Composition and microbiological quality of edible insects subjected to two drying methods

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ABSTRACT

Objective: To compare the chemical composition and microbiological quality of *escamoles* (*Liometopum apiculatum* Mayr) and white maguey worm (*Aegiale hesperiaris* W.) subjected to freeze-drying and oven drying.

Design/Methodology/Approach: The presence of *Escherichia coli*, molds, and yeasts was quantified. In addition, titratable acidity, free fatty acids (FFA), carbohydrates (CHO), fat, moisture, protein, and ash contents were determined in both edible insect species.

Results: *Escamoles* showed the highest content of FFA (0.65±0.05%), titratable acidity (5.2±0.2%), and protein (17.53±0.64%), while the white maguey worm recorded the highest ash content (25.4±1.51%). Oven drying reduced the presence of *E. coli*, molds, and yeasts to levels that comply with the Official Mexican Standard NOM-210-SSA1-2014.

Study Limitations/Implications: Edible insects contain significant amounts of proteins, minerals, and vitamins that can contribute to improved human nutrition. The food industry has shown interest in processing these insects into flour, in order to include it into other products; however, information regarding their microbiological quality remains limited.

Findings/Conclusions: These results highlight the importance of applying appropriate heat treatments to guarantee the safety of edible insects, particularly if they are used as food ingredients by the industry. The combination of high nutritional value and adequate microbiological quality positions these insects as a promising and safe alternative for the development of innovative food products.

Keywords: protein, microbiological quality, *Liometopum apiculatum* Mayr, *Aegiale hesperiaris* W., drying.

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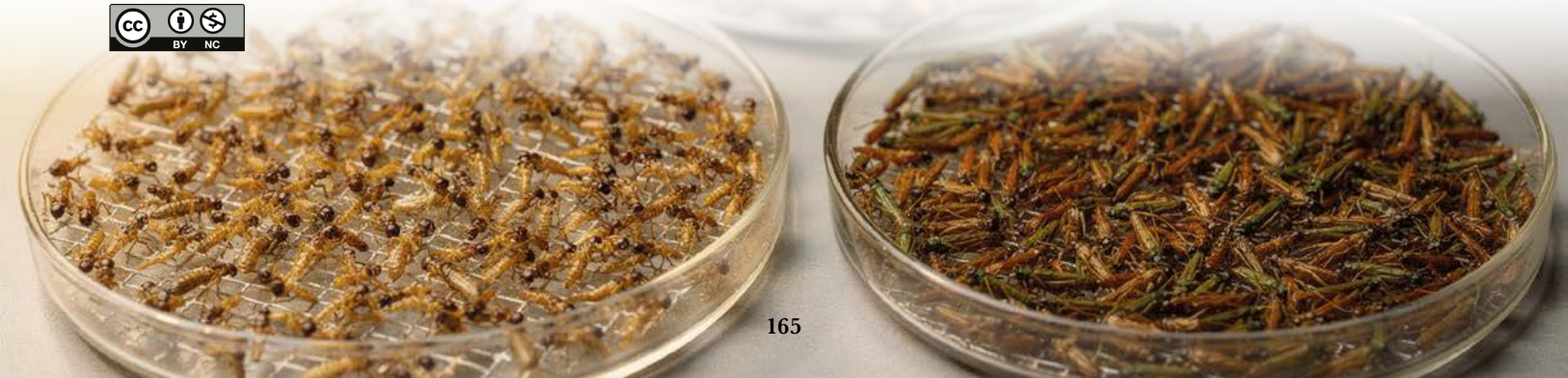
INTRODUCTION

The UN (ONU, 2025) estimates that the world population will reach 10.3 billion people by 2080. This steady growth will increase the demand for resources and exacerbate malnutrition problems. Therefore, the search for sustainable protein sources has become critically important (Doğan and Çekal, 2022). Edible insects have been consumed in Mexico since ancient times (Melgar *et al.*, 2019). In the Potosino-Zacatecano Highlands, the consumption of *escamoles* (*Liometopum apiculatum* Mayr) and white maguey worm (*Aegiale*



hesperiaris W.) has social, economic, and environmental significance (Cruz-Labana *et al.*, 2018). However, studies addressing the microbiological quality of edible insects remain scarce (Arango *et al.*, 2004).

