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Nutrient and oil profile of Escamol, an edible larva of ants *(Liometopum apiculatum Mayr)*

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The object of this study was to evaluate nutrient content and oil profile in Escamol, which are edible ants native to Mexico. These are dehydrated and butter-fried, and they are commercially available in Mexico. The nutrient content was analysed by using proximal analysis, while the oil profile and its quality were analysed using a refractive index and physicochemical analysis, respectively. The results of the proximal chemical analysis of fresh Escamol showed moisture (56.00±0.00 %), protein (15.30±0.50 %), lipids (20.05±0.37 %), ash (1.91±0.12 %), and carbohydrates (6.73±0.00 %) percentages fall within the parameter reported for the order Hymenoptera ($p < 0.05$) compared with butter-fried Escamol ($p < 0.05$). The moisture (1.53 %) in Escamol oil accelerates the degradation of the triacyl-glycerides, producing free fatty acids (17.48 % oleic acid). At the same time, frying increases lipids with double linkages (133. 79 cg I2 g-1) and causes oxidation products (3.60 meq O2 kg-1 of oil). The Escamol oil extracted from the dehydrated and butter-fried sample showed a refractive index similar to beeswax (1.442) and pure edible coconut oil (1.447), respectively. Therefore, they would mainly present fatty acids as the lauric acid [C12:0 (41.00-56.00 %)], monounsaturated: palmitoleic acid [C16:1 (12.00 %)] and oleic acid [C18:1 (3.50-11.00 %)] and polyunsaturated: linoleic acid [C18:2 (1.00-2.5 %)]. The frying has a minimal effect on the chemical composition of the oil and the fatty acids in Escamol.

1. Introduction

Insects are part of the human diet in many parts of the world (Melgar-Lalanne *et al.,* 2019). These are processed in a number of ways (steaming, roasting, smoking, stewing, curing, and frying, to mention a few) (Ramos-Elorduy *et al.,* 2007) in order to improve their sensory and nutritional qualities as well as their shelf-life (Melgar-Lalanne *et al.,* 2019). The orders of species with greater consumption are Orthoptera, Hemiptera, Coleoptera, and Hymenoptera. The Hymenoptera order includes ants from the Formicidae family, whose diversity is defined by latitude and altitude features (Kusnezov, 1975). The greatest distribution of ants takes place in tropical and subtropical forests of low altitude, as well as in the deserts throughout the world (Brown *et al.,* 1973).

In Mexico, there are five edible ants: Atta cephalotes, Atta mexicana, Myrmecosistus melliger, Myrmecosistus mexicanus, and *Liometopum apiculatum* Mayr (Ramos-Elorduy, 2009). The ant species *Liometopum apiculatum* Mayr is generally obtained during the pupae or larvae stage, known as Escamol or in plural, Escamoles, which are the immature stages of the breeding castes of drones or princesses (Lara-Juárez *et al.,* 2015). The pupae or larvae are eaten in several parts of Central Mexico (Ramos-Elorduy *et al.,* 2007), due to their exquisiteness and nutritional value (Ladrón *et al.,* 1995). They represent a part of the diets and economic incomes of rural communities, and the regional price range between $ 960 pesos ($ 49 USD) and $ 4800 pesos ($ 243 USD) per kilo (Ramos-Elorduy, *et al.,* 2006). Therefore, this study aims to evaluate nutrient content and oil profile of Escamol to ascertain its quality, using proximal chemical analysis and instrumental techniques, respectively.

2. Materials and methods

2.1 Samples

Escamoles (500 g each) were purchased fresh and fried (seasoned with butter), such as they are consumed by the people of the cities of the State of Hidalgo, Mexico. The samples of fresh Escamoles were frozen (-30 °C) and stored until ready for use. Subsequently, these Escamoles samples were thawed at 25 °C. They were dehydrated in a drying oven (Terlab trademark, México) at 70 °C for 4 h. The three Escamoles samples (fresh, dehydrated and butter-fried) were homogenised separately using a blender (Oster Besto2-E01, United States) until a particle size of 250 µm was reached. The Escamoles homogenised samples (fresh, dehydrated and butter-fried) proximal chemical analyses were defined.

2.2 Proximal analysis

The homogenised samples (fresh, dehydrated and butter-fried) Escamoles were analysed to define their moisture (method 926.08), proteins (method 955.04), lipids (method 945.16), and ashes (method 900.02), content according to the methods reported by the Official Association of Analytical Chemistry (AOAC, 2019). The amount of carbohydrates was obtained by the difference. The oil moisture was also determined, and each of the treatments was performed in triplicate.

