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11 Characterization of cooked meat models using grasshopper (*Sphenarium purpurascens*)

12

soluble protein extracted by alkalisation and ultrasound as meat-extender.

13 Abstract

14 The most abundant Orthoptera in Mexico is a small grasshopper (*Sphenarium purpurascens*) 15 which is considered a food source with increased nutritional value due to its high protein content. 16 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 17 18 substituting meat with a soluble protein extract from grasshopper obtained by alkalisation or 19 alkalisation-piezoelectric ultrasound, on the techno-functional, physicochemical, and sensory characteristics of cooked meat models (sausages). The soluble protein was extracted in 20 21 NaHCO₃ 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 22 characterised for their rheological behaviour, emulsion stability, weight loss by cooking, total 23 24 protein content, colour, and texture. Sensory evaluation was conducted with consumers using a 25 test involving check-all-that-apply and overall liking. The alkalisation-piezoelectric ultrasound method improved the solubility and the techno-functional properties of the soluble grasshopper 26 27 protein when applied in sausages at maximum levels of 10% meat substitution. The sensory 28 evaluation indicated that the formulation with 5% meat substitution exhibited the same 29 acceptability as the control sample. Given these results, the soluble protein treated with 30 alkalisation and piezoelectric ultrasound could be used as an extender in meat products.

31

32 *Keywords*: edible insect, soluble protein, functional properties, sausages, sensory.

33

35 Introduction

Around 2100 insect species have been identified worldwide as food products (Jongema, 2017). 36 37 One of the most common are grasshoppers, locusts, and crickets (13%) (Van Huis et al., 2013). 38 The grasshopper Sphenarium purpurascens (SP) of the order Orthoptera is endemic of Mexico 39 and it is distributed in the States of Oaxaca, Chiapas, Puebla, Mexico, Hidalgo, Queretaro, and 40 Tlaxcala. It is often known as saltamontes or chapul in de la milpa due to its abundance in agro-41 ecosystems where maize is grown (Serrano-Limón and Ramos-Elorduy, 1989; Torruco-Uco et 42 al., 2019), and is considered a plague by farmers due to the floral and foliar damage it inflicts 43 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., 44 45 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 (EAA) and 66.48 to 46 68.93 g non-essential amino acids (NAA). In addition, 11.04 to 24.89 g chitin, 6.02 to 14.86 g 47 48 crude fat, 15.59 to 30 g carbohydrates and 10 to 31.81 g crude fibre. The micronutrients found 49 in this product are 34.61 to 37.64 mg sodium, 1007 to 1028 mg potassium, 201 to 235 mg 50 calcium, 17.84 to 17.98 mg zinc, 13.33 to 18.29 mg iron, 124 to 131 mg magnesium, 0.27 mg 51 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 1736.28 kJ (Ibarra-Herrera et al., 2020; Kosečková et al., 2022; Melo-Ruiz et 52 53 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
(Smarzyński et al., 2019; Mishyna et al., 2021; Van Huis, 2020).

The Orthoptera order has high concentrations of protein; however, its digestibility varies 62 63 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 64 65 pulverising the whole insect. The main methods used are alkaline extraction, isoelectric precipitation, and ultrasound (Choi et al., 2017a; Kim et al., 2019; Mishyna et al., 2019; 66 67 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 68 ultrasound-assisted alkaline extraction obtaining average yields of 56% from both insects. Choi 69 70 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 71 72 (Bombyx mori). The proposed methodologies for protein extraction from each insect are 73 particular to each species and geographic region depending on the structure and functionality 74 of the proteins (Kim et al., 2019).

75 While there is little bibliographic information on Orthoptera in general, in the case of SP the 76 only information available concerns its nutritional value and provides no scientific evidence on 77 protein extraction methods. On the other hand, the meat industry has a huge interest in reducing 78 production costs by incorporating alternative ingredients, such as high-protein non-meat 79 ingredients as insect proteins whose aim is to substitute the meat content of a product, or to 80 extend the amount of meat used (extenders). Some orthopterans, such as Gryllidae sp., 81 Gryllodes sigillatus, Locusta migratoris, Schistocerca gregaria, Acheta domesticus, and Sphenarium purpurances have demonstrated to have techno-functional properties. These 82 83 properties can be grouped in water absorption capacity (WAC), oil absorption capacity (OAC), 84 water solubility capacity (WSC), emulsifying capacity (EC), foaming capacity (FC) and foam 85 stability (FS) (Da Silva Lucas et al., 2020; Mishyna et al., 2019; Purschke et al., 2018; Torruco-86 Uco et al., 2019). Due to the functionality that these insects have shown, some authors have 87 used those protein extracts to replace a portion of meat in processed meats like sausages. 88 Tenebrio 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 89 90 at a similar level to the control sample (50% pork ham) maintained the quality of this type of 91 products (Choi et al., 2017b). Other researchers have used the whole insect in the form of flour 92 as the house cricket Acheta domesticus (Kim et al., 2017), silkworm pupae Bombyx mori (Park 93 et al., 2017) and superworm Zophobas morio larvae (Scholliers et al., 2020).

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.

101 Materials and methods

102 Grasshopper powder

The ready-to-eat grasshoppers *Sphenarium purpurascens* 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) (Rodr íguez-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 110 determined using a conversion factor of N_{Kjel} 4.5 (Janssen et al., 2017; Mishyna et al., 2019).

111 The powder was obtained from SP previously cleaned of foreign matter and subsequently dried

112 in an oven (Felisa model FE-291AD, Jalisco, Mexico) at 105 °C for 24 h.

