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English version

Nutritional and techno-functional properties of maguey white worm flour for food product development

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Abstract

The present study evaluates the nutritional and techno-functional potential of *Aegiale hesperiaris* (maguey white worm) flour, an endemic species traditionally consumed in Mexico. Larvae were manually collected, lyophilized, and processed to obtain both defatted and non-defatted flours. Proximate composition was determined on a dry weight basis using a nitrogen-to-protein conversion factor of 4.76 to prevent overestimation. Functional properties such as water- and oil-retention capacities, emulsifying capacity and stability, foaming capacity and stability, and protein solubility as a function of pH were also evaluated. Defatted flour showed higher water retention ($2.82 \text{ g} \cdot \text{g}^{-1}$) and oil retention capacities ($2.82 \text{ g} \cdot \text{g}^{-1}$), as well as superior emulsifying properties (EC: 60.83 %, ES: 89.36 %). In contrast, the non-defatted flour showed greater foaming capacity. Protein solubility was higher in the defatted flour across the entire pH range evaluated, reaching a maximum value of 95.82 % at pH 12. These results suggest that defatting enhances the functional properties of the flour, improving its potential for use in processed foods. Therefore, *A. hesperiaris* flour presents an adequate nutritional profile and versatile functional properties, supporting its potential as an alternative ingredient for the development of innovative and sustainable food products.

► **Keywords:** *Aegiale hesperiaris*, edible insects, flour, proteins, techno-functionality.

Introduction

The growing global demand for sustainable protein sources is driven by the rapid increase in population. According to FAO projections, by 2050 the world population is expected to reach approximately 9.7 billion people, placing significant pressure on current food systems (FAO, 2013; Van Huis, 2013). This scenario has prompted the search for alternatives that are both nutritionally efficient and environmentally responsible. In this context, edible insects have emerged as a viable option due to their high nutritional value and sustainable production (Abril et al., 2022; Wade & Hoelle, 2020). Many insects are notable for their high protein content, beneficial lipids, dietary fiber, and essential micronutrients such as zinc, iron, and calcium, making

them nutrient-dense sources (Nowakowski et al., 2022). Comparative studies have shown that some insects, such as mealworms, have nutritional profiles comparable to, or even surpassing, those of conventional meats (Costa et al., 2020). Furthermore, insect farming involves lower greenhouse gas emissions, reduced land and water use, and outstanding efficiency in converting feed into edible biomass compared to traditional livestock production. These advantages position insects as a sustainable and promising alternative for enhancing global food security (Belluco et al., 2023; Rumpold & Schlüter, 2013; Van Huis, 2013). The practice of consuming insects, known as entomophagy, has a long history across diverse cultures. It is estimated that more than 2 billion people include insects in their traditional diets (Khan et al., 2020), and over 2 100 edible species have

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been documented worldwide. Regions of Asia, Africa, and Latin America have deeply rooted entomophagy traditions; for example, in Mexico, insect consumption date back to the pre-Hispanic times and remains prevalent in many communities (Rodríguez-Ortega et al., 2020; Rostro et al., 2012). With more than 549 records species, Mexico has one of the highest diversities of edible insects. In states such as Oaxaca and Hidalgo, species like grasshoppers, *escamoles*, and agave worms are highly valued, both in traditional cuisine and contemporary gastronomy (Ronquillo-de Jesús et al., 2024).

However, in Westernized societies, the acceptance of insects as food has been limited due to cultural barriers and food neophobia (Siddiqui et al., 2022). Nevertheless, in recent years, interest in edible insects outside their traditional context has grown, driven by their nutritional and ecological benefits. Regulatory frameworks, such as those established by the European Union, have even begun to create standards legitimizing their human consumption (Lähteenmäki-Uutela et al., 2017). Currently, insects are often used in the form of flour or powders, which facilitates their use in processed foods and improves consumer acceptance by eliminating visually recognizable insect characteristics. These flours are rich in proteins, minerals, and vitamins, and can serve as ingredients in the development of food products. However, their industrial utilization requires the analysis of their physicochemical and techno-functional properties, which may vary depending on the species (Devi et al., 2022; Pincirolì, 2011).