2.3 Oil extraction

2.3.1 Extraction by Soxhlet

Since the dehydrated and butter-fried Escamoles samples are the two cooking methods most commonly consumed in Hidalgo State, each one of these was placed in an extraction thimble-holder (1 g) to compare its oil. For the oil extraction, the thimble-holder with each one of the Escamoles samples was placed inside the Soxhlet extractor with petroleum ether at 40 °C during 4 h (method 945.16) (AOAC, 2019). The petroleum ether was poured into a round bottom flask attached to an extractor and flask as well as a condenser. The round bottom flask with petroleum ether was heated (40 °C) and this solvent was volatilised. The petroleum ether condensate was poured into the extractor with the thimble-holder. During the extraction, the extractor was gradually filled with condensed solvent. When the solvent reached an overflow level, it produced a siphon with analytes from the entire contents of the thimble-holder and discharged them back into the round bottom flask, and the cycle repeats. The process was run for 4 h (AOAC, 2019). The solvent and oil mixture were stripped using a rotary vacuum evaporator under vacuum at a temperature of 50 °C at a half rotational rate (Büchi, Heating Bath B-490, Mexico) coupled to a vacuum pump (Büchi, Vacuum Rump V-700, Mexico).

2.3.2 Physicochemical analysis of the oil

The quantification of the quality of Escamoles oils was carried out under the Mexican standards such as moisture NOM-116-SSA1-1994 (NMX 1994), acidity NMX-F-101-SCFI-2012 (NMX 2012), iodine NMX-F-152-SCFI-2011 (NMX 2011a), and peroxide NMX-F-154-SCFI-2010 (NMX 2010). Each of the analyses was performed in triplicate.

2.4 Fatty acid identification

In order to identify the fatty acids in dehydrated and butter-fried Escamoles, an Abbe refractometer (ATAGO, Master-RI, USA) was used. The results were compared consulting the table of refractive index of the oils included in the equipment, and the percentages were searched in the corresponding bibliography. The refractive index is the ratio of the sine of the angle of incidence and the sine of the angle of refraction, angles that are formed when passing a light beam from the air into another medium, in which the light propagates at different rates. Each analysis was performed in triplicate.

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2.5 Statistical analysis

The results of the proximal analyses, chemical indexes and refractive indexes are expressed as mean and standard deviation applying a Student T-test for independent samples with a 95% confidence range. The results of dehydrated and butter-fried Escamoles were compared using the statistical software IBM SPSS Statistics version 22 (IBM Corp. Released 2013. Armonk, NY: IBM Corp).

3. Results

3.1 Proximal analysis

Table 1 shows the results of the proximal chemical composition of the Escamoles samples.

The Escamoles results on a wet basis were lower when comparing this percentage with those reported by Ramos et al. (2012) with 60.57-71.90% analysed in the Municipalities of San Juan Teotihuacán and Otumba in Mexico. Hence, the moisture content in the insect depends on climatic and geographic conditions (Juárez *et al.,* 2010). The difference ($p < 0.05$) in the results on a wet basis in the percentage of protein, lipids, ash and carbohydrates is due to the stage of development of the insect (Ramos *et al.,* 2012).

The results of dehydrated Escamol are shown in Table 1, where the changes in its composition are due to the insect's physiology and diet (Ramos *et al.,* 2012). Comparably, butter-fried Escamol samples where the value increased for some parameters reflect the properties of butter, which are 16.17 %, 0.85 %, 81.11 % and 85.11 mg 100 g-1 of moisture, protein, lipids, and ashes respectively (USDA 2018).

3.2 Oil extraction and physicochemical analysis

Table 2 shows the results of the physicochemical analyses of the oils extracted from Escamoles.

The results of the moisture percentage in the dehydrated Escamol oil was high, due to the insect anatomy (Ghosh et al. 2017).

Table 1: Results in percentage of the proximal chemical analysis carried out in the Escamoles

Analysis (%)		Fresh *Escamoles*
Moisture		56.00 ± 0.00
Crude protein		15.30 ± 0.50
Lipids		20.05 ± 0.37
Ash		1.91 ± 0.12
Carbohydrates		6.73 ± 0.00
Dehydrated (D) and Butter-fried (BF) *Escamoles* in dry basis		
Analysis (%)	Cooking methods	Escamol
Moisture	D	1.89 ± 0.38[b]
	BF	2.84 ± 0.00[a]
Protein	D	34.78 ± 1.15[a]
	BF	32.86 ± 0.61[a]
Lipids	D	45.57 ± 0.85[b]
	BF	56.26 ± 1.79[a]
Ash	D	4.35 ± 0.28[a]
	BF	4.38 ± 0.04[a]
Carbohydrates	D	13.41 ± 0.00[a]
	BF	3.66± 0.00[b]

Note: Average ± SD. Different superscript lowercase letters in the same column indicate significant difference ($p < 0.05$) between D: Dehydrated *Escamoles* and BF: Butter-Fried *Escamoles.*

The results of acidity index percentage in the dehydrated oil sample was high due to the moisture percentage in the Escamoles (Rivera *et al.,* 2014). In the determination of the iodine index in the oil obtained from the butter-fried Escamol this value was increased, compared with the oil obtained from the dehydrated Escamol. In the other hand, the peroxide index value of the oil obtained from dehydrated Escamol is within what is represented as maximum value for vegetable oils under the Mexican Standard NMX-F-808-SCFI-2018 with 2.0 meq O2 kg-1 (NMX, 2018). Therefore, the frying affects unsaturated fatty acids, producing free fatty acids and hydroperoxides.