113 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).

121 **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 3000 x 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.

128 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 NaHCO₃ 3% (p/v) at pH 8.0 and divided into 2 equal parts to assess the different extraction methods: 1) Alkaline extraction (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) Alkalisation-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.

141 **Preparation of meat models with the soluble protein as meat extender**

142 Table 1 shows the seven formulations made with the two protein extracts: ALK and PUP, that 143 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 144 145 make the sausages, the meat was manually minced into small pieces of about 7 x 7 cm, removing 146 the bone. Before the process, the ice was divided into three equal parts approximately and the 147 phosphates into two equal parts. Later, it was mixed with 20 g of frozen lard (pork back fat) in 148 an immersion blender to maximum power (Hamilton Beach, Virginia, USA). The powdered 149 ingredients were then added (0.3 g curing salts, 0.25 g phosphates and the grasshopper protein 150 ALK or PUP, according to Table 1), in addition with 10 g of ice. The ingredients were 151 homogenised for 1 min without pausing the blender and another 10 g of ice was added to the 152 mix. The mix was homogenised for an additional 2 min and the remaining phosphate mixture 153 was added (0.25g). Lastly, approximately 9.2 g of ice was added to the mixture until a 154 homogeneous emulsion or paste was obtained. The emulsion was stuffed into 22 mm cellulose 155 sleeves and cooked at 80°C until the sausages reached an internal temperature of 75°C, at which 156 they were immediately placed in cold water for 30 min, until the internal temperature dropped 157 to 15°C. Finally, the product was vacuum packaged employing a vacuum machine (EVD4 158 Torrey, México City, Mexico) in oxygen-impermeable bags.

159 Evaluation of the physicochemical characteristics of meat models

160 A texture profile analysis (TPA) of the sausages was performed in a Brookfield CT3 Texture 161 Analyzer, using a TA3/100 cylindrical probe 2.5 mm in diameter. The sausages were cut into 162 slices 20 mm wide and 10 mm thick. Data for hardness, adhesiveness, brittleness, cohesiveness, 163 elasticity, and firmness were obtained. Crude protein content was determined by the Kjeldahl 164 method (method 981.10) (AOAC, 2000), using a conversion factor of N_{Kiel} 6.25 (Mæ hre et al., 165 2018). The pH was determined according to Choi et al. (2017b) with a previously calibrated 166 electronic potentiometer 120. The colour of each sausage formulation was determined using a previously calibrated colorimeter (ColorFlex-HunterLab, Virginia USA), with a 19.1 mm 167 aperture, Illuminate D65 and 10° standard observer. The determinations were carried out in 168 169 quadruplicate. The parameters measured were CIELab* (Urbina et al., 2021).

170 Evaluation of the techno-functional properties of meat models

171 The stability of the meat emulsions with and without SP protein extract was determined as 172 reported by Choi et al. (2017b), with some modifications. Screw top tubes of 50 mL that were 173 modified with a mesh at the bottom were filled with 20 g of the meat batter and placed in a hot 174 water bath at 75°C, where they were kept for 30 min. After this time, the samples were cooled 175 to 4° with ice water. The water and fat content found in the bottom of the tube was quantified 176 and the stability of the emulsion was reported as total expressible fluids in mL/batter. Cooking 177 loss of the meat batters was evaluated by the weight difference before and after the heat 178 treatment (Park et al., 2017). The viscoelastic properties were evaluated according to Gibis et 179 al. (2017) with some modifications. The meat batters were analysed after heat treatment with 180 an MCR 300 rheometer (Paar Physica Messtechnic GmbH, Stuttgart, Germany) with a striated 181 PP 50/P2 geometry (25 mm diameter), with a 1 mm gap for uncooked samples and 9 mm gap 182 for cooked samples, using approximately 10 g of sample for each determination. Frequency 183 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 °C. We obtained graphs of the storage modulus (G '), loss modulus (G '') and absolute viscosity n*. The data were analysed with US200/32 Rheometer V2.50 software.

188 Sensory evaluation of sausages made with SP protein

189 Sensory evaluation was applied to the formulations that presented the best techno-functional 190 and physicochemical characteristics (T1 and T2) and these were compared to the control (100% 191 meat). The sensory analysis was done by consumers (n = 100) aged between 19 and 40 years. 192 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 193 194 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). 195 196 Finally, the check-all-that-apply (CATA) test was applied, in which consumers chose the 197 descriptors that apply to the sample from a list of 34 sensory attributes related to taste, smell, 198 texture and appearance (Ares et al., 2014; Jaeger et al., 2020). All participants agreed to 199 participate in the sensory analysis of this research and signed the Informed Consent Form. This 200 work is part of the divisional project "Techno-Biofunctional and Sensory properties of 201 Biomolecules and their Application in Food" and it has the approval of the Ethics Committee 202 of UAM-Iztapalapa under the number 1913.

203 Statistical analysis

All determinations were made in triplicate and the results are presented as the average with standard deviation. 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).

