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Insect protein concentrates from Mexican edible insect: structural and functional characterization

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Abstract

Industrial valorization of proteins from traditional edible insects requires knowledge of their nutritional, chemical, technological characteristics. Therefore, these characteristics in two Mexican edible insects (*A. cordifera* and *B. mellifica*) were studied and compared to commercial insect species (*H. illucens*). The nutritional attributes of insect protein concentrates (PCs) were determined by their protein content, amino acid and polyphenol compositions. The chemical structure of the proteins was determined by Fourier-Transform Infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). Moreover, the surfactant properties of insect-PC (emulsions) were evaluated. *B. mellifica* PC showed the highest content of protein content among the studied insects (57.1%). In contrast, *H. illucens* showed the highest polyphenols (12.7 mg/g). The emulsions prepared with insect-PC from *A. cordifera* and *B. mellifica* showed higher stability than *H. illucens*, where a bigger droplet size and a rapid phase separation occurred. The decrease in emulsification properties of black soldier fly larvae was attributed to a possible interaction between polyphenols and proteins that hinders emulsification.

This study increases the understanding of the structural and techno-functional characteristics of insect-PC of commercial and local insect species.

Keywords: Novel proteins, novel food ingredient, *Edessa cordifera, Hermetia illucens, Brachygastra mellifica*

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1. Introduction

Insect consumption is a traditional practice for several population groups. Mexico stands out as the country with more than 500 identified edible insect species (Ramos-Elorduy, Pino Moreno, & H., 2012; Ramos-Elorduy & Viejo Montesinos, 2007). Therefore, entomophagy is a culturally accepted habit in Mexico, as evidence by the fact that several insect species are included in gastronomic dishes. These edible insects are consumed with minimal processing, as is the case in most countries with a long history of entomophagy. Although the nutritional value of these traditionally consumed species has been reported, information concerning the functionality of their major nutrients, such as proteins and fats, is scarce in the scientific literature.

Concerns about the environmental impact of livestock production, sustainability, and food security have promoted the inclusion of insects as a sustainable protein source for food and feed (Sedaghat Doost, Van Camp, Dewettinck, & Van der Meeren, 2019). Insects contain nutritional compounds such as proteins, ω-3 fatty acids, vitamins, and minerals valuable for human food and animal feeding (for livestock, aquaculture, poultry, and pets, among others) (Tzompa-Sosa & Fogliano, 2017; Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014). This new protein source triggered the establishment of several insect rearing companies located mainly in Europe and in North America. These enterprises have produced insect flours primarily from six insect species, namely black soldier fly larvae (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus spp*.) (Derrien, 2017) for their use as food and feed.

Insect protein concentrates (PC) are not yet available as a commercial food ingredient, but its use is the next step in the valorisation of insects. Therefore, it is necessary to explore the nutritional value and functionality of the PC from commercially reared insect species as well as of other traditional edible insect species with no industrial production, but with potential for industrial valorisation.

Proteins are amphipathic molecules formed by polar and nonpolar groups, providing surfactant activity. These surfactant compounds decrease the interfacial tension between hydrophilic and

hydrophobic immiscible phases enabling protein interactions with the hydrophobic side and water (Kralova & Sjöblom, 2009). Biosurfactants such as proteins are widely used as functional ingredients in several industrial sectors such as food and pharmaceutical, since they stabilize these systems (Foegeding, 2015). For instance, in an O/W emulsion, dispersed oil droplets are formed when the unfolded proteins coat them at the interface by exposing their hydrophilic groups towards the continuous phase (Sedaghat Doost, Dewettinck, Devlieghere, & Van der Meeren, 2018). This protein interfacial layer forms an energy hindrance, preventing coalescence by an electrostatic stabilization mechanism (depending on pH and ionic strength), leading to the desired stability (Dickinson, 2009). The structural characteristics, such as, conformation, charge, and hydrophobicity define the functionality of proteins in complex food systems (Delahaije, Wierenga, Giuseppin, & Gruppen, 2015). These structural characteristics depend on intrinsic (composition of protein) and extrinsic (extraction parameters) factors causing changes among protein. Therefore, it is relevant to investigate the structural characteristics of novel protein sources to identify potential sources of proteins with desirable functionalities in food.

The aim of this study was to extract PC from two Mexican edible insect species, namely adult jumil also known as stinkbug (*Ascra cordifera,* previously classified as *Edessa cordifera*), and wasp larvae (*Brachygastra mellifica).* We studied their protein structure and functionality (emulsifying capacity) and compared it with that of black soldier fly larvae (BSF) PC. This insect was used as a reference since it has been widely studied and is the most produced insect species in Europe (Caligiani et al., 2018; Derrien, 2017; Janssen, Vincken, van den Broek, Fogliano, & Lakemond, 2017). The nutritional composition of jumil and wasp larvae has been studied before (Ramos-Elorduy et al., 1997; Ramos-Elorduy, Pino Moreno, & Marquez Mayaudon, 1984). However, the structural characteristics of their protein concentrate and its functionality have not been investigated.

2. Materials and methods

2.1. Materials

Adult jumil and wasp larvae were purchased in a local market in Guerrero and Yucatán (states), Mexico, respectively. ProEnto S.A.P.I. de C.V. (Queretaro, Mexico) reared larvae of BSF and kindly provided them for this study. Polyphenol standards (β-carotene, caffeic acid, chlorogenic acid, epicatechin, gallic acid, kaempferol, and p-coumaric acid) were purchased fromSigma-Aldrich (St. Louis, MO) and they were used to prepared stock and calibration curve solutions.

2.2. Proximate analysis of the whole insect

Protein content analysis was performed by the micro-Kjeldahl method according to Ergan, H *et al.* (1981). A nitrogen-to-protein conversion factor of 4.76 was used (Janssen *et al*., 2017). Fat content analysis was performed using the Weibull method (Egan *et al*., 1981). The total dietary fiber (TDF) content was determined by the enzymatic and gravimetric method using the reagent kit Bioquant® (Merck, Germany) according to the official method from the German federal office of consumer protection and food safety (Amtliche Sammlung von Untersuchungsverfahren, 1997). Ash content was determined gravimetrically by drying and incinerating the samples at 525 °C overnight. All analyses were performed in triplicate.

