 <sup>AB</sup>
T2	0.87±0.17 <sup>AB</sup>	0.33±0.07 <sup>AB</sup>	0.96±0.12 <sup>A</sup>	3.61±0.02 <sup>AB</sup>	0.77±0.04 <sup>A</sup>	0.74±0.12 <sup>AB</sup>
T3	1.00±0.16 <sup>B</sup>	0.28±0.05 <sup>AB</sup>	1.12±0.06 <sup>A</sup>	3.42±0.01 <sup>A</sup>	0.75±0.01 <sup>A</sup>	0.84±0.06 <sup>BC</sup>
T4	0.67±0.03 <sup>A</sup>	0.25±0.02 <sup>A</sup>	0.98±0.28 <sup>A</sup>	3.73±0.02 <sup>B</sup>	0.69±0.12 <sup>A</sup>	0.62±0.02 <sup>A</sup>
T5	0.88±0.01 <sup>AB</sup>	0.25±0.01 <sup>A</sup>	1.01±0.05 <sup>A</sup>	3.71±0.18 <sup>B</sup>	0.80±0.10 <sup>A</sup>	0.81±0.14 <sup>AB</sup>
T6	0.95±0 <sup>AB</sup>	0.27±0.02 <sup>AB</sup>	1.11±0.12 <sup>A</sup>	3.63±0.02 <sup>B</sup>	0.77±0.04 <sup>A</sup>	0.85±0.05 <sup>BC</sup>

All values are mean±SD of three replicates (n=9).

<sup>A-C</sup> Different letters in the same column means significant differences between samples at  $p < 0.05$ .

Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat+7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat+2.5% ALK); T5, sausages with 10% meat substitution (45% pork meat+5% ALK); T6, sausages with 15% meat substitution (42.5% pork meat+7.5% ALK).

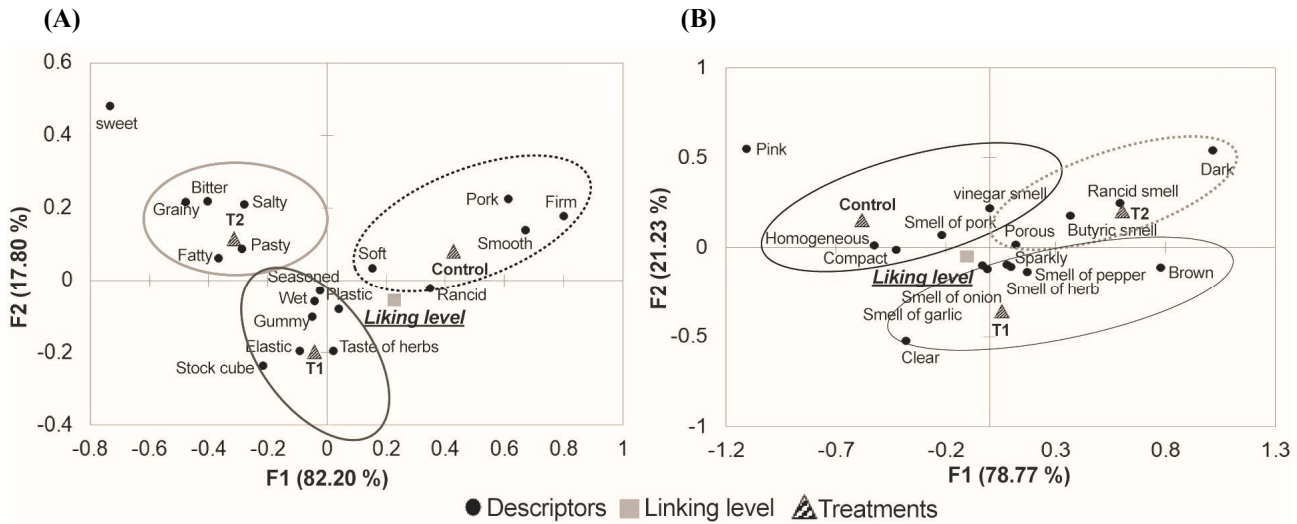
SP, *Sphenarium purpurascens*.