212 **Results and Discussion**

213 **Protein quantification of grasshopper**

214 Sphenarium purpurascens (SP) showed a total protein content using the N_{Kiel} factor 4.5 of 39.39 215 \pm 0.84%. This value is lower when compared to other type of grasshopper which is not *ready* 216 to be consumed (seasoned and roasted), making them eligible to be considered as fresh insects 217 such as the case of Schistocerca spp., Melanoplus femrubrum, Shpenarium histrio (Melo-Ruiz et al., 2015) and Sphenarium purpurascens (Ibarra-Herrera et al., 2020; Rodríguez-Miranda et 218 219 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, 220 221 season, age, gender, processing, and method of determination (Kouřimská and Adámková, 222 2016; De Carvalho et al., 2020). The most described method in the literature is that of Kjeldahl, 223 which uses a protein conversion factor depending on the protein source. Nevertheless, for 224 insects, a N_{Kjel} factor ranging from 4.67 to 5.62 has been reported (Janssen et al., 2017), while 225 in the case of Orthoptera such as grasshoppers, the N_{Kiel} factor has been established at 4.5 based 226 on amino acid analysis (Mishyna et al., 2019). However, some authors point out that this varies 227 because these insects have non-protein nitrogen in their structure, as is the case of excretion 228 products in the intestinal tract (ammonia) and chitin which forms part of the exoskeleton in 229 ratios of 5.3 to 6.6% (Janssen et al., 2017). The protein concentration found in SP is higher 230 when compared with the protein of beef (18.4%), chicken (22%) and fish (18.3%) (Yi et al., 231 2017). Although there is little information in the literature on total protein determination in 232 Orthoptera using the N_{Kiel} factor 4.5, among the data reported and with similar results is the 233 desert locust Schistocerca gregaria that presents 30.1% protein (Mishyna et al., 2019). 234 Likewise, the orthopter are considered a good source of protein such as a desert locust 235 Schistocerca gregaria, nymphs of the migratory locust Locusta migratoris, crickets Gryllus 236 bimaculatus, Schistocerca spp., Melanoplus femrrubrum and Shpenarium histrio (Melo-Ruiz 237 et al., 2015; Mishyna et al., 2019; Udomsil et al., 2019). But because of its structure it produces 238 neophobia to some people (Sogari et al., 2019), some authors have indicated that concentrates 239 or isolates of protein can be obtained from insects, promoting the acceptance of these novel 240 foods with added value (Shelomi, 2016). Currently, there is no research reported in literature 241 that mentions how to obtain protein concentrates from SP, the information that is published 242 corresponds mainly on the way of using the complete insect for edible purposes (Cruz-López 243 et al., 2022; Cuj-Laines et al., 2018).

244 **Protein solubility**

245 Some techno-functional properties of proteins such as foaming properties, emulsion capacity 246 (EC) and gel formation (GF) are dependent on the degree of protein solubility (Jeong et al., 247 2021; Torruco-Uco et al., 2019). The solubility of proteins is also influenced by the structure 248 of their molecules and the ratio of polar to non-polar groups, making pH an important parameter 249 to change the solubility of the proteins (Jeong et al., 2021). Fig. 1A shows the solubility profile 250 of proteins present in the DGP. The solubility of the proteins is observed to increase 251 significantly at pH values ranging 7.0 and 9.0, reaching a maximum solubility at pH 9.0 (19.33) 252 \pm 0.45 mg soluble protein/g DGP). Meanwhile, at a pH between 7.0 and 8.0 no significant 253 difference (p>0.05) was found in the soluble protein content. These results are similar to those 254 reported by using alkaline pH values between 10.0 and 12.0 to solubilise insect proteins with 255 high yields (Bubler et al., 2016; Mishyna et al., 2019; Purschke et al., 2018; Udomsil et al., 256 2019; Yi et al., 2016; Zhao et al., 2016). In acidic conditions (pH 1.0 - 5.0), lower soluble 257 protein concentrations were achieved, being pH 3.0 the one that presented the lowest value $(3.61 \pm 0.23 \text{ mg soluble protein/g PDC})$, suggesting that the isoelectric point (pI) of these 258

259 proteins is between pH 2.0 to 4.0. These results are similar to those found for other insect protein 260 sources, which in acid conditions (pH 4.0 - 5.0) decrease the solubility of their proteins, as 261 reported for silkworm pupae Bombyx mori (Kim et al., 2016); crickets such as Gryllus 262 bimaculatus and Acheta domesticus (Udomsil et al., 2019); grasshoppers Schistocerca gregaria; western honeybees Apis mellifera (Mishyna et al., 2019), migratory locust Locusta migratoria 263 264 (Purschke et al., 2018); mealworm larvae Tenebrio molitor, and black soldier fly Hermetia 265 illucens (Bubler et al., 2016). It is surmised that SP grasshopper proteins are more soluble in 266 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 267 268 result, an increase in the surface charge leading to a greater electrostatic repulsion, which in 269 turn increases the solubility of the proteins in the supernatant phase (Yi et al., 2016). Until now, 270 the characterization of proteins in SP have not been reported; however, some authors have noted 271 the presence of structural and globular proteins in Orthoptera such as Acheta domesticus, which 272 are soluble in saline or low alkaline solutions like actin and myosin (Montowska et al., 2019).

273 Extraction of SP soluble protein

274 Proteins play an important role in food technology, and the extraction method is different 275 according to the protein characteristics and their extraction source. In the case of grasshoppers 276 some authors mentioned that the insect protein extract has a high particle size with a granular 277 texture, which is not pleasant to the palate when incorporated into food products (Cruz-López 278 et al., 2022), also for some consumers the appearance of grasshoppers causes neophobia (Sogari 279 et al., 2019). On other hand, there are no reports regarding the extraction method of Sphenarium 280 purpurascens protein or their techno functional properties. Ultrasound has been widely used in 281 protein extraction or in changing the structural characteristics of proteins, decreasing particle 282 size, improving rheological properties, solubility, and emulsifying activity (Wang et al., 2021).