3.3 Fatty acid identification

The identification of the fatty acids in Escamoles oil samples is presented in Table 3.

The Escamol oil extracted from the dehydrated sample presented a refractive index similar to that reported for beeswax. Whereas the Escamol oil extracted from the butter-fried sample had a refractive index similar to that represented for pure edible coconut oil, Table 4 shows its content of fatty acids (NMX, 2011c).

The oil in both Escamol samples had a higher content of saturated fatty acids, so it is suggested that it should be consumed in moderation.

4. Discussion

4.1 Proximal analysis

Table 2: Physical and chemical analyses of the oils extracted from Escamol

Index	**Cooking methods**	***Escamol***
Oil moisture (%)	D	1.53 ± 0.25^a
	BF	0.04 ± 0.05^b
Acidity index (% oleic acid)	D	17.48 ± 0.00^a
	BF	3.38 ± 0.00^b
Iodine index (cg I_2 g^{-1})	D	112.00 ± 8.59^b
	BF	133.79 ± 0.72^a
Peroxide index (meq O_2 kg^{-1})	D	0.80 ± 0.00^b
	BF	3.60 ± 1.69^a

Note: Average ± SD. Different lowercase superscript letters in the same column indicate significant difference ($p < 0.05$) between D: Dehydrated Escamoles and BF: Butter-Fried Escamoles.

Table 3: Results of the determination of refractive index in the oils Escamoles

Escamoles	**Refractive index**
Dehydrated	1.442 ± 0.00^b
Butter-Fried	1.447 ± 0.00^a

Note: Average ± SD. Different lower case superscript letters in the same column indicate significant difference ($p < 0.05$) between Dehydrated Insect Oil and Butter-fried Insect Oil.

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Due to its moisture content, Escamol favours the growth of microorganisms and biological reactions, resulting in the acceleration of the enzymatic activity, hydrolytic reactions, non-enzymatic darkening and lipid oxidation, so it is necessary to establish a preservation method such as dehydration or frying (Badui, 2013). Escamol in the pupa stage contains more lipids than during other stages, and this is similar to other insects in the pupa stage (Rumpold & Schlüter, 2013). The ash and carbohydrate percentage are affected by the insect diet (Ramos *et al.,* 2012) as shown in Table 1.

Table 1 shows that the percentage of protein in Escamol samples are within the parameter indicated for the order Hymenoptera (bees, wasps, and ants) of 10.00-81.00% and a percentage higher compared to beans 23.00% and lentils 27.00%. Also, it presents a lower percentage compared with chicken 43.00%, soy 44.00%, egg 46.00% and beef 54.00% (Conconi, 1993). Therefore, the protein found in Escamol, can be an alternative to meet protein requirements. On the other hand, the percentage of proteins in the butter-fried Escamol sample was lower compared to the dehydrated samples. However, this difference was not statistically significant. Besides, during frying, the absorbed fat increases until the food is saturated and present minimal losses of proteins, carbohydrates, and minerals (Bognár, 1998). These losses are due to long frying times and the use of temperatures above boiling temperatures that cause a decrease in the protein content (Zhang *et al.,* 2014) or the loss of nitrogen in the form of volatile nitrogen compounds (Yu *et al.,* 1993).

As for the percentage of Escamol lipids, its concentration was lower than those reported for the Hymenoptera order with 55.10 % (Rumpold & Schlüter, 2013). In another study carried out in Hidalgo, Estado de México and Puebla, Mexico, a percentage of 29.45-47.98 g 100 g-1 on a dry basis was reported (Ramos *et al.,* 2012). Hence, its concentration depends on factors such as species, reproductive stages, age (life stage), the season of year, habitat and diet (Raksakantong *et al.,* 2010). On the other hand, Table 1 shows that the lipids percentage increased due to the thermal process. During this process, a heat transfer occurs (Dana & Saguy, 2006), which causes dehydration (bark) and a wet core that when it cools, water vapour condenses inside the food, affecting the decrease in inner pressure, which exerts a vacuum effect, thus absorbing the lipids (Cortés *et al.,* 2014).