2.3. Insect PC extraction and characterization

2.3.1. Insect-PC extraction

Insects (adult jumil, wasp larvae, and BSF larvae) were dried in a freeze-dryer (Labconco, FreeZone Triad Cascade Benchtop) and ground into a very fine powder using a Moulinex grinder (AR110830, Belgium). This flour was defatted twice by mixing with hexane (in 1:10 ratio) for two hours. Afterward, the dispersion was centrifuged at 4911 *g* for 30 min (20 °C), the supernatant was discarded and the pellet was kept in the fume hood until the hexane was completely evaporated. The defatted flour was dissolved in a 0.05 M NaOH solution (pH 12) at a 1:3 ratio and stirred for six h at 20 °C. This solution was centrifuged at 3978 g at 20°C for 20 min (Hettich, Rotina 380R, Tuttlingen, Germany). The soluble proteins in this solution were recovered from the supernatant by acid precipitation at pH 4 using a 0.1 M HCl solution. The obtained insect-PC was washed three times using demineralized water before

freeze-dried. The extracts were kept at -20°C until further characterization. Three extractions per insect species were performed.

2.3.2. Protein content

The crude protein content of insect-PC was determined by the Kjeldahl method as described above. The determination was performed on the three extractions per insect species. The nitrogen-to-protein conversion factor used for insect-PC was 5.6 as suggested by Janssen, et al., (2017).

2.3.3. Structural characterization

Structural characterization of insect-PC was performed by FTIR. FTIR data of insect-PC were recorded in the region between 400-4000 cm-1 with an Agilent Cary 630 spectrophotometer coupled with ATR ZNSe. The absorbance spectra were analyzed using Microlab PC software (Agilent Technologies Inc., Santa Clara, CA, USA). The determination was performed on the three extractions per insect species.

2.3.4. Amino acid profile of insect-PC

Protein was hydrolyzed according to the EC (CommisionDirective98/64/EC, 1988). Basic hydrolysis was performed according to AOAC Official Method 988.15 (AOCS, 1988). Primary amino acids were derivatized with ortho-phtaldialdehide (OPA) and the secondary amino acids (such as proline) were derivatised using 9-fluorenylmethyl chloroformate (FMOC); hence, two-excitation and emission wavelengths were used (from 16.3 to 25 min for secondary amino acids). The amino acids were then profiled according to (Henderson, Ricker, Bidlingmeyer, & Woodward, 2000; Schuster, 1988) using an HPLC with an autosampler (Agilent 1100 series, Santa Clara, CA, USA) coupled with a Fluorescence detector (Agilent 1200 series, Santa Clara, CA, USA) as described by (Kerkaert et al., 2011). The separation was performed on an Agilent ZORBAX Eclipse Plus C18 column (95Å, 4.6 x 150 mm, 3.5 µm) with precolumn. Mobile phase A consisted of phosphate/borate buffer at pH 8.2. Mobile phase B consisted of acetonitrile/methanol/water (45/45/10, v/v/v). A gradient elution, at a flow rate of 1.5 mL/min, was performed. The determination was performed on the three extractions per insect species.

135 **2.3.5. Thermal stability of insect-PC**

136 The denaturation behavior of the dry PC was evaluated with a differential scanning calorimeter (DSC)
137 (TA Q1000, TA Instruments, New Castle, USA) with a refrigerated cooling system. The DSC was
138 calibrated with indium (TA Instruments, New Castle, USA), azobenzene (Sigma– Aldrich, Bornem,
139 Belgium) and undecane (Acros Organics, Geel, Belgium). Nitrogen was used to purge the system. The
140 sample weighed into an aluminum pan (Tzero pan; TA Instruments Ltd., New Castle, USA) was adjusted
141 depending on the amount of crude protein per sample and was set to 2.9 ± 0.1 mg of protein. The
142 aluminum pan was hermetically sealed with an aluminum lid (Tzero lid; TA Instruments Ltd., New
143 Castle, USA) and weighed. As a reference, an empty sealed pan was used. The applied time-
144 temperature program was as follows: equilibration a 20°C for 1 min and heating from 20 °C to 180 °C
145 at 2 °C/min. The denaturation process was quantified by the thermal transition midpoint (Tm) and
146 enthalpy of unfolding (ΔH). The analysis was performed on the three extractions per insect species.

147 **2.3.6. Polyphenols profile of insect-PC**

148 Insect-PC was prepared by dissolving 10 mg of PC in 1 mL demineralized water. Afterward, samples
149 were filtered through a 0.2 µm nylon syringe filter before injection. Liquid chromatography–
150 electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) (Waters, Mildford, MA, USA) was
151 used for the identification and quantification of polyphenols. A Quattro Premier XE triple quadrupole
152 mass spectrometer fitted with ESI source was used in positive mode for, epicatechin, and kaempferol.
153 In contrast, the negative method was used for caffeic acid, chlorogenic acid, epicatechin gallate, gallic
154 acid, and p-coumaric acid. Mass spectrometer optimization was conducted by direct infusion of 50
155 mg/L stock standard solutions to select the ionization mode and precursor and ions production. An
156 Acquity UPLC BEH C18 (2.1 x 100 mm, 1.7 µm) column was used for compounds separation. Mobile
157 phases were 0.1 % (v/v) formic acid in demineralized water (A) and 0.1 % (v/v) formic acid in methanol
158 (B). A flow rate of 0.45 mL/min and an injection volume of 5 µL were used. Total run time of 14.5 min

with 99 % A at 0.5 min, 20 % A at 13.5 min, and 99 % A at 14 min. The column was maintained at 40 °C. Samples were performed on the three extractions per insect species.

2.3.7. Emulsifying capacity and characterization of insect –PC emulsions

The emulsifying characteristics of the extracted insect-PC was performed in a pool sample of the extracts. Insect -PC solutions (0.4 %) were stirred overnight prepared in sodium phosphate buffer (pH 8, 0.1 M). These PC solutions were mixed with 4% tricaprylin oil (surfactant to oil ratio of 0.1) using an Ultra-Turrax (Ultra-Turrax, type S 50N – G 45 F, IKA®-Werke, Germany) for 2 min at 24000 rpm. The pre-emulsified mixtures were further passed through a Microfluidizer (M110-S, Microfluidics Corp., Newton, MA) 10 times at 84 MPa at 20 °C cooled down by placing the heat exchanger coil into a water bath.