283 The establishment of the best pH extraction condition for soluble proteins was based on the 284 suitability of the SP proteins to perform as a good meat extender and contribute to the protein 285 content of the product from an unconventional source of protein. Preliminary assays showed 286 that soluble protein extracted at pH 9 did not produced meat emulsions with adequate stability 287 during sausage stuffing (data not shown), so it was decided to try different pH conditions for 288 extracting the soluble protein in SP. In agreement to the protein solubility described in previous 289 section, at pH 7 and 8 the solubility of SP protein did not display significant differences, 290 nevertheless, in accordance with the normative regulations on the addition of acidity regulators 291 in food products (Codex Alimentarius, 1995), the use of NaHCO₃ (pH 8 at 3% w/v) presents a higher acceptability and compatibility in meat-like products, preferably than NaOH commonly 292 293 used for reaching more alkaline conditions (pH> 9), allowing to extract high yields of soluble 294 protein from SP. Therefore, the soluble protein recovery was done at pH 8 by using two 295 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 296 297 (p>0.05) and it was lower than 10%. The maximum recovery was 25% and it was obtained for 298 the PUP method after 20 min, without significant difference (p>0.05) for longer times. The 299 results indicate that the application of ultrasound by sonotrode increased the yield 2.5-fold 300 compared to the alkalisation method. The result of PUP method is in accordance with crude 301 protein recovery percentages of 17 to 23% for insects such as mealworms *Tenebrio molitor*; 302 crickets Acheta domesticus and Acheta diaperinus; beetles Zophobas morio and cockroaches 303 Blaptica dubia (Yi et al., 2013). These results are similar to those reported by Mishyna et al., 304 (2019) for the protein extracted using an ultrasound-assisted alkaline method on grasshoppers 305 Schistocerca gregaria yielding for 19.4%. Nevertheless, the protein percentages with PUP 306 recovery using the sonication method were relatively lower than those reported in *Tenebrio* 307 molitor larvae (94%), crickets Gryllus bimaculatus (34%) and silkworm pupae Bombyx mori 308 (28%) (Choi et al., 2017a). These differences in the protein yielding when using PUP may be 309 due to the extraction conditions, the type of ultrasound device and configuration (bath or 310 piezoelectric sonotrode), surface area of the pre-treated samples, residual fat percentage of the 311 powder, and the presence of chitin in the case of Orthoptera (Choi et al., 2017a). Moreover, 312 because of the high energy addition to the protein molecules due to ultrasound application, 313 variations on the protein recovery are also attributed to changes in the surface hydrophobicity 314 of proteins, due to splitting and fractionation of the protein structure due to the cavitation 315 phenomenon, which leads to changes in the conformation of the secondary, tertiary, and 316 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 317 318 aggregates with low dispersibility in aqueous media, but when ultrasound is applied, a large 319 number of cavitation bubbles are produced, which cause a rapid increase in local temperature 320 and pressure at the neiborhood of the collapsing bubbles. This cavitation causes the disruption of hydrogen bonds, hydrophobic interactions and peptide bonds by hydrolysis mechanisms, 321 322 provoking the unfolding of protein structure (Jambrak et al., 2009), dissociating the former 323 protein aggregates, reducing the particle size, and the exposing greater number of inner 324 sulfhydryl (SH) groups, and therefore increasing the surface area and particle charge (Jeong et 325 al., 2021; Téllez-Morales et al., 2020) which contributes to stronger protein-water interactions 326 and improving the protein solubility (Zhang et al., 2017). Some works state that during 327 ultrasound application on protein samples, covalent bonds are not broken, but instead small 328 changes in the secondary structure of the protein are occurred; inducing a decrease in α -helix 329 content and increasing the ß-laminar structure, besides the increase in free SH groups causes 330 changes in the tertiary structure with significant effect on the protein solubility (Jeong et al., 331 2021; Téllez-Morales et al., 2020). Additional factors that contribute to modifying the protein

- 332 solubility include the amino acid composition, three-dimensional structure in native proteins,
- pH, temperature, and ionic strength (Su et al., 2021; Téllez-Morales et al., 2020).

334 Physicochemical characteristics of meat models

335 The results for pH, colour, and total protein of the sausage like m; eat products are given in 336 Table 2. The pH values among treatments presented significant differences when compared to 337 the control. The pH value increased accordingly to the percentage of meat substitution when 338 compared to the control. These results may be attributed to the pH of the meat ranging 5.5-6.0 339 and the SP protein extracts having a pH of 8.0 due to the extraction method. Urbina et al. (2021) 340 observed that the final pH of the cooked meat emulsions incorporated with extract from the cricket Acheta domesticus depends on the type of extraction used, in acid conditions, it 341 342 presented an acidic final pH of 5.0 to 6.5 and the extracts obtained under basic conditions had 343 a final pH of 8.0 to 9.0.

344 The characteristics colour of the cooked meat models decreased in terms of the CIEL* values compared to the control, while the CIEa* and CIEb* parameters increased across all treatments 345 346 prepared with SP protein extract. It is important to mention that no colourants were used in any 347 of the formulations. Significant differences (p<0.05) were observed in all the colour parameters 348 compared to the control, although there was no significant difference among treatments. Based 349 on the results, the cooked meat models with SP extract tend towards red (CIEa*) and yellow 350 (CIEb*) in darker tones (CIEL*). These results may be due to the protein extraction method 351 with no significant differences between them: ALK CIELab* 57.70 \pm 0.30, 5.74 \pm 0.01, 20.99 352 \pm 0.07 and PUP CIELab* 60.36 \pm 0.04, 6.01 \pm 0.37, 22.2 \pm 0.41. The colour values obtained in the a* parameters may be due to the roasting of the SP for consumption, which may promote 353 354 Maillard reactions due to the presence of amino acids, sugars and proteins causing darkening 355 of the grasshoppers (Kinyuru et al., 2009). Another factor, which may enhance red and yellow 356 tones in insect extracts, is the oxidation of pigments such as melanin and primarily pheomelanin 357 (Kim et al., 2020; Urbina et al., 2021). The results of pH and colour coincide to those reported 358 by other authors who incorporated insect protein extracts such as Tenebrio molitor larvae or 359 silkworm Bombyx mori pupae in cooked emulsified products (Park et al., 2017; Kim et al., 360 2020). The total protein content in the sausages in the treatments T1, T2, T4 and T5 present no 361 significant difference (p>0.05) compared to the control. Treatments T3 and T6 with 15% meat 362 substitution present the highest percentage of total protein. The results obtained agree to 363 previous observations where an increase in the percentage of protein of *Bombyx mori* (Park et 364 al., 2017) and Tenebrio molitor (Choi et al., 2017b) in meat batters, and Acheta domesticus crickets in pork pate (Smarzyński et al., 2019). In addition, according to Ibarra-Herrera et al. 365 (2020) the Sphenarium purpurascens protein is considered highly digestible (85 to 90%) and 366 367 comparable to meat (89.6%), as well as having concentrations of essential and non-essential 368 amino acids comparable to egg.

369 Viscoelastic properties

The dynamic oscillatory rheology for the meat batters with ALK and PUP as meat substitutes 370 371 are shown in Fig 2. The moduli G' and G" for the different treatments present a frequency-372 dependent behaviour, where the elastic component (G') is above the viscous component (G'') 373 throughout the frequency interval (Fig. 2A-B), indicating the formation of ordered and elastic 374 gel structures (Li et al., 2020). This behaviour is characteristic of weak viscoelastic materials, 375 which tend to exhibit a solid-like behaviour where elasticity predominates over viscosity (Gibis 376 et al., 2017; Kim et al., 2022; Scholliers et al., 2020a). There are no studies in the literature 377 regarding the viscoelasticity of meat sausages using grasshopper Shepenarium purpurances 378 protein as meat extenders. However, the rheological behaviour obtained for the different 379 treatments of this research is in accordance with some other authors, which used other insects 380 in their studies. Scholliers et al. (2020a, 2020b) evaluated the effect of heating temperature (70 381 to 90 °C) on the gelation of different ratio solutions of Zophobas morio larvae protein and pork 382 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 383 384 slight frequency-dependence. Kim et al. (2022) compared rheological properties among 385 thermal-induced gels using porcine myofibril protein and five different edible insect species: 386 Tenebrio molitor L., Protaetia brevitarsis, Allomyrina dichotoma, Gryllus bimaculatus and 387 Oxyachinensis sinuosa, where samples exhibited solid-like behaviour, and G' was greater than 388 G" approximately at 50°C due to the formation of a rigid structure. In contrast, some authors 389 obtained different results in emulsified systems using Tenebrio molitor larvae as partial 390 substitutes for myofibrillar protein since the G' and G" moduli are not grouped between 391 treatments (Kim et al., 2020). The differences could be attributed because a meat matrix is more 392 complex in comparison to controlled systems in terms of pH, temperature, and protein 393 concentration. The control sample profiles were higher among treatments, indicating that SP 394 protein does not have the same capacity to form gels as meat protein. The replacement of meat with SP protein affects the apparent viscosity (η^*) of the cooked sausages, the η^* of the control 395 396 was higher in comparison with all treatments with SP protein. This could be explained, because 397 edible insect protein has the capacity of reducing water and fat binding capacities (Choi et al., 398 2017b; Kim et al., 2016; Kim et al., 2020). The control and all the treatments with SP protein 399 presented a thixotropic behaviour, with η^* values that decreased with increasing rotation time 400 (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 η^* than the control 401 402 when comparing sausages with 5 and 10% meat substitute (Choi et al., 2017b; Kim et al., 2020). 403 On the other hand, the η^* of the formulation with meat substitution of 5 and 10% PUP were 404 similar to that displayed by the control treatment (Fig. 2C), whereas all treatments with the 405 ALK method have approximately a viscosity 10-fold lower when compared to the control (Fig. 406 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).

413 Cooking loss and emulsion stability

414 Cooking loss (CL) and emulsion stability (ES) of the different meat models are shown in Fig. 415 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 416 417 control. The treatments with PUP extract (T1-T3) and the control showed significant 418 differences (p < 0.05) with the formulations with ALK extract T5 and T6. These treatments with 419 ALK extract showed that when increasing protein concentration in the formulations, the 420 cooking loss and pH of meat models (T3-T6) increased when compared to the control, but the 421 viscoelastic properties decreased with respect to control. Different results were reported by Park 422 et al. (2017) showed that the decrease of cooking loss for meat batter added with silkworm 423 powder has an inverse relation with pH and viscosity. Therefore, when CL decreases the 424 viscosity and pH of the meat batter are increased when compared to the control. Results are 425 similar to those reported by Choi et al. (2017b) that when substituting meat with at least 15% 426 protein from Tenebrio molitor, the pH and cooking loss increased. This behaviour was 427 explained due to the denaturation of the insect's built-in protein due to the drying process of the 428 insect, which could clarify that CL is not a factor dependent on the increase in pH. On the other 429 hand, the increased CL in sausages with ALK extract could be attributed to the loss stability of 430 the emulsion due to the decrease of myofibrillar protein or possibly because the grasshopper 431 protein has a higher proportion of hydrophobic groups that do not allow a good water absorption 432 causing an increase in the cooking loss. The results indicated that the ALK extract does not 433 have a high-water holding capacity, although some authors have reported that the cooking loss 434 is improved with the insect protein (Pintado et al., 2020). Torruco-Uco et al. (2019) reported 435 that Sphenarium purpurascens 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 436 437 and other insects or their extracts would be due to different protein contents and/or its different 438 extraction methods (Kim et al., 2017). On the other hand, PUP treatments showed a different 439 behaviour in comparison to ALK treatments, where an increasing meat substitution and high 440 pH in the meat model resulted in a decrease in cooking loss (T2 y T3) and these do not present 441 significant differences with the control (p>0.05). These results coincide with those reported by 442 Kim et al. (2016), Kim et al. (2017), Park et al. (2017) and Scholliers et al. (2020); that established that there is an inverse relation between CL with respect to a higher concentration 443 444 of insect and the pH, which is observed in mealworm larvae Tenebrio molitor, silkworm pupae 445 Bomboxy mori and Acheta domesticus. The reduction of CL in the treatments with PUP extract 446 could be due to the decrease of moisture, which could be explained by the increased solid 447 content which took place by replacing pork meat portion with grasshopper protein (Kim et al., 448 2017; Park et al., 2017). Also, the results obtained could indicate that the grasshopper protein 449 obtained by sonication method may have changes in its structure, such as surface charge and 450 exposure of hydrophilic or hydrophobicity groups present in the protein. These changes 451 contributed to improve the solubility and CL in comparison with ALK extract, making their 452 behaviour similar to the control (without substitution) (Mishyna et al., 2019; Su and Cavaco-453 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 emulsion stability (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 457 458 treatments and the control. These results indicate that the SP protein extract has functional 459 properties that help to stabilise the emulsion formed in the meat models, even at 15% meat 460 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. 461 (2016) observed no difference in the emulsifying capacity of the control with meat batters that 462 463 incorporated 10% of *Tenebrio molitor* and *Bomboxy mori* as a meat substitute. Finally, the SP 464 protein that was obtained by ultrasound method presented cooking loss and emulsion stability properties like the control. The results obtained can be attributed to the PUP method of 465 extraction that improved functional properties with ultrasound treatment, which can bind to 466 467 water and avoid cooking loss. Furthermore, some studies have shown that the ultrasound 468 method applied during pre-treatment of insect proteins such as Schistocerca gregaria, Apis 469 mellifera (Mishyna et al., 2019), Clanis Bilineata Tingtauica Mell (Wang et al., 2021), and 470 Hermetia illucens (Mintah et al., 2019) has different effects. For example, it modifies particle 471 size, solubility, increases sulfhydryl content, increases surface hydrophobicity and rheological 472 properties in proteins extracted due to its physical effects such as capillary surface waves and 473 acoustic cavitation. Also, Majzoobi et al. (2012) reported that higher protein solubility would 474 increase protein adsorption and protein migration rate when considering the water-oil interface, 475 thereby increasing the emulsion properties of proteins, which can cause a low loss due to 476 cooking.

477 *Texture*

The results of the texture profile analysis 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 482 authors who noted no difference in these parameters (Kim et al., 2016; Kim et al., 2020; Park 483 et al., 2017). The extraction method does not influence these parameters. Regarding the 484 firmness, only the treatments with higher percent of 15% substitution, with both extraction 485 methods (T3 and T6), did not show significant differences (p>0.05) with the control treatment. 486 By other hand, hardness was not significantly different (p>0.05) between treatments and the 487 control samples, except for T4 treatment. T4 had the lowest value of hardness, even when CL 488 and ES were significantly equal (p>0.05) to control. This behaviour in texture properties has 489 been described previously during the incorporation of *Tenebrio molitor* larvae flour in 490 frankfurters and emulsion systems, where concentrations increase from 10 to 20% caused the 491 decrease in hardness, springiness, cohesiveness, gumminess and chewiness (Choi et al., 2017b; 492 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 493 494 different meat substitutions. These results are in accordance with lower cooking loss, higher 495 emulsion stability, and higher viscoelastic properties, when compared to these characteristics in the ALK extract treatments. Wang et al. (2022) explained that the insect protein isolates when 496 497 submitted to high ultrasound power treatment (400 W), could present the unfolding of its 498 protein chains, resulting beneficial to the stability of the gel structure in meat and fluid-type 499 emulsions, favouring the development of stronger gel-like structure in the sausages. Contrary 500 to our observations, the gels obtained with PUP extract did not show differences with treatments 501 made with ALK extract. This behaviour could be explained by the presence of polyphenols in 502 the crude protein extracts obtained from SP. Some authors have demonstrated that defatted 503 flours obtained by A. dosmesticus, T. molitor, Z. morio, and R. ferrugineus (Botella-Martínez 504 et al., 2021) exhibit antioxidant activity, while Cuaxospa-Xolalpa (2021) found that extracts 505 from Sphenarium purpurascens 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 506

507 these results, it is possible that some phenolic compounds could be extracted under the 508 conditions used for the protein SP extraction, influencing the physicochemical properties, 509 including the gel-like structure, of myofibrillar protein (MP) in SP through both covalent and 510 noncovalent interactions (reversible or irreversible pathways) resulting in the blockage of 511 exposed hydrophobic sites, reducing the surface area, lowering the concentration of MP 512 available to interact in the formation of the gel-like structure and affecting the texture properties. 513 In addition, the lack of improvement on the texture properties in treatments where PUP extract 514 was used may be attributed to the increase in free SH groups content, and their prompt to be 515 attacked by the phenol ring structure (quinone) forming protein-quinone complexes, altering 516 the gelation capability of proteins, which is the most important texture property in meat 517 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.