Table 4. Fatty acid content in pure edible coconut oil

Fatty acids	Percentage
Saturated fatty acids	
Caproic (C6:0)	0.00-1.2
Caprylic (C8:0)	5.50-9.50
Capric (C10:0)	4.50-9.50
Lauric (C12:0)	41.00-56.00
Myristic (C14:0)	13.00-19.00
Palmitic (C16:0)	7.50-12.00
Stearic (C18:0)	1.00-4.00
Monounsaturated fatty acids	
Oleic (C18:1)	3.50-11.00
Polyunsaturated fatty acids	
Linoleic (C18:2)	1.00-2.5

Content in percentage according to the Mexican standard (NMX, 2011c).

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The ash values of the Escamol were similar, and are within the percentage indicated for the Hymenoptera order with 0.71-9.31 % (Rumpold & Schlüter, 2013) and the following minerals have been indicated: calcium (174.8 µg g-1), magnesium (455.3 µg g-1), sodium (823.6 µg g-1), potassium (3182.7 µg g-1), zinc (52.7 µg g-1), copper 9.3 (µg g-1) and iron (0.8 µg g-1) (Ramos *et al.,* 2012). Therefore, the Escamoles could be a source of mineral for diet. The results of the carbohydrate percentage in dehydrated Escamoles were high than the results reported in Hymenoptera order 7.15 % (Sun *et al.,* 2007). It is observed that in the butter-fried Escamol, this compound decreased, due to the Maillard reaction during frying (Torres-Cifuentes *et al.,* 2015).

4.2 Oil extraction and physicochemical analysis

The moisture percentage is a factor that accelerates the degradation of the unsaturated fatty acids, so it is necessary to reduce it, using a pre-treatment or during the oil refinement (degumming method) (Ariza *et al.,* 2004). According to the Mexican Standard for an edible vegetable oil NMX-F-808-SCFI-2018, it should have a maximum moisture concentration of 0.05 % (NMX 2018) (Table 2), and the butter-fried Escamol oil sample had a lower percentage due to this process (Dana & Saguy, 2006).

The results of the acidity index in the Escamol showed there are degraded chains of carbon atoms from long to short carbon structures (Rangaswamy & Nasirullah, 2016). Compared with butter-fried Escamol, this value is due to the interaction of lipids with degradation products with degradation products of the Maillard reaction, which cause the formation of various volatile chemical compounds (Yu *et al.,* 1993; Badui, 2013; Zhang *et al.,* 2014). It has been reported that an excess of free fatty acids is related to resistance to insulin, fatty liver disease, atherosclerosis and myocardial dysfunction (Vilarrasa, 2014).

The iodine index determined in the Escamoles oils extracted from butter-fried was high, probably due to the presence of vitamin A (684 µg 100 g-1) and vitamin E (2.32 mg 100 g-1) in the butter (USDA, 2018). The peroxide index in oil extracted from dehydrated Escamol indicated that this oil presented minimal changes in its composition of unsaturated fatty acids compared with oil obtained from the butter-fried Escamol. So, this oil is not suitable for consumption due to the formation of peroxides, which causes the presence of rancidity and free radicals that can increase the risk of developing degenerative diseases (Okparanta & Solomon, 2018).

4.3 Fatty acid identification

The Escamol oil extracted from the dehydrated sample presented a refractive index similar to that reported for beeswax, in that it could contain a high concentration of saturated fatty acids (85%), compared to monounsaturated fatty acids: palmitoleic acid [C16:1 (12.00 %)] and oleic acid [C18:1 (5.00 %)] (Jackson & Eller, 2006; Trillo, 2017). On the other hand, the Escamol oil extracted from the butter-fried sample is similar to that reported for pure edible coconut oil (NMX, 2011c). As observed in Table 4, it could contain mainly saturated fatty acids that are necessary for metamorphosis (Ying *et al.,* 2009).

Escamol oil contains saturated fatty acids, most of which are C12:00 and C14:0. The fatty acid C12:0 has antiviral and antibacterial health benefits for the human body (Abbas *et al.,* 2017). C14:0 can slightly decrease the ratio between total cholesterol and high-density lipoprotein cholesterol (Orsavova *et al.,* 2015). Among the unsaturated fatty acids that we can find in Escamol oil are C18:1 and C18:2. The former, C18:1 reduces the plasma concentrations of total cholesterol, and at low concentrations, C18:2 does not have an inflammatory effect (Nagy & Tiuca, 2017).

5. Conclusion

The butter-frying process in the Escamoles increased the percentage of lipids and decreased the percentage of proteins and carbohydrates, which caused changes in the oil, increasing the concentration of hydroperoxides. The dehydrated Escamoles presented high concentrations of saturated fatty acids, so it is suggested to use another type of lipid for frying.

Conflict of interest

The authors declare no conflict of interest.

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