The volume-weighted mean droplet diameter (d43) was determined using a Malvern Mastersizer (Model 3000 Hydro MV, Malvern Instruments, UK). The samples were measured at a refractive index of 1.47 for oil droplets and 1.33 for demineralized water with an absorption coefficient of 0.01. A few droplets of the emulsion were drizzled into a wet dispersion unit to reach an obscuration value between 5-10%. The measurements were performed on days 0, 1 and 9. Each measurement was the average of three replicates. For determination of accelerated emulsion stability, the Lumifuge® 116 (LUM GmbH, Germany) analyzer with a CCD-line sensor was used. Measurements were performed at room temperature (20°C) during centrifugation at 786 g for 40 min. The creaming rate was calculated using Sedaghat Doost, Sinnaeve, De Neve, and Van der Meeren (2017) method. Moreover, emulsion stability was visually followed until day 9. The zeta potential (ζ) of the emulsion droplets (was determined using a Zetasizer 2c (Malvern Ltd, UK) by measuring the electrophoretic mobility, using the Helmholtz-Smoluchowski approximation. The emulsions were diluted with phosphate buffer at pH 8 before being analyzed. The reported values are the mean of three repetitions.

2.4. Statistical analysis

Analysis of variance was carried out on the components of the entire insect, the protein content of insect-PC, polyphenols and emulsion capacity characteristics. Assumptions of normality and equality of variance were tested prior to the analysis. Where assumptions were fulfilled, a post-hoc Tukey's test was used to investigate significant differences among insect species. A significance level of 5% was used in this study. ANOVA was performed using SPSS Statistics 26.

3. Results and discussion

3.1. Proximate analysis of whole insects

The proximate analysis of the whole insects and insect-PC are summarized in table 1. Wasp larvae showed the highest crude protein content in whole insects and insect-PC followed by BSF larvae and jumil. The protein content found for the whole insects in this study is lower than reported values in the literature, probably because in this study a corrected protein conversion factor was used (4.76), which is lower than the typical protein conversion factor. In case of wasp larvae, the reported protein content was around 70 % (Ramos-Elorduy et al., 1984) and for BSF larvae and two stink bug species (*Atizies taxcoensis A* and *Fuchistus sufultus S*) it ranged between 39-43 % and 40-43 % respectively (Melo-Ruiz, 2004; Spranghers et al., 2017).

Table 1

We found that the fat content of the whole insects was significantly higher for jumil (42.4%) compared to BSF larvae (24.3 %) and wasp larvae (14.3%). These values were also higher than other stink bug species previously reported (~31-32 %) and most reared insect species (Tzompa-Sosa et al., 2014). This fat fraction can be valorised in several applications like food, feed, biodiesel, among others. The total dietary fiber content (water-soluble and -insoluble) for all the evaluated insects ranged from 20.6% to 25.5%. These amounts corresponded to values reported for BSF larvae fed with chicken feed (Spranghers et al., 2017). In the case of wasp larvae, lower fiber contents for Edessa genus stinkbugs (11-18%) were reported by (Ramos-Elorduy et al., 1997). This difference in the reported values may be related to the fact that it is based on one type of fiber (crude fiber) and not on the total dietary fiber,

209 which refers to both water-soluble and -insoluble types. To our knowledge, this is the first time that a
210 proximate analysis is carried out specifically on jumil.

211 **3.2. Insect-PC characterization**

212 **3.2.1. Amino acids profile of insect-PC**

213 The amino acid profile of the three insect species studied is shown in table 2. The amount of negatively
214 charged amino acids was higher in jumil-PC and wasp larvae-PC (~ 180 mg/g) compared to BSF (~140
215 mg/g). Cysteine, a sulfur-containing amino acid, was not detected in jumil-PC or in BSF larvae-PC. For
216 wasp larvae-PC, cysteine was present at low amounts and no detected in BSF and jumil. Other studies
217 have also reported low concentrations of cysteine (<1 mg/g of protein) or its absence in BSF larvae
218 (Firmansyah & Abduh, 2019; Janssen et al., 2017). Even though cysteine is a non-essential amino acid,
219 it is listed by FAO/WHO as one of the required amino acids of adults and children. Cysteine and
220 methionine are listed in the same category, as both are sulfur amino acids. From this point of view,
221 despite the fact that cysteine is not present, the methionine content in BSF larvae-PC (21 mg/g) and
222 jumil-PC (18.5 mg/g) was similar to the sulfur amino acids recommendation for adults (22 mg/g) (FAO,
223 2011).
224 The total content of essential amino acids in the insect-PC in the present study ranged from 306 mg/g
225 to 329 mg/g. These values were similar to other insect species, namely banded cricket (Gryllodes
226 sigillatus) and desert locust (Schistocerca gregaria) (300 mg/g and 311 mg/g, respectively). Lilian, N.S.
227 et al. (2017) found a slightly higher essential amino acid content for BSF larvae (365 mg/g). Moreover,
228 the total content of essential amino acids in jumil-PC and wasp larvae -PC was higher than that
229 reported in *T. molitor* (231 mg/g) (Azagoh et al., 2016), which is another commercial insect species
230 valorised as food and feed (Tang et al., 2019). To our knowledge, no values have been reported for
231 jumil and wasp larvae species either in the entire insect or in their protein concentrate. The
232 concentration of essential amino acids in all three species corresponds to the values established for a
233 good nutritional quality of proteins, which is 260 mg/g (FAO/WHO/UNU, 2007). Previous studies have
234 shown that the insect feed has a substantial effect on the amount of crude protein and the amino acid

content found in insects (Oonincx, van Broekhoven, van Huis, & van Loon, 2015). In our study, both jumil and wasp larvae insects were purchased in a local market (wild and naturally feeding), whereas BSF larvae were reared under controlled conditions within an industrial facility (controlled feeding using fodder). Therefore, it is likely that the total protein content of jumil and wasp larvae could increase with a formulated diet, which could possibly affect its amino acid composition. However, this possibility is yet to be explored in these species. From the functional point of view, it has been observed two main phenomena involve in specific for emulsifying properties and emulsions stability. On the one hand, the amino acids composition, which determines not only the formation of the secondary structure but also the protein surface charge. The exposition of buried hydrophobic groups enhances the interaction with the oil phase and negatively charged amino acids with the increment of electrostatic repulsion between oil droplets which avoids the coalescence (Sedaghat Doost, Nikbakht Nasrabadi, et al., 2019). In our case, no significant differences in the number of hydrophobic amino acids in insect protein concentrates were found (~ 300mg/g). However, glutamic and aspartic acid (negatively charged amino acids) were higher in jumil and wasp (180 and 179 mg/g) than in BSF (166 mg/g). Secondary structures, mainly (α-helices and β-sheets), have also been related to emulsions stability. Emulsions stability using soy protein have shown a negative correlation with the presence of higher α-helices and vice versa (Zhao, Dong, Li, Kong, & Liu, 2015). Some amino acids propensity for these structures conformation, jumil and wasp larvae showed more β-structure formers amino acids (Gln, Ser, Tyr, and Trp) than BSF (Fujiwara, Toda, & Ikeguchi, 2012).