influence these parameters. Regarding the firmness, only the treatments with higher percent of 15% substitution, with both extraction methods (T3 and T6), did not show significant differences ( $p>0.05$ ) with the control treatment. By other hand, hardness was not significantly different ( $p>0.05$ ) between treatments and the control samples, except for T4 treatment. T4 had the lowest value of hardness, even when CL and ES were significantly equal ( $p>0.05$ ) to control. This behaviour in texture properties has been described previously during the incorporation of *T. molitor* larvae flour in frankfurters and emulsion systems, where concentrations increase from 10% to 20% caused the decrease in hardness, springiness, cohesiveness, gumminess and chewiness (Choi et al., 2017b; Kim et al., 2020). Despite no significant differences among treatments T1, T2, T3, T5, and T6 were found, the average value of hardness is greater in treatments with PUP extract in the different meat substitutions. These results are in accordance with lower cooking loss, higher ES, and higher viscoelastic properties, when compared to these characteristics in the ALK extract treatments. Wang et al. (2021) explained that the insect protein isolates when submitted to high ultrasound power treatment (400 W), could present the unfolding of its protein chains, resulting beneficial to the stability of the gel structure in meat and fluid-type emulsions, favouring the development of stronger gel-like structure in the sausages. Contrary to our observations, the gels obtained with PUP extract did not show differences with treatments made with ALK extract. This behaviour could be explained by the presence of polyphenols in the crude protein extracts obtained from SP. Some authors have demonstrated that defatted flours obtained by *A. dosmesticus*, *T. molitor*, *Z. morio*, and *R. ferrugineus* (Botella-Martínez et al., 2021) exhibit antioxidant activity that extracts from SP not only present antioxidant activity, but also report a concentration of total polyphenols of 27 mg of gallic acid equivalent/g of extract. According to these results, it is possible that some phenolic compounds could be extracted under the conditions used for the protein SP extraction, influencing the physicochemical properties, including the gel-like structure, of MP in SP through both covalent and noncovalent interactions (reversible or irreversible pathways) resulting in the blockage of exposed hydrophobic sites, reducing the surface area, lowering the concentration of MP available to interact in the formation of the gel-like structure and affecting the texture properties. In addition, the lack of improvement on the texture properties in treatments where PUP extract was used may be attributed to the increase in free SH groups content, and their prompt to be attacked by the phenol ring structure (quinone) forming protein–quinone complexes, altering the gelation capability of proteins, which is the most important texture property in meat products (Guo et al., 2021).

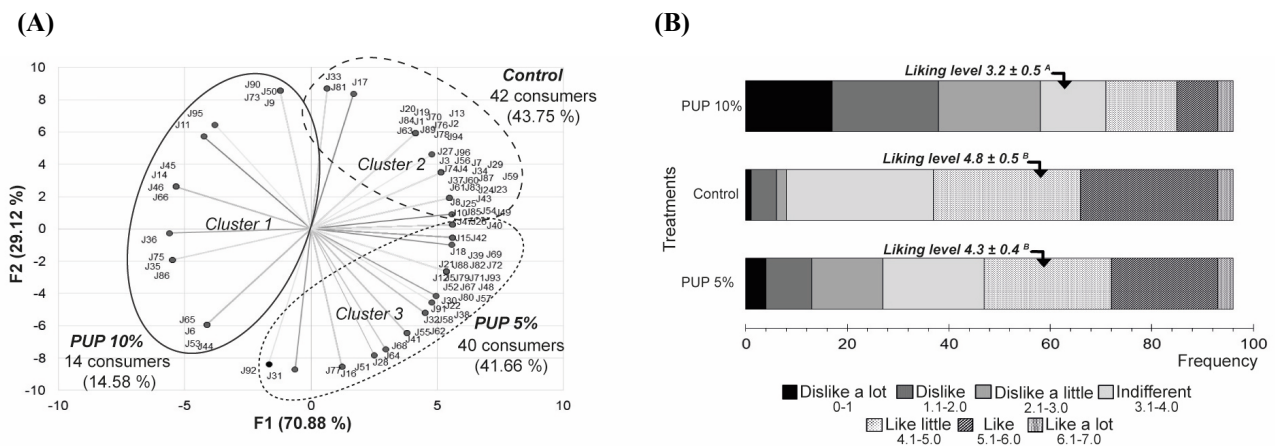
The results suggest that SP protein, even at low substitution concentrations, can be equivalent to a 100% meat product. Based on the above results, the formulations with 5% and 10% meat substitution (T1–T2) with the soluble protein obtained by the ultrasound method (PUP) showed no significant difference compared to the control ( $p>0.05$ ) in parameters such as texture, absolute viscosity, cooking loss and total protein. Finally, the PUP extract can be considered as a meat extensor when using a 10% as substituting meat according to the obtained results.