524 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 532 were to the liking of consumers. It should be noted that no spices were added to the formulations 533 and the descriptors identified are associated with the SP extract. Fig. 5A shows the preference 534 map. Control was preferred by 42 consumers and had the highest percentage 43.75% and 40 535 consumers with a slightly lower percentage of 41.66% favoured PUP 5%. The sample least 536 preferred was PUP 10% with only 14 consumers that liked it (14.58%). Fig. 5B shows the 537 results of the hedonic scale used to find the overall liking of consumers. The control and PUP 538 5% showed no significant differences between them in the level of liking (p>0.05) but did show 539 a significant difference regarding PUP 10%, according to the Friedman test. In addition, the 540 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 541 542 of 3.229 (indifferent). The results obtained are similar to those obtained incorporating Tenebrio 543 molitor into sausages (Choi et al., 2017b) and crickets into pork pate (Smarzyński et al., 2019), 544 they observed that at higher substitution concentrations, acceptance was lower. Some authors 545 mention that the acceptability of products that incorporate insects is multifactorial; taste and 546 smell depend on the insects' pheromones, whose concentration in turn depends on the 547 environment where the insects feed and develop. The type of insect and its food can also affect 548 taste, as can the type of process the insect undergoes before or during incorporation into a food, 549 and the tradition of insect consumption in the region (Van Huis, 2020). Finally, regarding colour, 550 it is necessary to continue improving the extraction process, perhaps with the incorporation of 551 an enzyme complex that lessens the darkening of protein extracts, thus improving colour, a 552 highly important sensory attribute for consumers.

553 Conclusion

The alkalisation combined with ultrasound method improved techno-functional properties of the *Sphenarium purpurascens* (SP) protein in cooked meat models at meat substitution levels below 10%, equating to the control (100% meat) in physicochemical properties. The sensory

557 tests detected descriptors such as rancid smell and taste, seasoned and with herbal taste in the 558 PUP samples as well as a brown colour; these aspects can be attributed to the SP protein extract 559 since no colourants or spices were added to the formula. The hedonic scale and preference map 560 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 561 products. However, further work is recommended to incorporate different types of 562 563 hydrocolloids and spices that contribute to the formulation of a more acceptable product with 564 high benefit.

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Ingradiants (0/)	Treatments ¹							
ingreatents (%)	Control	T1	T2	Т3	T4	T5	T6	
Pork meat	50	47.5	45	42.5	47.5	45	42.5	
Grasshopper protein (ALK)*	-	-	-	-	2.5	5	7.5	
Grasshopper protein (PUP)**	-	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	

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

760 *PUP (Alkalisation-ultrasound extraction of grasshopper protein)

761 **ALK (Alkaline extraction of grasshopper protein)

762	¹ Control, sausages without meat substitution (50% pork meat + 0% grasshopper protein); T1,
763	sausages with 5% meat substitution (47.5% pork meat + 2.5% PUP); T2, sausages with 10%
764	meat substitution (45% pork meat + 5% PUP); T3, sausages with 15% meat substitution (42.5%
765	pork meat + 7.5% PUP); T4, sausages with 5% meat substitution (47.5% pork meat + 2.5%
766	ALK); T5, sausages with 10% meat substitution (45% pork meat + 5% ALK); T6, sausages
767	with 15% meat substitution (42.5% pork meat + 7.5% ALK).

	Parameters					
	Treatments ¹	Total protein ²	рН		Colour	
		(70)		L*	a*	b*
	Control	$13.13\pm0.41^{\rm A}$	$6.35\pm0.03^{\rm A}$	$74.25\pm3.50^{\mathrm{B}}$	$1.76 \pm 1.03^{\rm A}$	$13.18\pm0.11^{\rm A}$
	T1	$12.54 \pm 1.24^{\text{A}}$	7.21 ± 0.03^{B}	$59.48\pm4.54^{\rm A}$	$4.83\pm1.06^{\text{B}}$	18.40 ± 0.20^{B}
	T2	$13.13\pm0.41^{\rm A}$	$7.13\pm0.07^{\rm B}$	$56.56\pm3.97^{\rm A}$	$5.26\pm0.13^{\rm B}$	20.47 ± 0.10^{B}
	Т3	$14.88\pm0.41^{\rm B}$	$8.71\pm0.05^{\text{E}}$	$50.63\pm1.71^{\rm A}$	$5.36\pm0.22^{\text{B}}$	$20.49\pm0.13^{\rm B}$
	T4	$11.96\pm0.41^{\rm A}$	$7.50\pm0.04^{\rm C}$	$58.18\pm7.77^{\rm A}$	$5.11\pm0.75^{\text{B}}$	$20.92 \pm 1.41^{\text{B}}$
	T5	$13.13\pm0.41^{\rm A}$	$8.42\pm0.06^{\text{D}}$	$56.38 \pm 1.13^{\rm A}$	$5.22\pm0.31^{\rm B}$	$20.75\pm1.34^{\rm B}$
	T6	$15.17\pm0.83^{\rm B}$	$8.68\pm0.19^{\text{E}}$	$56.60\pm8.18^{\rm A}$	$5.36 \pm 1.14^{\text{B}}$	19.83 ± 2.44^{B}
779	¹ Control, saus	ages without me	at substitution	(50% pork meat	+ 0% grasshopp	per protein); T1,
780	sausages with	5% meat substi	tution (47.5% ₁	pork meat + 2.5%	PUP); T2, sau	sages with 10%
781	meat substituti	ion (45% pork m	eat + 5% PUP);	; T3, sausages wit	h 15% meat sub	stitution (42.5%
782	pork meat + 7	7.5% PUP); T4,	sausages with	5% meat substitu	tion (47.5% po	rk meat + 2.5%
783	ALK); T5, sau	usages with 10%	b meat substitu	tion (45% pork n	neat + 5% ALK	X); T6, sausages
784	with 15% mea	t substitution (42	2.5% pork mea	t + 7.5% ALK).		
785	² Kjeldahl N x	6.25				
786	All values are	mean ± standard	l deviation of th	nree replicates (n=	-9).	
787	Different letter	rs in the same co	olumn mean sig	nificant differenc	es between sam	ples at <i>p</i> <0.05.
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Table 2. Physicochemical parameters of meat models formulated with SP protein