Table 2

3.2.2. Structural characterization of insect-PC by FTIR and DSC

The secondary structure of insect-PC observed by FTIR is shown in Figure 1. In BSF larvae –PC, we found higher intensity peaks for amide I (C=O stretching bonds), and amide II (N-H bonds) (wavelength 1700-1600 cm-1 and 1600-1500 cm-1, respectively), in comparison to jumil and wasp larvae-PC. β-sheets, α-helices and random coil structures represent the amide I region, which are stabilized by hydrogen

bonds (Chirgadze, Brazhnikov, & Nevskaya, 1976). Hence, a highly preserved secondary structure may be present in BSF larvae protein samples. Additionally, this PC showed more pronounced absorbance peaks in the ranges of ~3000-2960 cm-1 (which could represent C-H vibrations from aromatic rings, alkenes and alkanes) and 2934-2950 cm-1 related to aromatic, aliphatic, and charged amino acids (Howell, Arteaga, Nakai, & Li-Chan, 1999). It is known that the secondary protein structure is mainly stabilized by hydrogen bonds (Pace et al., 2014). Therefore, reduced absorbance peaks at these regions (amide I and II) could be denoting some protein unfolding. α-helices and β-sheets have been reported to improve the interfacial interactions with non-polar compounds forming intermolecular structures. However, it is important to expose these structures by unfolding the proteins (total or partially) to allow molecular interactions (Howell, Herman, & Li-Chan, 2001). During the heating of insect proteins, enthalpic peaks were observed for the studied samples (Figure 2). A lower protein denaturation temperature for wasp larvae-PC and jumil -PC (76 and 82 °C, respectively) was found compared to BSF, i.e., 94 °C. Although that there are no values reported for the same type of protein used in this study, similar values (73-78 °C) are shown for some plant (pea, 72 °C) and animal (egg white and whey protein ~82 °C) proteins (Martin, Nieuwland, & de Jong, 2014). Higher enthalpy values were also found for BSF larvae-PC (219 J/g) as compared to jumil-PC and wasp larvae-PC (195 J/g). The plausible cause for this observation is that a higher number of hydrogen bonds were broken in BSF larvae during protein unfolding than the other insect species. This effect was also confirmed by FTIR as BSF larvae- PC showed more hydrogen-bond stabilized structures (amide I region) reflected by higher enthalpies. Moreover, highly stable conformations in BSF larvae protein could also be related to the higher denaturation temperature observed. In contrast with the other two species under study, wasp larvae-PC showed a second enthalpic peak at 130°C, which could be related to a second protein fraction being denatured at higher temperatures.

3.2.3. Polyphenol profile in insect-PC

The polyphenol profile of insect-PC is shown in table 3. BSF larvae-PC showed the highest content of total polyphenols (~12.8mg/g) in comparison to wasp larvae-PC (~10 mg/g) and jumil-PC (~3.9 mg/g).

The main difference was seen in the content of epicatechin gallate (~11.1 mg/g) where the difference between jumil-PC and BSF larvae-PC was almost seven-fold. Catechins are flavanols (a type of flavonoid) normally present in tea, chocolate, and red wine. They have excellent scavenging capacity due to their phenolic hydroxyl groups (Harbowy & Balentine, 1997). Moreover, polyphenols interact with proteins via covalent and non-covalent bonds (Frazier et al., 2010; Stojadinovic et al., 2013). This interaction is possible since structural elements in proteins such as free amino acids, thiols, and specific amino acid residues (tryptophan) act as reactive sites for phenolic compounds, which are enhanced by extrinsic factors such as pH, temperature, or oxygen. These interactions between proteins and phenolic compounds could be affecting the conformation of proteins in our insect-PC and, thus, some functional properties such as solubility and hydrophobicity (Rawel, Czajka, Rohn, & Kroll, 2002; Yuksel, Avci, & Erdem, 2010). Previous studies demonstrated the disturbance of intermolecular beta-sheet hydrogen bonds of β-Lactalbumin in the presence of epigallocatechin-3-gallate (Hasni et al., 2011; Zorilla, Liang, Remondetto, & Subirade, 2011). They observed that when these interactions were present, changes in casein protein structure occurred (lower α-helix and β-sheet) due to tea polyphenols, specifically by flavanols.

Table 3

3.4. Emulsifying capacity

The physical stability of the emulsions stabilized by insect proteins was examined based on the zeta-potential, creaming rate (CR), and droplet size. Larger negative zeta potential was obtained for jumil-PC emulsions as compared to wasp larvae and BSF larvae (Table 4). On the other hand, emulsions prepared with BSF larvae had a larger droplet size and higher creaming rate, resulting in lower physical stability. This could be related to the differences in the superficial features, as systems with enough electrostatic repulsion (usually indicated by ζ potential values below −30 mV) have less probability of suffering from coalescence. Emulsions stabilized with jumil-PC and wasp larvae-PC indeed had a more negative zeta-potential, than emulsions stabilized with BSF larvae-PC.

Table 4

Although the average particle size of jumil and wasp larvae-PC-stabilized emulsions was similar (monomodal distribution) at day 0, after nine days, bigger droplets were found in wasp larvae emulsions (1.87 µm) as compared with jumil (1.14 µm). In jumil emulsions, the droplet size reached a maximum size after one day and remained unchanged during the next nine days. However, phase separation was seen after nine days of storage (Figure 3). BSF larvae-PC stabilized emulsions showed a bimodal particle size distribution being unstable within an increase in droplet size after 24 h storage and doubling its size after nine days of storage (Table 5). Moreover, phase separation was observed already two hours after preparation.

Previous studies have shown that smaller particle size and homogeneous distribution can be obtained from insect-PC from yellow mealworm (*T. molitor)* compared with whey protein (Gould & Wolf, 2018). This difference can be related to the intrinsic structural differences of yellow mealworm protein or the difference in emulsion preparation. Gould and Wolf (2018) used higher protein concentrations in their study (0.44, 088, 1.75, and 2.63 %) than our study concentrations. Although the emulsion properties vary depending on the parameters used, it is relevant to note that in both studies, we were able to create stable emulsions from insect-PC.