In this context, the use of edible insects provides an opportunity for the supplementation of balanced diets and for the whole gastronomy sector (Guiné *et al.*, 2021; Aigbedion-Atalor *et al.*, 2024).

Edible insects contain high levels of proteins, vitamins, minerals, amino acids, and essential amino acids (Doğan and Çekal, 2022; Papastavropoulou *et al.*, 2022). In addition, their production may be more sustainable than other protein sources, due to lower greenhouse gas emissions, lower land use, and potentially lower water footprint (Guiné *et al.*, 2021; Nowakowski *et al.*, 2022; Mishyna *et al.*, 2023).

The inclusion of edible insect flours into food products has increased iron and zinc contents (Ochieng *et al.*, 2023) and improved the texture of baked products such as cookies (Bottle *et al.*, 2024). However, prior drying of edible insects is essential, since molds, yeasts, and other pathogenic microorganisms have been detected in products made from these flours (Ibarra, 2017; Ochieng *et al.*, 2023).

Recently, interest in the processing of edible insects has increased in developing countries, as they can contribute to the improvement of human nutrition (Imathiu, 2020) and food quality. Nevertheless, due to harvesting processes and secondary contamination, pathogens such as *Salmonella* spp. and *Escherichia coli* have been detected in edible insects (Pal *et al.*, 2024). These agents are responsible for approximately 4% of all foodborne illnesses worldwide (Kapoor *et al.*, 2023). Therefore, the chemical composition and microbiological quality of *Liometopum apiculatum* Mayr and *Aegiale hesperiaris* W. were evaluated under two drying methods in order to determine their nutritional value and specific microbial load.

MATERIALS AND METHODS

The research was conducted in the Chemistry, Biochemistry, and Microbiology Laboratory of the Coordinación Académica Región Altiplano Oeste of the Autonomous University of San Luis Potosí, Mexico (22° 38’ 28.5” N, −101° 42’ 10.0” W).

Biological materials

Escamoles (*Liometopum apiculatum* Mayr) and white maguey worms (*Aegiale hesperiaris* W.) were used in this study. One kg of specimens of each species was provided by local collectors from Salinas, San Luis Potosí, Mexico, between March and June 2023 (Figueroa-Sandoval *et al.*, 2018). The insects were washed with purified water to remove impurities and stored frozen at −16 °C in polyethylene bags.

Variables and treatments

A proximate chemical analysis of the two edible insect species was performed. The presence of *Escherichia coli*, molds, and yeasts in insects subjected to freeze-drying and oven drying was compared with the microbial load found in fresh insects.

Moisture

Moisture content was determined in 5 g of fresh weight (FW) of each insect species using a Radwag® MA 50/1. R thermobalance, following the method described by Ileleji *et al.* (2010).

Free fatty acids (FFA)

Twenty-five mL of neutralized alcohol solution were added to 5 g (FW) of each insect species. Subsequently, 1 mL of phenolphthalein indicator was added and the solution was titrated with 0.01 N NaOH (AOCS, 2004). The FFA content was calculated using the following equation:

$$FFA = S \times N \times 28.2 \times W^{-1} \quad (1)$$

where: *FFA* is the free fatty acid content expressed as oleic acid (%); *S* is the volume of sodium hydroxide consumed (mL); *N* is the normality of sodium hydroxide (0.1 N); and *W* is the sample weight (g).

Titratable acidity

The percentage of titratable acidity was determined by titration. Ten grams of each insect sample were weighed and mixed with 90 mL of distilled water adjusted to a pH of 7 (10% sample). The mixture was homogenized in an Oster® BLST4655-013 blender and filtered through cheesecloth. Ten mL of the filtrate were titrated with 0.1 N NaOH using two drops of phenolphthalein as indicator (Melia *et al.*, 2022). The acidity percentage was calculated as follows:

$$Acidity(\%) = v \times N \times m_{eq} \times 100 \times m^{-1} \quad (2)$$

where: v is the volume of sodium hydroxide used (mL); N is the normality of NaOH (0.1 N); m_{eq} is the equivalent weight of lactic acid; and m is the mass (g) of the titrated sample.