	Texture Parameters					
Treatments ¹	Hardness	Adhesiveness	Brittleness	Elasticity	Cohesiveness	Firmness
	(Kg)	(mJ)	(Kg)	(mm)		(Kg)
Control	$1.07\pm0.20^{\rm B}$	0.26 ± 0.05^{AB}	$1.19\pm0.16^{\rm A}$	3.60 ± 0.13^{AB}	$0.80\pm0.05^{\rm A}$	$0.95 \pm 0.07^{\circ}$
T1	0.84 ± 0.03^{AB}	$0.37\pm0.08^{\rm B}$	$1.01\pm0.08^{\rm A}$	$3.66\pm0.02^{\rm B}$	$0.77\pm0.01^{\rm A}$	0.78 ± 0.05^{AB}
T2	0.87 ± 0.17^{AB}	0.33 ± 0.07^{AB}	$0.96\pm0.12^{\rm A}$	$3.61\pm0.02^{\rm AB}$	$0.77\pm0.04^{\rm A}$	0.74 ± 0.12^{AB}
T3	1.00 ± 0.16^{B}	$0.28\pm0.05^{\rm AB}$	$1.12\pm0.06^{\rm A}$	$3.42\pm0.01^{\rm A}$	$0.75\pm0.01^{\rm A}$	0.84 ± 0.06^{BC}
T4	$0.67\pm0.03^{\rm A}$	$0.25\pm0.02^{\rm A}$	$0.98\pm0.28^{\rm A}$	$3.73\pm0.02^{\rm B}$	$0.69\pm0.12^{\rm A}$	$0.62\pm0.02^{\rm A}$
T5	0.88 ± 0.01^{AB}	$0.25\pm0.01^{\rm A}$	$1.01\pm0.05^{\rm A}$	$3.71\pm0.18^{\rm B}$	$0.80\pm0.10^{\rm A}$	0.81 ± 0.14^{AB}
T6	0.95 ± 0^{AB}	0.27 ± 0.02^{AB}	1.11 ± 0.12^{A}	3.63 ± 0.02^{B}	$0.77 \pm 0.04^{\rm A}$	0.85 ± 0.05^{BC}

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

 $\overline{}^{1}$ 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

801 with 15% meat substitution (42.5% pork meat + 7.5% ALK).

802 All values are mean \pm standard deviation of three replicates (*n*=9).

803 Different letters in the same column means significant differences between samples at p < 0.05.

818 Figure Legends



Fig. 1. 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) ± the standard deviation. Different capital letters indicate significant differences (p < 0.05) with respect to time using ALK extraction. Different lowercase letters indicate significant differences (p < 0.05) with respect to time using PUP extraction. Asterisk (* or **) indicates significant differences (p < 0.05) between treatments ALK and PUP evaluated at the same time.

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Fig. 2. Dynamic oscillatory rheology of meat models with grasshopper protein extracted by 830 831 PUP (A, C) or ALK (B, D). Storage modulus G' and loss modulus G" (Pa) and complex viscosity η^* (Pa s). Control, sausages without meat substitution (50% pork meat + 0%) 832 grasshopper protein); T1, sausages with 5% meat substitution (47.5% pork meat + 2.5% PUP); 833 834 T2, sausages with 10% meat substitution (45% pork meat + 5% PUP); T3, sausages with 15% 835 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); 836 837 T6, sausages with 15% meat substitution (42.5% pork meat + 7.5% ALK).

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841 Fig. 3. Emulsion stability and cooking loss of meat batters formulated with various levels of 842 soluble protein extracts from SP extracted by ALK or PUP. Control, sausages without meat 843 substitution (50% pork meat + 0% grasshopper protein); T1, sausages with 5% meat 844 substitution (47.5% pork meat + 2.5% PUP); T2, sausages with 10% meat substitution (45% 845 pork meat + 5% PUP); T3, sausages with 15% meat substitution (42.5% pork meat + 7.5% 846 PUP); T4, sausages with 5% meat substitution (47.5% pork meat + 2.5% ALK); T5, sausages 847 with 10% meat substitution (45% pork meat + 5% ALK); T6, sausages with 15% meat 848 substitution (42.5% pork meat + 7.5% ALK).

- All values are represented as the mean value and the vertical bars show the standard deviation
- 850 of three replicates (n=9).
- 851 Different letters mean significant differences between treatments for each variable at p < 0.05.
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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.



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

867 the Liking level indicate significant differences between treatments (p < 0.05).