The fact that jumil-PC resulted in the most stable emulsion during storage could be due to the compositional and structural characteristics of the protein. Hereby, the stability results corresponded well with the zeta-potential values. Jumil insect-PC contained more negatively charged amino acids conferring repulsive forces contributing to the stability of the system. In BSF larvae-PC, the presence of a higher concentration of polyphenols (mainly epicatechin gallate) could have resulted in more interactions between proteins and polyphenols impairing the amino acid's interactions in the oily-phase.

4. Conclusions

In this study, the chemical and structural characteristics and emulsifying capacity of insect-PC of two Mexican edible insects (wasp larvae and jumil) and one reared insect (*H. illucens*) were studied. Wasp

larvae showed the highest crude protein content in whole insects and insect-PC, whereas the protein content of jumil was comparable with that of BSF larvae. All three PC obtained from these insects were considered to have a good nutritional quality since their concentration of essential amino acids was within the recommendation established by the FAO/WHO for children. The emulsions stabilized by PC from jumil-PC and wasp larvae-PC showed higher physical stability than those from BSF larvae-PC. In the latter case, larger droplet size and phase separation were observed two hours after preparation. Furthermore, O/W emulsions produced with jumil-PC showed the highest physical stability after nine days of storage. This could be explained by the abundance of negatively charged amino acids compared with the other two species in this study. This type of amino acids confers repulsive forces contributing to the stability of the system.

To our knowledge, this is the first time that the nutritional quality and the techno-functional characteristics of jumil and wasp larvae protein concentrates have been studied. Moreover, we increase the knowledge of BSF larvae-PC. Although BSF is the most important commercial insect species, knowledge of its PC functionality is scarce. The increased understanding of the structural and techno-functional characteristics of commercial and local insect species could stimulate their valorization. Moreover, the study of non-commercial insect species is necessary to broaden our knowledge of insects with potential industrial valorization.

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363 **References**

364

365 Amtliche Sammlung von Untersuchungsverfahren, B. (1997). Enzymatic-gravimetric method
366 for the determination of Total Dietary Fiber in foods, L.00.00-18 § 35 LMBG.
367 Darmstadt, Germany.
368 AOCS. (1988). AOAC 988.15-1988, Tryptophan in Foods and Food and Feed Ingredients.
369 Azagoh, C., Ducept, F., Garcia, R., Rakotozafy, L., Cuvelier, M. E., Keller, S., . . . Mezdour, S.
370 (2016). Extraction and physicochemical characterization of Tenebrio molitor proteins.
371 *Food Research International, 88*, 24-31. doi: 10.1016/j.foodres.2016.06.010
372 Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S.
373 (2018). Composition of black soldier fly prepupae and systematic approaches for
374 extraction and fractionation of proteins, lipids and chitin. *Food Research International,*
375 *105*, 812-820.
376 Chirgadze, Y. N., Brazhnikov, E. V., & Nevskaya, N. A. (1976). Intramolecular distortion of the
377 alpha-helical structure of polypeptides. *J Mol Biol, 102*(4), 781-792. doi: 10.1016/0022-
378 2836(76)90291-6
379 CommisionDirective98/64/EC. (1988). Methods of analysis for the determination of
380 aminoacids, crude oils and fats, and olaquindox in feedingstuffs and amending
381 Directive 71/393/EEC (pp. L257/214-L257/228).
382 Delahaije, R. J. B. M., Wierenga, P. A., Giuseppin, M. L. F., & Gruppen, H. (2015). Comparison
383 of Heat-Induced Aggregation of Globular Proteins. *Journal of Agricultural and Food*
384 *Chemistry, 63*(21), 5257-5265.
385 Derrien, C. (2017). *Insects – from curious to serious business – Status of the European industry*
386 *and development of the Law*. Paper presented at the IPIFF 2017 International
387 Conference, Brussels, Be.
388 Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids,*
389 *23*(6), 1473-1482.
390 Egan, H., Kirk, R. S., Sawyer, R., & Pearson, D. (1981). *Pearson's chemical analysis of foods* (8th
391 ed ed.). Edinburgh ; New York: Churchill Livingstone.
392 FAO. (2011). *Dietary protein quality evaluation in human nutrition: Report of an FAO Expert*
393 *Consultation*. Auckland, New Zealand: Food and Agriculture Organization of the
394 United Nations.
395 FAO/WHO/UNU. (2007). Protein and amino acid requirements in human nutrition *WHO*
396 *Technical Report Series 935* (pp. 265). Geneva, Switzerland: FAO/WHO/UNU
397 Firmansyah, M., & Abduh, M. Y. (2019). Production of protein hydrolysate containing
398 antioxidant activity from Hermetia illucens. *Heliyon, 5*(6). doi: ARTN e02005
399 10.1016/j.heliyon.2019.e02005
400 Foegeding, E. A. (2015). Food Protein Functionality-A New Model. *Journal of Food Science,*
401 *80*(12), C2670-C2677.
402 Frazier, R. A., Deaville, E. R., Green, R. J., Stringano, E., Willoughby, I., Plant, J., & Mueller-
403 Harvey, I. (2010). Interactions of tea tannins and condensed tannins with proteins.
404 *Journal of Pharmaceutical and Biomedical Analysis, 51*(2), 490-495. doi:
405 10.1016/j.jpba.2009.05.035
406 Fujiwara, K., Toda, H., & Ikeguchi, M. (2012). Dependence of α-helical and β-sheet amino acid
407 propensities on the overall protein fold type. *BMC structural biology, 12*, 18-18. doi:
408 10.1186/1472-6807-12-18