Carbohydrates

To determine carbohydrate content, 5 g of FW of insects were mixed with 10 mL of distilled water, macerated in a mortar, and filtered through a cloth filter (cheesecloth). The samples were centrifuged at 4,000 rpm for 5 min (Hettich®, Universal 320 R). Subsequently, 1 mL of the filtered sample was transferred to test tubes and 3 mL of sulfuric acid were added. The mixture was shaken for 30 s and then cooled in an ice bath for 2 min. Finally, absorbance was measured at 315 nm using a spectrophotometer (MAPADA, VISIBLE V-1600/1600 PC). Distilled water was used as blank. A standard calibration curve was prepared using D (+)-xylose (Millipore, 58-86-6) at concentrations of 1.25, 2.5, 5, and 10 mg/L. Results were expressed as xylose equivalents (%), according to Albalasmeh *et al.* (2013).

Fat

Fat content was determined using the AOAC method 920 (1990). Five g of previously dried and ground insect samples were placed in a porous extraction cartridge. Extraction was performed using a Soxhlet apparatus. The cartridge was placed in the extractor chamber. A total of 250 mL of hexane was heated at 130 °C. The extraction process was repeated for 8 h until complete extraction of the analytes from the sample. The solvent was evaporated at 100 °C, to obtain the dried hexane extract. Fat content was calculated using the following equation:

$$Fat = 100 \times \left(W_1 - W_2\right)\left(W^{-1}\right) \quad (3)$$

where: *Fat* is the fat content (%); W is the weight of the sample used; W_1 is the weight of the container with the dried hexane extract (fat residue); and W_2 is the weight of the empty container.

Protein

Protein content was determined using the Kjeldahl method 2.062 (AOAC, 1984). A 3 g insect sample and 1 g of protein catalyst were placed in a Büchi digestion unit. Subsequently, 2.5 mL of concentrated sulfuric acid were added until the sample changed from a dark to a clear color. Subsequently, 10 mL of distilled water were added to the sample, which was transferred to a tube in the distillation unit. Then, 10 mL of 60% NaOH were added. The distillate was collected in an Erlenmeyer flask, mixing 5 mL of saturated boric acid and two drops of methyl red indicator. Distillation stopped until the flask reached a final volume of 50 mL. Finally, the distillate was titrated with 0.125 N HCl. The crude protein percentage was calculated with the following equation, using the 6.25 factor:

$$Protein\ (\%) = 14.007\left(v - b\right) \times \left(N\right)\left(factor\right)\left(100\right)\left(m\right)^{-1} \quad (4)$$

where: v is the volume of HCl used (mL); b is the blank volume (mL); N is the normality of HCl; and m is the mass of the sample (g).

Ash

Ash content was determined following the AOAC procedure 923.03 (AOAC, 1990). Five grams of fresh insect samples were weighed and placed in crucibles previously brought to constant weight. Samples were initially incinerated at low flame in a fume hood to avoid losses caused by smoke or liquid spillage. The crucibles were then placed in a Thermo Scientific® Thermolyne muffle furnace preheated at 550 °C for 30 min. Samples were maintained at this temperature for 2 h. Finally, the crucibles were transferred to a desiccator until constant weight was achieved. Ash content was calculated using the following equation:

$$Ash\ (\%) = 100 \times \left(W_2 - W_1\right) \times \left(W_0\right)^{-1} \quad (5)$$

where: W_0 is the sample mass (g), W_1 is the crucible mass (g), and W_2 is the mass of the crucible with ash (g).

Microbiological analysis

The presence of *E. coli*, molds, and yeasts was quantified in both fresh and dried edible insect species. The inocula were prepared following the Mexican Official Standard NOM-110-SSA1-1994 with four repetitions. One g of insect sample and 9 mL of diluent were placed in test tubes containing nine glass beads. The tubes were stirred in a Vortex® XH-D for 2 min and allowed to settle for 5 min. Monobasic sodium phosphate buffer (NaH_2PO_4, 95%) was used as diluent for mesophilic molds and yeasts. The pH was adjusted at 7.2 with NaOH (0.01 N) and the solution was sterilized at 121 °C for 15 min.

Mesophilic molds and yeasts were quantified using potato dextrose agar (PDA, Difco®, Detroit, MI, USA) according to NOM-110-SSA1-1994. One mL of each inoculum was placed at the center of 90-mm Petri dishes containing agar acidified with 10% tartaric acid. Subsequently, the inocula were spread using a Drigalski spatula with six movements from the center toward the edge of the plate. Inoculated plates were incubated at 25±2 °C (Yamato Scientific®, IN-804-115V). After 72 h of incubation, colonies were counted and microbial density was calculated as CFU g^{-1} using the following equation:

$$CFU \times mL^{-1} = A \times F \times B^{-1} \quad (6)$$

where: A is the number of colony-forming units; B is the inoculated volume (mL); and F is the dilution factor.

For the preparation of the *E. coli* inoculum, peptone water at pH 9 was used as diluent according to NOM-110-SSA1-1994. Detection was carried out using MacConkey selective agar (BD Bioxon™, Mexico City, Mexico) following the procedure described by Cardona-López *et al.* (2020). Twenty-five mL of agar was poured into each sterile Petri dish and sealed with Parafilm® plastic film. Before inoculation, a 1:10 dilution was prepared by mixing 1 mL of inoculum with 9 mL of peptone water. One mL of the dilution was poured and spread over the agar surface using a Drigalski spatula as previously described. The plates were incubated at 35 °C for 24 h in a Yamato Scientific® IN-804-115V incubator. After incubation, Petri dishes showing intense pink colonies with precipitation halos were selected. Colony counts were performed using an UNICO PRO® 1001439 colony counter.

Data analysis

The chemical variables of both insect species were compared using a t-test (α=0.05). Microbiological counts were subjected to an analysis of variance (ANOVA), followed by Tukey's multiple comparison test (α=0.05). All analyses were conducted in R-project® v. 4.4.1 using the RStudio® interface v. 2024.04.2.

RESULTS AND DISCUSSION

Chemical composition

No significant differences were found in the moisture ($p=0.261$), carbohydrates ($p=0.480$), or fat content ($p=0.601$) of both edible insect species (Table 1). Their moisture content was similar to that reported by Rostro *et al.* (2012) for escamoles (71.90%) and white maguey worms (70.78%) collected in Teotihuacán, Mexico.

Edible insects showed a carbohydrate content close to 10% (Mayack and Naug, 2010). Melo-Ruíz *et al.* (2016) determined that escamoles contain relatively high carbohydrate levels (6.8-18.27%), which may be the result of the conversion of proteins into carbohydrates through the gluconeogenesis pathway. According to Rostro *et al.* (2012), the fat content of white maguey worms ranges from 8.64 to 9.80%, which matches the results obtained in this study (9.5±2.86%). Rostro *et al.* (2012) also reported that the fat content of escamoles ranges from 10.39 to 12.40%. In contrast, the results of this study were lower (7.9±3.92%).

Significant differences ($p\leq0.05$) were found in the content of free fatty acids (FFA), titratable acidity, protein, and ash between insect species (Table 1). Escamoles showed higher FFA content than white maguey worms. Nevertheless, these values were lower than those reported by Atowa *et al.* (2021), who found levels ranging from 24.91 to 36.57%. According to Ewald *et al.* (2020), the content of vaccenic acid ranges from 0.8 to 1.5%; and values above 1% suggest the presence of beneficial fatty acids, for human growth and development. The percentage of titratable acidity in both edible insect species fell within the range reported for preserving sausages (5.07-6.2%) (Capita *et al.*, 2006).

Escamoles stood out for their protein content (17.53±0.64%) compared with white maguey worms (10.80±0.26%). Nevertheless, both values were higher than those reported by Rostro *et al.* (2012), who indicated that white maguey worms contain between 8.64 and 9.80% protein, while escamoles contain between 10.39 and 12.40%. White maguey worms showed the highest mineral content, reaching 25.40±1.51%, which exceeded the values reported by Rostro *et al.* (2012) (1.00-1.05%). The ash content of escamoles (13.20±1.06%) was also higher than the percentages (6.53-7.85%) reported by Melo-Ruíz *et al.* (2016) (Table 1).

Table 1. Comparison of the composition of two species of edible insects.

Constituents	*L. apiculatum* Mayr.	*A. hesperiaris* W.
Moisture (%)	60.91±11.04[a]	70.78±0.66[a]
Free fatty acids (%)	0.65±0.05[a]	0.40±0.13[b]
Titratable acidity (%)	5.20±0.20[a]	4.20±0.20[b]
Carbohydrates (%)	8.64±1.02[a]	9.14±0.21[a]
Fat (%)	7.90±3.92[a]	9.50±2.86[a]
Protein (%)	17.53±0.64[a]	10.80±0.26[b]
Ashes (%)	13.20±1.06[b]	25.40±1.51[a]

¥ % on a wet basis. Means with the same superscript within each row are not significantly different ($\alpha=0.05$).

Microbiological quality

The drying method had a significant effect ($p<0.001$) on the growth of molds and yeasts after 72 h of incubation (Figure 1). Oven drying resulted in the lowest number of colony-forming units (CFU), but this pattern was not impacted by the edible insect species (Table 2). Both species in their fresh and freeze-dried states exceeded the limit for molds and yeasts (150 CFU g^{-1}) established by the Mexican Official Standard NOM-111-SSA1-1994. In contrast, oven-dried samples had 0.33 CFU g^{-1}, which made them microbiologically safe.

Edible insects are feasible option for human consumption; however, briefly heating crickets and cricket flour can reduce some bacterial populations, although *Bacillus cereus* may survive (Pal *et al.*, 2024), potentially explaining the reduction of the microbial load found in oven-dried insects (Table 2). Kolakowski *et al.* (2021) indicated that raw, unprocessed insects contain more bacteria than conventional meat samples; therefore, proper sterilization and handling are essential for both the food industry and consumers.

After 24 h of incubation (Figure 2), no significant differences ($p=0.409$) were found in the number of colony-forming units between species. The values recorded in this study for freeze-dried white maguey worms (244.67±201.00 CFU g^{-1}) and for *escamoles* (335.00±135.60 CFU g^{-1}) can potentially exceed the maximum limit (250 CFU g^{-1}) established by the Mexican Official Standard NOM-210-SSA1-2014 (Table 2). The presence of bacteria such as *E. coli* and *Salmonella* spp. poses a risk to human health, as they may cause foodborne diseases (Guragain *et al.*, 2024).

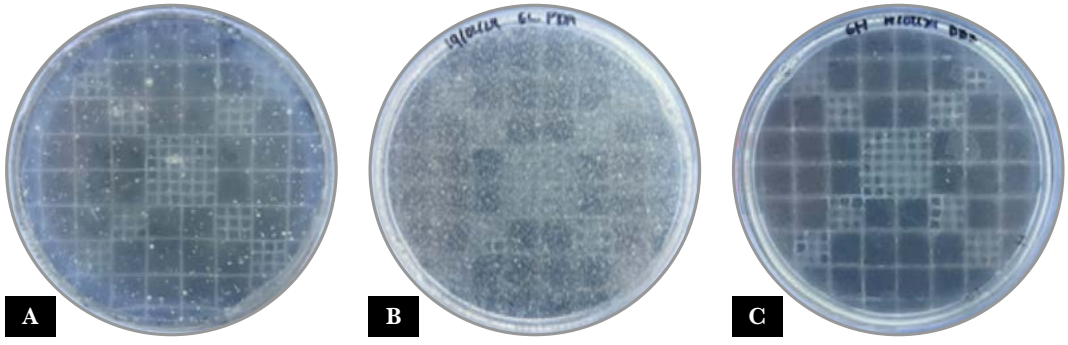


Figure 1. Growth of molds and yeasts in white maguey worms (*A. hesperiaris* W.): (A) fresh, (B) freeze-dried, and (C) oven-dried.

Table 2. Presence of molds, yeasts, and *E. coli* in two species of edible insects.

Species	Sample preparation	Molds and yeasts (CFU g^{-1})	*E. coli* (CFU g^{-1})
L. apiculatum Mayr.	Fresh	305±68.2^{a}	336.67±163.61^{ab}
	Freeze-dried	271±60.3^{a}	244.67±201.00^{ab}
	Oven-dried	0±0.0^{b}	0.67±1.16^{b}
A. hesperiaris W.	Fresh	371±136.0^{a}	405.00±134.81^{a}
	Freeze-dried	299±87.6^{a}	335.00±135.60^{ab}
	Oven-dried	0.7±1.2^{b}	1.00±1.00^{b}

*Values with the same superscript within each column are not significantly different ($\alpha=0.05$).

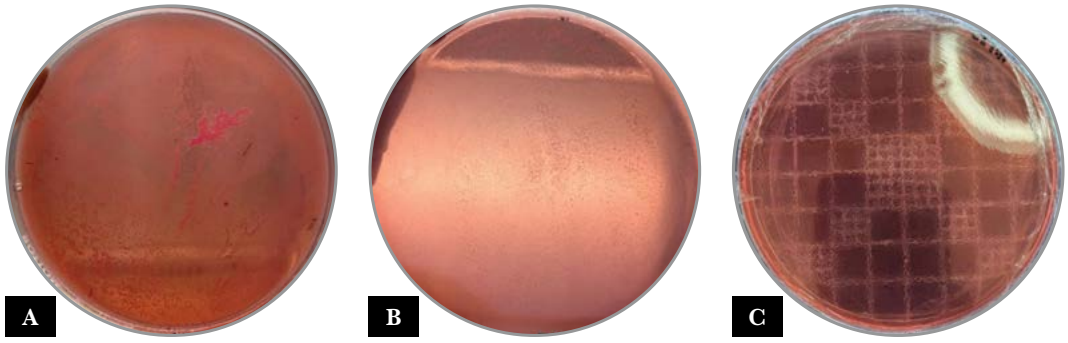


Figure 2. *E. coli* growth in escamoles (*L. apiculatum* Mayr): (A) fresh, (B) freeze-dried, and (C) oven-dried.

Significant differences ($p<0.001$) were observed depending on the condition of the edible insects. Fresh samples showed the highest content of *E. coli* colony-forming units (370.83 ± 139.00 CFU g^{-1}), followed by freeze-dried insects (289.83 ± 161.00 CFU g^{-1}), and oven-dried insects (0.83 ± 0.98 CFU g^{-1}). These results suggest that the oven-drying method results in a microbiologically safe product, following the Mexican Official Standard NOM-210-SSA1-2014. According to Hernández-Álvarez *et al.* (2021), fresh edible insects pose a potential microbiological risk; however, this risk can be reduced through drying processes. These results are similar to the findings of this study. Fresh white maguey worms showed the highest *E. coli* colony-forming units (405.00 ± 134.81 CFU g^{-1}) compared with oven-dried insects, which recorded 1.00 ± 1.00 CFU g^{-1} and 0.67 ± 1.15 CFU g^{-1} for white maguey worms and *escamoles*, respectively (Table 2).

CONCLUSIONS

Escamoles (*L. apiculatum* Mayr) showed high free fatty acids, titratable acidity, and protein content. Meanwhile, white maguey worms (*A. hesperiaris* W.) were characterized by their higher ash content. The fresh samples of both insects indicated the presence of *E. coli*, molds, and yeasts at levels that may pose potential health risks. Oven drying significantly reduced the microbial load, proving its effectiveness as a hygienization method. Therefore, the implementation of this type of thermal processing is essential to guarantee the safety of these traditional foods.

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