Therefore, this study proposes to evaluate the nutritional and techno-functional properties of the white maguey worm (*Aegiale hesperiaris*) as part of the material characterization. This approach will enable a comprehensive assessment of its potential as a functional food ingredient, providing scientific evidence to support its incorporation into sustainable and innovative food systems.

Materials and Methods

Sample

A. hesperiaris (white maguey worm, WW) was collected at the larval stage directly from the leaves of its host plant, *Agave salmiana*, in the Actopan region, Hidalgo, Mexico. The larvae were euthanized by freezing and then lyophilized using a laboratory-scale freeze dryer (Triad™ Labconco, 103 USA). Subsequently, the sample was ground using a Nutribullet® food processor at 10 000 rpm for 15 s to achieve a particle size of < 0.15 mm. To prevent deterioration of the resulting flour, the sample was stored in black hermetic stand-up pouches at -20 °C until further use.

Defatted white maguey worm flour (WW-D) was prepared following the method of Kim et al., (2021) with minor modifications. Flour-solvent suspensions (hexane) were made at 20 % (w/v) in 50 mL tubes and mixed on an analog

roller mixer (Cole-Parmer SRT6D, Fisher Scientific, UK) at 60 rpm and 24 °C for 6 h. The fat-containing solvent was removed by decantation, and the extraction was repeated three times to ensure complete defatting.

Proximate Analysis and Caloric Contribution

Moisture, nitrogen, lipids, and ash contents were determined following the official methods recommended by the Association of Official Analytical Chemists (AOAC, 2012). Lipid content was measured using a Goldfish extractor (E-500 Büchi, Flawil, Switzerland) with hexane as the solvent. Total nitrogen was determined using the micro-Kjeldahl method (AOAC 945.01). To avoid overestimating the protein content of WW, a nitrogen-to-protein conversion factor of 4.76, as suggested by Janssen et al. (2017), was applied. The gross energy (kcal · 100g⁻¹) of WW flour was determined using an oxygen bomb calorimeter (model 6050, Parr Instrument Company, USA) with benzoic acid as the standard.

Techno-Functional Properties

Solubility

The protein content in the solubilized fractions of the defatted (WW-D) and non-defatted (WW-ND) flour was determined using the method described by Bradford (1976). For this purpose, 1.0 g of flour was mixed in 10 mL of 20 mM phosphate buffer. The pH of each sample was adjusted from 2 to 12 by adding 0.1 M HCl or 0.1 M NaOH. The suspensions were incubated at 37 °C for 45 min and subsequently centrifuged at 6 000 rpm for 15 min at 4 °C. The protein content in the supernatant was measured by mixing 20 µL of the sample with 200 µL of Bradford reagent. Absorbance was read at 595 nm using a UV-Vis microplate spectrophotometer (Multiskan Sky, Thermo Scientific, Singapore). Quantification was performed using a standard curve prepared with bovine serum albumin (BSA, 0-1 mg · mL⁻¹). The percentage of solubility was calculated using the following equation:

$$\text{Solubility (\%)} = \frac{\text{Protein content of the supernatant}}{\text{Total protein content in the sample}} \times 100 \quad (1)$$

Water and Oil Retention Capacity

The method for evaluating the water retention capacity (WRC) and oil retention capacity (ORC) of WW-D and WW-ND flours is based on the ability of the samples to absorb water or oil over a set period. To assess WRC, 0.2 g of flour was re-suspended in mL of distilled water. The mixture was vortexed for 60 s and then centrifuged at 3000 rpm for 20 min. The supernatant was removed by decantation and further extracted using a micropipette. WRC was expressed as the mass of water (g) per unit mass of flour (g). ORC was determined using the same procedure as WRC, substituting water with mL of canola oil. ORC was expressed as the mass of oil (g) per unit mass

of flour (g). WRC and ORC were calculated using the following equation.

$$WRC \text{ or } ORC = \frac{P_f - P_i}{P_i} \times 100 \quad (2)$$

Where: P_i is the initial mass of the sample (g) and P_f is the final mass of the sample (g).

Emulsifying Capacity and Stability

The emulsifying capacity and stability of WW-D and WW-ND flours were evaluated volumetrically, following the method described by Vanqa et al. (2022) with minor modifications. Flour-water suspensions (0.1 g in 10 mL) were prepared, and 10 mL of canola oil was added. The mixtures were homogenized using an IKA T25 Ultra-Turrax homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10 000 rpm for 3 min. The samples were then centrifugated at 4 000 rpm for 5 min. The volume of the emulsified layer was measured volumetrically and expressed as a percentage. Emulsion stability was assessed after allowing the samples to stand for 30 min at room temperature (22 °C), evaluating the stability of the emulsified layer. Emulsifying capacity and stability (EC and ES) were calculated using the following equation.

$$\text{Emulsifying capacity (EC)} = \frac{V_{em}}{V} \times 100 \quad (3)$$

$$\text{Emulsion stability (ES)} = \frac{V_{em30}}{V_{em}} \times 100 \quad (4)$$

Where: V_{em} is the volume of the emulsified layer, and V is the total volume; V_{em30} is the volume of the emulsified layer after 30 min.

Foaming Capacity and Stability

Foaming capacity (FC) and foam stability (FS) were determined according to Vanqa et al. (2022) with minor

modifications. Flour-water suspensions (20 % w/v) were prepared and then homogenized at 10 000 rpm for 4 min. The whipped sample was transferred to a graduated cylinder, and the foam volume was measured immediately (time 0) and after 30 min. of homogenization. Foaming capacity and foam stability (FC and FS) were calculated using the following equation:

$$\text{Foaming capacity (FC)} = \frac{V_{esp} - V}{V} \times 100 \quad (5)$$

$$\text{Emulsion stability (ES)} = \frac{V_{em30}}{V_{em}} \times 100 \quad (6)$$

Where: V_{esp} is the volume of the foam immediately after whipping, V_{esp30} is the volume of the foam after 30 min.

Statistical analysis

All experimental data are presented as mean \pm standard deviation. One-way ANOVA and significant differences (Tukey's test) were performed using Minitab 18® software (Minitab Inc., USA). Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

As mentioned previously, the insects were manually collected from the host plant of the white maguery worm (WW) in the Actopan region (20.287683, -98.912330), Hidalgo, Mexico. The process began with the visual identification of infested plants, characterized by irregular holes at the base of the leaves, typically associated with plant exudates and scorching on the leaf surface). These signs indicate that the larva has penetrated the plant tissue and is actively feeding within the leaf. Once an infested leaf was located, the tissue was carefully cut using manual tools such as knives or machetes, thereby exposing the internal galleries where the larva resides. In some cases, multiple larvae were collected from a single plant, depending on the degree of infestation (Figure 1).



Figure 1. Collection process to produce white maguery worm (*A. hesperiaris*) flour.

Table 1 shows the proximate composition of WW flour expressed on a dry matter basis. The protein content was 28.11 %, while the fat content was 32.63 % ($P \leq 0.05$). The ash content was 9.60 and the crude fiber content was 27.41 %. The nitrogen-free extract obtained by subtraction represented 27.41 % of the sample. The estimated energy value was $645.95 \text{ kcal} \cdot 100 \text{ g}^{-1}$.

The results of the proximate composition of WW flour were expressed on a dry matter basis, allowing for a more accurate comparison with other studies that have reported the nutritional value of edible insects processed under similar conditions. The protein content in this study was estimated from the total nitrogen content using a specific conversion factor of 4.76, instead of the conventional value of 6.25 commonly employed in bromatological analyses. This choice was based on the work of Janssen et al. (2017), who proposed this factor for insects, suggesting that the use of 6.25 tends to overestimate the actual protein content due to the presence of non-protein nitrogen sources such as chitin, nucleotides, and nitrogenous metabolites. Therefore, the value reported in this study provides a more accurate estimation of the actual protein content of WW. The protein content obtained (28.11 %) is consistent with the findings of Escamilla-Rosales et al. (2022), who reported values ranging from 27.0 to 33.5 % for dehydrated WW. Melo-Ruiz et al. (2011) reported a protein content of 30.88 % for the same species; however, it is likely that they used the conventional conversion factor of 6.25, which may have resulted in an overestimation.

The high fat content observed (32.63 %) falls within the range reported in the literature for this species (27–58 %) and contributes to the calculated energy of $645.95 \text{ kcal} \cdot 100 \text{ g}^{-1}$. The presence of lipids has also been highlighted as a distinctive characteristic of lepidopteran compared with other edible insects, such as *Tenebrio molitor*, whose fat proportion is generally lower (Martins da Silva et al., 2024). Regarding the ash content (2.25 %), this value suggests a moderate presence of minerals,

similar to the findings of Rodríguez-Ortega et al. (2020), who reported an ash content of 0.82 % in *Agathymus remingtoni* larvae, while Melo-Ruiz et al. (2011) reported values up to 1.35 % in other Latin American edible insect species. These differences may be attributed to species variation, type of pre-processing, developmental stage of the insects, and even environmental and dietary factors. Nevertheless, the value reported here for *Aegiale hesperiaris* highlights its relative mineral richness and potential as a complementary source of micronutrients in food products.

The crude fiber content (9.60 %) is relatively high for an insect, which may be attributed to the inclusion of chitin in the sample, as previously documented in whole-body insect analyses. However, according to Rostro et al. (2012), fiber content can vary significantly depending on the insect's development stage (egg, pupa or larva), with reported values ranging from 3.6 to 7.23 %. In the same study, variations in crude fiber were also documented among different species with a range between 1.1 to 9.5 %. Thus, the proximate profile obtained supports the potential of *A. hesperiaris* white worm flour as a functional ingredient in the development of enriched food products, contributing both to dietary diversification and to the utilization of endemic resources of high cultural and nutritional value.

Table 2 shows the techno-functional properties of non-defatted (WW-ND) and defatted (WW-D) white worm flours. The defatted flour (WW-D) had significantly higher values ($P \leq 0.05$) for water retention capacity (WRC) and oil retention capacity (ORC), with values of $2.82 \text{ g} \cdot \text{g}^{-1}$ in both cases. In contrast, the non-defatted flour (WW-ND) showed values of 1.75 y $1.85 \text{ g} \cdot \text{g}^{-1}$ for WRC and ORC, respectively. Regarding emulsifying capacity (EC), WW-D also demonstrated a significantly higher value (15.83 %) compared to GB-ND. Similarly, emulsion stability (ES) increased following the defatting process, reaching 89.36 % for WW-D versus 85 % for WW-ND. On the other hand, foaming capacity (FC) and foam stability (FS) were

Table 1. Proximate composition and caloric contribution of *A. hesperiaris* flour (% dry basis).

Sample	% Protein	% Fat	% Ash	% Fiber crude	% NFE	Energy (kcal·100g ⁻¹)
WW	28.11 ± 0.4 b	32.63 ± 0.2 a	2.25 ± 0.7 c	9.60 ± 0.1 d	27.41*	645.95 ± 0.1

WW: White worm; ELN: Nitrogen-free extract (obtained by difference). Values with different lowercase letters in the same row are significantly different ($P \leq 0.05$).

Table 2. Techno-functional properties of non-defatted and defatted *A. hesperiaris* flour.

Sample	WRC (g·g ⁻¹)	ORC (g·g ⁻¹)	EC (%)	ES (%)	FC (%)	FS (%)
WW-ND	1.75 ± 1.0 ^a	1.85 ± 0.2 ^a	50.00 ± 0.3 ^a	85.00 ± 0.3 ^a	5.50 ± 0.5 ^a	9.42 ± 0.5 ^b
WW-D	2.82 ± 0.7 ^b	2.82 ± 0.1 ^b	60.83 ± 2.8 ^b	89.36 ± 3.1 ^b	2.03 ± 0.2 ^b	6.49 ± 2.5 ^a

WW-ND: Non-defatted white worm flour; WW-D: Defatted White worm flour, WRC: water retention capacity, ORC: oil retention capacity, EC: Emulsifying capacity, ES: emulsion stability, FC: foaming capacity and FS: Foam stability. Values with different lowercase letters in the same column indicate a significant difference ($P \leq 0.05$).

significantly higher in the non-defatted flour (WW-ND), with values of 3.47 and 2.93 %, respectively), compared to the defatted flour (WW-D).