409 Gould, J., & Wolf, B. (2018). Interfacial and emulsifying properties of mealworm protein at the
410 oil/water interface. *Food Hydrocolloids, 77*, 57-65. doi:
411 10.1016/j.foodhyd.2017.09.018
412 Harbowy, M. E., & Balentine, D. A. (1997). Tea chemistry. *Critical Reviews in Plant Sciences,*
413 *16*(5), 415-480. doi: Doi 10.1080/713608154
414 Hasni, I., Bourassa, P., Hamdani, S., Samson, G., Carpentier, R., & Tajmir-Riahi, H. A. (2011).
415 Interaction of milk α- And β-caseins with tea polyphenols. *Food Chemistry, 126*(2), 630-
416 639. doi: 10.1016/j.foodchem.2010.11.087
417 Henderson, J. W., Ricker, R. D., Bidlingmeyer, B. A., & Woodward, C. (2000). Rapid, Accurate,
418 Sensitive, and Reproducible HPLC Analysis of Amino Acids. *Application note 5980-*
419 *1193E Agilent Technologies*.
420 Howell, N. K., Arteaga, G., Nakai, S., & Li-Chan, E. C. Y. (1999). Raman spectral analysis in the
421 C-H stretching region of proteins and amino acids for investigation of hydrophobic
422 interactions. *Journal of Agricultural and Food Chemistry, 47*(3), 924-933. doi: DOI
423 10.1021/jf981074l
424 Howell, N. K., Herman, H., & Li-Chan, E. C. Y. (2001). Elucidation of protein-lipid interactions
425 in a lysozyme-corn oil system by Fourier transform Raman spectroscopy. *Journal of*
426 *Agricultural and Food Chemistry, 49*(3), 1529-1533. doi: 10.1021/jf001115p
427 Janssen, R. H., Vincken, J. P., van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M.
428 (2017). Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio
429 molitor, Alphitobius diaperinus, and Hermetia illucens. *Journal of Agricultural and*
430 *Food Chemistry, 65*(11), 2275-2278. doi: 10.1021/acs.jafc.7b00471
431 Kerkaert, B., Mestdagh, F., Cucu, T., Aedo, P. R., Ling, S. Y., & De Meulenaer, B. (2011).
432 Hypochlorous and peracetic acid induced oxidation of dairy proteins. *J Agric Food*
433 *Chem, 59*(3), 907-914. doi: 10.1021/jf1037807
434 Kralova, I., & Sjöblom, J. (2009). Surfactants used in food industry: a review. *Journal of*
435 *Dispersion Science and Technology, 30*(9), 1363-1383.
436 Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbo, R., . . . Lock, E. J.
437 (2017). Modulation of nutrient composition of black soldier fly (Hermetia illucens)
438 larvae by feeding seaweed-enriched media. *Plos One, 12*(8).
439 Melo-Ruiz, V. (2004). Atizies taxcoensis A and Euchistus sufultus S, jumil bugs nutraceutic
440 foodstuff for iodine deficiency *Metal Ions in Biology and Medicine* (Vol. 8, pp. 324-329).
441 Budapest, Hungary OHN LIBBEY EUROTEXT.
442 Oonincx, D. G. A. B., van Broekhoven, S., van Huis, A., & van Loon, J. J. A. (2015). Feed
443 Conversion, Survival and Development, and Composition of Four Insect Species on
444 Diets Composed of Food By-Products. *Plos One, 10*(12). doi: DOI: ARTN e0144601
445 10.1371/journal.pone.0144601
446 Pace, C. N., Fu, H. L., Fryar, K. L., Landua, J., Trevino, S. R., Schell, D., . . . Grimsley, G. R. (2014).
447 Contribution of hydrogen bonds to protein stability. *Protein Science, 23*(5), 652-661.
448 doi: 10.1002/pro.2449
449 Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L., & De Guevara, O. L.
450 (1997). Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of*
451 *Food Composition and Analysis, 10*(2), 142-157. doi: 10.1006/jfca.1997.0530
452 Ramos-Elorduy, J., Pino Moreno, J. M., & H., M. C. V. (2012). Could Grasshoppers Be a Nutritive
453 Meal? *Food and Nutrition Sciences, 3*, 164-175. doi:
454 http://dx.doi.org/10.4236/fns.2012.32025

Ramos-Elorduy, J., Pino Moreno, J. M., & Marquez Mayaudon, C. (1984). Protein content of some edible insects in Mexico. *Journal of Ethnobiology, 4*(1), 61-72.

Ramos-Elorduy, J., & Viejo Montesinos, J. L. (2007). Los insectos como alimento humano: Breve ensayo sobre la entomofagia, como especial referencia a Mexico. *Boletin de la Real Sociedad Espanola de Historia Natural (Seccion Biologica), 102*((1-4)), 61-84.

Rawel, H. M., Czajka, D., Rohn, S., & Kroll, J. (2002). Interactions of different phenolic acids and flavonoids with soy proteins. *International Journal of Biological Macromolecules, 30*(3-4), 137-150. doi: 10.1016/S0141-8130(02)00016-8

Schuster, R. (1988). Determination of Amino-Acids in Biological, Pharmaceutical, Plant and Food Samples by Automated Precolumn Derivatization and High-Performance Liquid-Chromatography. *Journal of Chromatography-Biomedical Applications, 431*(2), 271-284. doi: Doi 10.1016/S0378-4347(00)83096-0

Sedaghat Doost, A., Dewettinck, K., Devlieghere, F., & Van der Meeren, P. (2018). Influence of non-ionic emulsifier type on the stability of cinnamaldehyde nanoemulsions: A comparison of polysorbate 80 and hydrophobically modified inulin. *Food Chemistry, 258*, 237-244. doi: https://doi.org/10.1016/j.foodchem.2018.03.078

Sedaghat Doost, A., Nikbakht Nasrabadi, M., Kassozi, V., Dewettinck, K., Stevens, C. V., & Van der Meeren, P. (2019). Pickering stabilization of thymol through green emulsification using soluble fraction of almond gum – Whey protein isolate nano-complexes. *Food Hydrocolloids, 88*, 218-227. doi: https://doi.org/10.1016/j.foodhyd.2018.10.009

Sedaghat Doost, A., Sinnaeve, D., De Neve, L., & Van der Meeren, P. (2017). Influence of non-ionic surfactant type on the salt sensitivity of oregano oil-in-water emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 525*, 38-48. doi: https://doi.org/10.1016/j.colsurfa.2017.04.066

Sedaghat Doost, A., Van Camp, J., Dewettinck, K., & Van der Meeren, P. (2019). Production of thymol nanoemulsions stabilized using Quillaja Saponin as a biosurfactant: Antioxidant activity enhancement. *Food Chemistry, 293*, 134-143. doi: https://doi.org/10.1016/j.foodchem.2019.04.090

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., . . . De Smet, S. (2017). Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture, 97*(8), 2594-2600.

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., & Cirkovic Velickovic, T. (2013). Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chemistry, 136*(3-4), 1263-1271. doi: 10.1016/j.foodchem.2012.09.040

Tang, C., Yang, D., Liao, H., Sun, H., Liu, C., Wei, L., & Li, F. (2019). Edible insects as a food source: a review. *Food Production, Processing and Nutrition, 1*(1), 8. doi: 10.1186/s43014-019-0008-1

Tzompa-Sosa, D. A., & Fogliano, V. (2017). Potential of insect derived ingredients for food applications *Insect Physiology and Ecology.* : In Tech.

Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. S., & Lakemond, C. M. M. (2014). Insect lipid profile: Aqueous versus organic solvent-based extraction methods. *Food Research International, 62*, 1087-1094. doi: DOI: 10.1016/j.foodres.2014.05.052

Yuksel, Z., Avci, E., & Erdem, Y. K. (2010). Characterization of binding interactions between green tea flavanoids and milk proteins. *Food Chemistry, 121*(2), 450-456. doi: 10.1016/j.foodchem.2009.12.064

Zhao, J., Dong, F., Li, Y., Kong, B., & Liu, Q. (2015). Effect of freeze–thaw cycles on the emulsion activity and structural characteristics of soy protein isolate. *Process Biochemistry, 50*(10), 1607-1613. doi: https://doi.org/10.1016/j.procbio.2015.06.021

Zorilla, R., Liang, L., Remondetto, G., & Subirade, M. (2011). Interaction of epigallocatechin-3-gallate with β-lactoglobulin: Molecular characterization and biological implication. *Dairy Science and Technology, 91*(5), 629-644. doi: 10.1007/s13594-011-0036-3

Table 1. Proximate analysis (expressed in % on dry weight basis) of adult jumil (*A cordifera*), wasp larvae (*B. mellifica*) and black soldier fly larvae (*H. illucens*) crude protein concentrates [1] (N = 3)

	Whole insect					Protein concentrate
Insect	Protein	Fat	Total dietary fiber	Ash	Other compounds	Protein
Jumil	32.03±0.03[a]	42.41±0.06[c]	25.47±0.44[c]	2.58±0.05[a]	2.49±0.15	49.42±0.04[a]
Wasp larvae	38.24±0.44[c]	14.26±0.[06a]	20.64±0.12[a]	4.10±0.03[b]	22.76±0.14	57.12±0.58[b]
BSF larvae	33.91±0.03[b]	24.36±0.19[b]	22.27±0.[15b]	8.32±0.06[c]	11.14±0.10	55.18±0.20[b]

[1] Values with different letters within a row are significantly different ($p > 0.05$)

Table 2. Mean value of amino acid profile[1] of protein concentrates from adult jumil (*A. cordifera*), wasp larvae (*B. mellifica*), black soldier fly larvae (*H. Illucens*) (N = 3).

Amino acid	Concentration (mg/g of protein concentrate)		
	Jumil	Wasp larvae	Black soldier fly larvae
Cysteine	ND	3.6	ND
Aspartate†	75.5	74.4	81.8
Glutamate†	105.2	105.2	85.0
Serine	31.6	33.0	30.1
Histidine*	16.5	19.5	19.7
Glycine	30.3	28.5	30.1
Threonine*	28.9	32.1	28.2
Arginine	56.6	47.6	53.9
Alanine	43.4	33.6	39.3
Tyrosine	36.4	44.5	51.1
Valine*	35.3	38.2	37.9
Methionine*	18.5	15.3	21.2
Tryptophan*	10.0	7.2	9.3
Phenylalanine*	35.1	33.4	43.6
Isoleucine*	38.7	38.4	39.5
Leucine*	67.3	62.2	64.3
Lysine*	57.2	59.3	66.1
Proline	31.0	31.8	30.0
Total essential a.a	307.2	305.5	329.3

1Limit of detection = 0.16 mg/g

*essential a.a

† negatively charged a.a

Table 3. Polyphenol profile of insect protein concentrate (PC). Specie- adult jumil (*A. cordifera*), wasp larvae (*B. mellifica*), black soldier fly larvae (*H. Illucens*)[1] (N = 3).

Polyphenol (mg/g)[2]	Jumil- PC	BSF larvae- PC	Wasp larvae- PC
3-Chlorogenic acid	0.377 ± 0.00[b]	0.054 ± 0.00[a]	ND
Epicatechin	0.155 ± 0.02	ND	0.134 ± 0.01
p-Coumaric acid	0.890 ± 0.02 [b]	0.477 ± 0.02[a]	1.655 ± 0.01[c]
Caffeic acid	0.534 ± 0.01 [b]	0.401 ± 0.02 [a]	1.056 ± 0.02[c]
Gallic acid	0.111 ± 0.01	0.152 ± 0.02	0.188 ± 0.02
Kaempferol	0.288 ± 0.00	ND	ND
Epicatechin gallate	1.607 ± 0.02[a]	11.088 ± 0.05[c]	4.277 ± 0.08[b]
Total polyphenols	3.960 ± 0.[10a]	12.777 ± 0.[13c]	10.090 ± 0.14[b]

[1] Values with different letters within a row are significantly different ($p > 0.05$)

[2] Results expressed in mg/g of dried insects. Data are presented as mean ± SD.

ND = Not detected.

Table 4. Creaming rate, zeta potential, and volume-weighted average droplet diameter for emulsions stabilized with adult jumil (*A. cordifera),* wasp larvae (*B. mellifica*) and black soldier fly larvae (*H. illucens*) protein concentrates[1].

Insect	ζ-potential (mV)	Creaming rate (mm/day)	D [4,3] (μm) Day 0	Day 1	Day 9
Jumil	-46± 2.58[a]	0.58 ± 0.01[a]	0.80±.0.02[a]	1.16±0.02[b]	1.14±0.03[a]
Wasp larvae	-41± 3.01[a]	0.82 ± 0.02[b]	0.71±0.04[a]	0.99±0.06[a]	1.87±0.01[b]
BSF larvae	-34± 2.63[b]	2.24 ± 0.22[c]	1.12±0.1[b]	1.55±0.03[c]	2.26±0.02[c]

[1] Values with different letters within a row are significantly different ($p > 0.05$)