### Sensory evaluation

The sensory evaluation of products with modified formulations can provide important information on consumer acceptance and highlight the attributes that can be altered to obtain a better final product. Some consumers could be disgruntled to see insect parts in their food, so it is important to evaluate sensory perception with insect extracts. The sensory descriptors obtained by CATA test show a 100% relationship between the samples and the sensory descriptors of taste-texture (Fig. 4A) and smell-appearance (Fig. 4B), also the liking level in both analyses is high between the control and the PUP 5%, indicating that both formulations were to the liking of consumers. It should be noted that no spices were added to the formulations and the descriptors identified are associated with the SP extract. Fig. 5A shows the preference map. Control was preferred by 42



**Fig. 4. Correspondence factorial analysis of the meat models descriptors.** (A) taste-texture; (B) smell-appearance. Control, sausages without meat substitution (50% pork meat+0% grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat+2.5% PUP); T2, sausages with 10% meat substitution (45% pork meat+5% PUP). In both graphs, the F1 and F2 axes explain 100% of all the data. PUP, alkalisation-ultrasound extraction.



**Fig. 5. Consumer acceptability of meat models.** (A) Preference map was made through principal component analysis (PCA) and hierarchical agglomeration (clustering), and it explains 100% of all data on F1 and F2 axes; (B) Hedonic scale, values marked with different capital letters in the Liking level indicate significant differences between treatments ( $p < 0.05$ ). PUP, alkalisation-ultrasound extraction.

consumers and had the highest percentage 43.75% and 40 consumers with a slightly lower percentage of 41.66% favoured PUP 5%. The sample least preferred was PUP 10% with only 14 consumers that liked it (14.58%). Fig. 5B shows the results of the hedonic scale used to find the overall liking of consumers. The control and PUP 5% showed no significant differences between them in the level of liking ( $p > 0.05$ ) but did show a significant difference regarding PUP 10%, according to the Friedman test. In addition, the control and PUP 5% have the same acceptability with a mean liking of 4.8 and 4.3 respectively, positioning them on the scale as “Like a little”, in contrast with PUP 10% with a mean liking of 3.229 (indifferent). The results obtained are similar to those obtained incorporating *T. molitor* into sausages (Choi et al., 2017b) and crickets into pork pate (Smarzyński et al., 2019), they observed that at higher substitution concentrations, acceptance was lower. Some authors mention that the acceptability of products that incorporate insects is multifactorial; taste and smell

depend on the insects' pheromones, whose concentration in turn depends on the environment where the insects feed and develop. The type of insect and its food can also affect taste, as can the type of process the insect undergoes before or during incorporation into a food, and the tradition of insect consumption in the region (van Huis, 2020). Finally, regarding colour, it is necessary to continue improving the extraction process, perhaps with the incorporation of an enzyme complex that lessens the darkening of protein extracts, thus improving colour, a highly important sensory attribute for consumers.

## Conclusion

The alkalisation combined with ultrasound method improved techno-functional properties of the SP protein in cooked meat models at meat substitution levels below 10%, equating to the control (100% meat) in physicochemical properties. The sensory tests detected descriptors such as rancid smell and taste, seasoned and with herbal taste in the PUP samples as well as a brown colour; these aspects can be attributed to the SP protein extract since no colourants or spices were added to the formula. The hedonic scale and preference map analyses indicate that PUP 5% formulation has the same acceptability and liking as the control. Given these results, SP soluble protein treated with ultrasound can be used as extender in meat products. However, further work is recommended to incorporate different types of hydrocolloids and spices that contribute to the formulation of a more acceptable product with high benefit.

## Conflicts of Interest

The authors declare no potential conflicts of interest.

## Author Contributions

Conceptualization: Alvarez-Cisneros YM. Data curation: Cruz-López SO, Alvarez-Cisneros YM. Formal analysis: Cruz-López SO, Escalona-Buendía HB, Alvarez-Cisneros YM. Methodology: Cruz-López SO, Escalona-Buendía HB, Román-Guerrero A, Domínguez-Soberanes J, Alvarez-Cisneros YM. Software: Cruz-López SO, Escalona-Buendía HB. Validation: Cruz-López SO, Escalona-Buendía HB, Román-Guerrero A, Domínguez-Soberanes J, Alvarez-Cisneros YM. Investigation: Cruz-López SO, Alvarez-Cisneros YM. Writing - original draft: Alvarez-Cisneros YM. Writing - review & editing: Cruz-López SO, Escalona-Buendía HB, Román-Guerrero A, Domínguez-Soberanes J, Alvarez-Cisneros YM.

## Ethics Approval

All participants agreed to participate for the sensory analysis of this research and signed the Informed Consent Form. This work is part of the divisional project "Techno-Biofunctional and Sensory properties of Biomolecules and their Application in Food" (Propiedades Tecno-Biofuncionales y Sensoriales de Biomoléculas y su Aplicación en Alimentos) and it has the approval of the Ethics Committee (Comisión Académica de Ética de la División de Ciencias Biológicas y de la Salud, UAM-Iztapalapa) under the number 1913 (dictamen 1913).

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