The techno-functional properties of WW flours evaluated in this study showed that the defatting process had a significant impact on key parameters such as water retention capacity (WRC), oil retention capacity (ORC), and emulsion capacity and stability (EC and ES), while notably reducing foaming capacity and stability.

In particular, the defatted flour showed a higher WHC (WW-D, $2.82 \text{ g} \cdot \text{g}^{-1}$) compared to the non-defatted flour (WW-ND, $1.75 \text{ g} \cdot \text{g}^{-1}$), a result with the findings of Cortazar-Moya et al. (2023) for *Arsenura armida*, where the defatted flour reached water retention values exceeding $2.75 \text{ g} \cdot \text{g}^{-1}$. This behaviour can be attributed to a greater exposure of polar groups following lipid removal, which enhances interactions with water molecules (Zhang et al., 2024). Similarly, the OHC was higher in the defatted flour ($2.82 \text{ g} \cdot \text{g}^{-1}$), consistent with studies on defatted *Gryllus bimaculatus*, where Jeong et al. (2021) reported that the use of ethanol as a solvent significantly improved both oil retention capacity and protein solubility. These findings reinforce that the partial removal of the lipid fraction increases the exposure of hydrophobic protein side chains, thereby enhancing their ability to retain lipid compounds.

In terms of emulsion capacity (EC), the defatted flour showed a significantly higher value (60.83 %) compared to the non-defatted flour (50.0 %). This is consistent with the findings of Lucas-González et al. (2019) in *Acheta domesticus* flour, where both lyophilization and defatting enhanced emulsion formation and stability. Furthermore,

Zielińska (2022) reported that defatted insect flour shows superior functional performance, emphasizing the influence of protein content and the reduced lipid interference in the formation of stable emulsions.

Conversely, the foaming capacity and stability (FC and FS) were higher in WW-ND, suggesting that the lipid fraction present in the not-defatted flour may contribute to the formation and maintenance of bubbles, thereby improving foam stability, possibly due to the formation of mixed protein-lipid films at the air-water interface. This effect has been reported by Mshayisa et al. (2022), who compared *Hermetia illucens* flours and protein concentrates and found that non-defatted flours showed greater foaming stability under certain conditions, supporting the hypothesis that residual lipids may play a structural role in foam stabilization.

Overall, these findings indicate that defatting WW flour enhances properties related to protein-water/oil interactions, which is advantageous for applications such as emulsions, dressings, or meat analogues. On the contrary, the non-defatted flour (WW-ND) may be more suitable for formulations that require some degree of foaming capacity, such as baked goods or aerated beverages. This functional differentiation is essential for defining specific technological applications for each fraction, taking into account their performance within food matrices, as well as the influence of pH conditions and protein concentration.

Figure 2 shows the influence of pH on the protein solubility of non-defatted (WW-ND) and defatted (WW-D) flours from *Aegiale hesperiaris*. In both samples, solubility showed a typical protein behavior, with an isoelectric

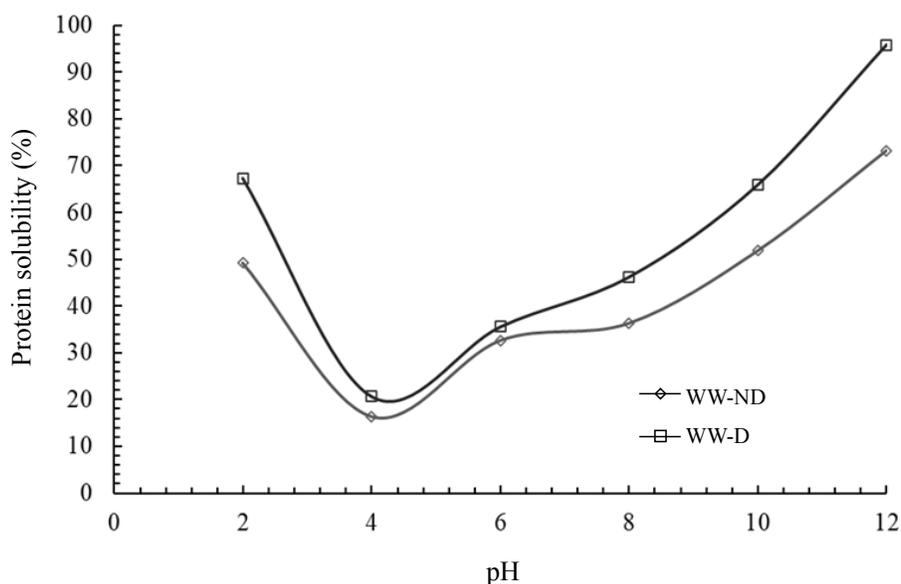


Figure 2. Effect of pH on the protein solubility of *A. hesperiaris*. WW-ND: non-defatted flour; WW-D: defatted flour.

point near pH, where the lowest solubility values were observed: 16.43 % for WW-ND and 20.68 % for WW-D. At extreme pH values (acidic and alkaline), protein solubility increased significantly. At pH 2, WW-D flour showed higher solubility (18.07 %) compared to WW-ND. This pattern persisted throughout the curve, becoming more pronounced under alkaline conditions, reaching maximum solubility values at pH 12: 95.82 % for WW-D and 73.24 % for WW-ND. This behavior can be attributed to the exposure of charged protein groups at pH values far from the isoelectric point, enhancing interaction with the aqueous medium. Additionally, the consistently higher solubility of WW-D suggested that lipid removal improves protein accessibility and dispersion, probably by reducing hydrophobic interactions that limit protein solvation.

Protein solubility as a function of pH showed the typical pattern of globular proteins, with a minimum near the isoelectric point (pH ~4) and a marked increase at extreme pH values. In this study, defatted flour (WW-D) showed higher solubility across the entire pH range, reaching a maximum of 95.82 % at pH 12, compared to 73.24 % for non-defatted flour (WW-ND). These results agree with Kim et al. (2017), who observed a significant increase in protein solubility of *Acheta domesticus* flour as pH moved away from the isoelectric point, with maximum values at extreme pH levels (60 and 120 % respectively). Similarly, Ndiritu et al. (2019), described a comparable behavior in *Acheta domesticus* protein concentrates, showing low solubility between pH 4-6 and a significant recovery of solubility above pH 8.

The higher solubility observed in WW-D flour in this study has also been reported in other species, such as *Gryllus bimaculatus* and *Tenebrio molitor*, where lipid removal improves protein accessibility to the aqueous medium and reduces internal hydrophobic interactions that limit protein dispersion in solution (Bußler et al., 2016; Jeong et al., 2021). Furthermore, the study by Villaseñor et al. (2022) highlights that defatting is a crucial step to enhance both the extraction yield and functionality of insect proteins, facilitating their application in functional foods. For *Aegiale hesperiaris*, these findings provide evidence of its high potential as a source of functional proteins, particularly when defatting is applied to improve solubility in food matrices with variable pH. This parameter is essential in the formulation of emulsified products, protein beverages, or dietary supplements, where adequate protein dispersion and stability are required.

Conclusion

White maguey worm (*A. hesperiaris*) flour showed a balanced nutritional profile and notable techno-functional properties, supporting its use as an ingredient in the development of functional foods. Defatting significantly enhanced water and oil retention capacities as well as

emulsifying properties, whereas the non-defatted flour showed superior foaming capacity. Additionally, protein solubility was higher in the defatted flour throughout the entire pH range evaluated, highlighting its versatility for various food applications. These results confirm the potential of this species as a functional protein source in sustainable food systems.

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