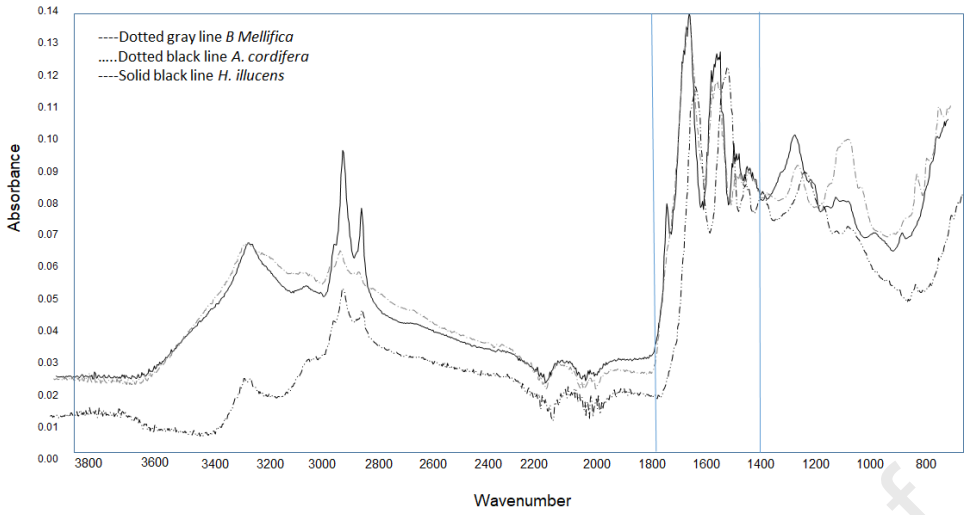

Figure 1. FTIR spectra for the protein concentrates of Black soldier fly larvae (*H. illucens)* (solid black line), wasp larvae (*B. mellifica)* (dotted grey line) and adult jumil (*A cordifera)* (dotted black line).

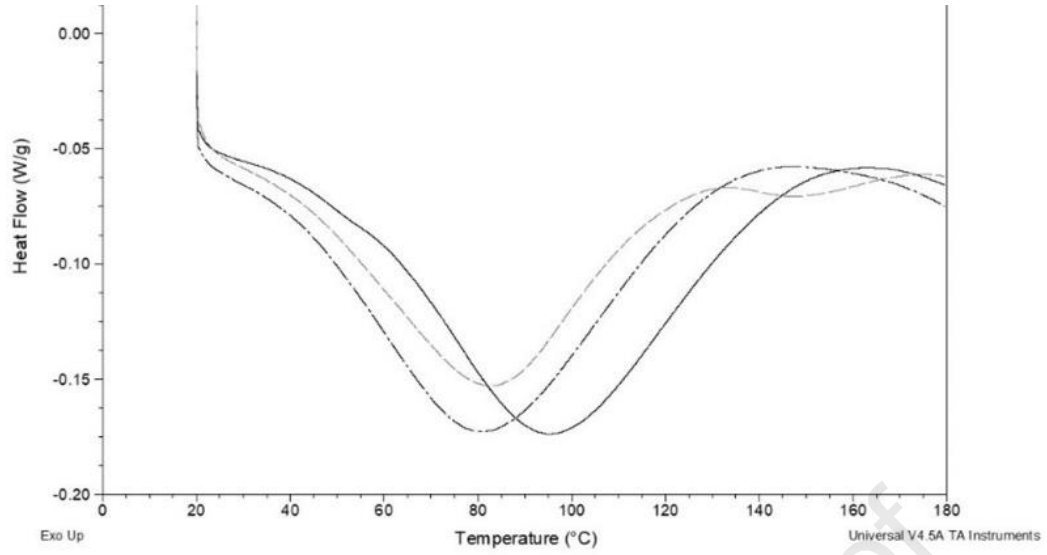

Figure 2. Thermograms for denaturation of protein concentrates from wasp larvae (*B. mellifica*) (dotted gray line), adult jumil *(A.cordifera)* (dotted black line) and black soldier fly larvae (*H. illucens)* (solid black line).

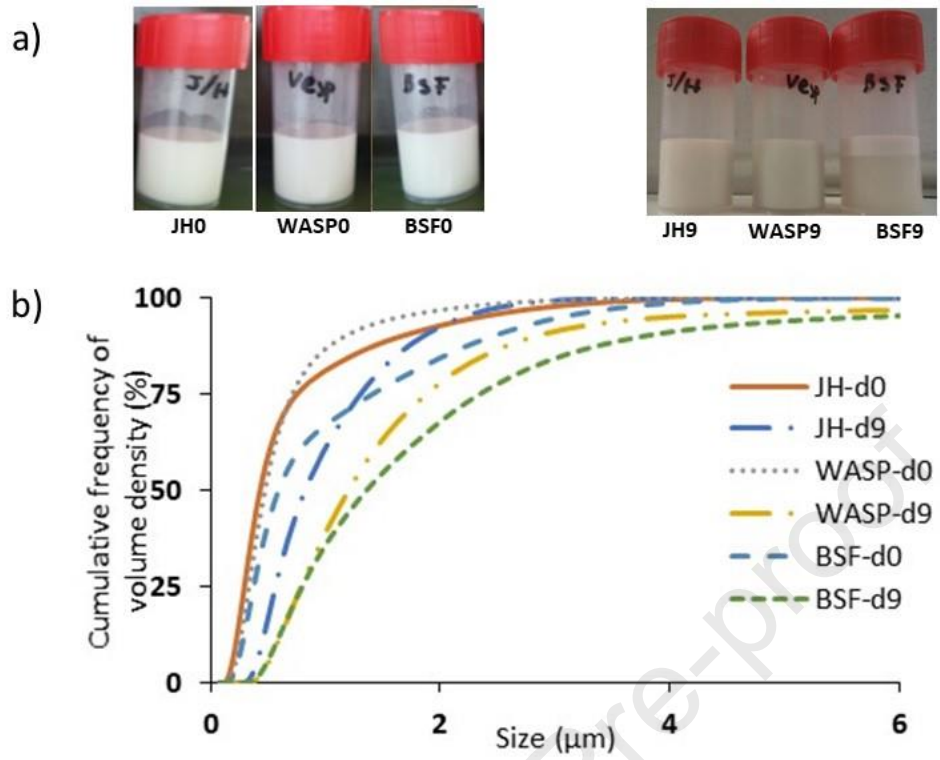

Figure 3. Visual appearance (a) of the emulsions stabilized by adult jumil (*A. cordifera*) (JH), wasp larvae (*B. mellifica*) (WASP) and black soldier fly larvae (*H. Illucens*) (BSF) at day 0 (left) and 9 (right). Cumulative frequency of volume density (b) of emulsions prepared with insect protein concentrate at day 0 and day 9.

Highlights

- Protein concentrates of jumil and wasp have higher emulsion capacity.
- BSF protein concentrate is abundant in polyphenols, mainly epicatechin gallate.
- Polyphenols in BSF protein concentrate could hinder its emulsion capacity.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: