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Consumption of Escamoles (*Liometopum apiculatum M.*): A Source of Vitamins A and E

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MRV and QBT designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GUC managed the literature searches. Author DGR managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess lipids and fat-soluble vitamins A and E in Escamoles and inform the population about the benefits the intake of these edible insects may provide for human health.
Study Design: A combined protocol with both sampling and analytical procedures was used.
Place and Analysis: Escamoles were collected at "El Cardonal", located at northwest of the State of Hidalgo, in spring (2014). Nutrient content analysis was performed according to AOAC methods and High Performance Liquid Chromatography (HPLC).
Methodology: Sample: Conventional sampling was performed during the second week of March, 2014. Escamoles analysis: Lipid content was determined according to AOAC methods by the semicontinuous solvent extraction method. Fat-soluble vitamin contents (A and E) were determined by High Performance Liquid Chromatography (HPLC) with a 5-point calibration curve with external standards (0.66 to 6.60 mg/L and 30.22-302.18 mg/L of all-trans-retinol and α-tocopherol, respectively).

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Results: The analysis showed that Escamoles contain: Lipids, 34.96 g/100 g dry sample; Vitamin A, Retinol, 0.3024 mg /100 g and Vitamin E, α-tocopherol, 3.29 mg/100 g.
Conclusion: Escamoles consumption, as a source of vitamins A and E may help populations living in rural and semi-arid zones, improve their health.

Keywords: Escamoles; edible insects; health; immune system; nutrition.

1. INTRODUCTION

The association between malnourishment and infectious disease epidemics has been reported throughout history; poorly nourished people are more suceptible to infectious disease, since malnourishment impairs the immune system, affecting fundamental immune functions that provide protection against pathogenic organisms [1], probably due to an insufficient intake of macronutrients or to specific-micronutrient deficiencies, such as vitamin A and E [2]. The impact of malnourishment is a great concern in developing and developed countries. The particular effect of each nutrient on different aspects of the immune function has been difficult to study; however, many nutrients have specific roles on the immune response and each nutrient has a different concentration range over which it provides an optimal function. Lower or higher levels of the needed nutrient might impair the immune function [3]; therefore, the adequate performance of the immune system is directly related to nutrient consumption, so a balanced diet is required to maintain adequate defenses against bacteria, viruses and parasites [4].

Consumption of edible insects in Mexico [5,6], practiced mostly by tradition, is increasing day by day not only in rural communities but also in urban areas; Escamol ant eggs *(Liometopum apiculatum M.)* can be found in arid zones; nests are found in an xerophyte thicket environment, nearby maguey *agave sp*. or nopal *opuntia sp* cacti, which are sources of sugars, aminoacids, and minerals. These insects are high demanded in high-social class restaurants and are known as the Mexican caviar due to their delicious taste and the great difficulty in harvesting; only the most skilled collectors can extract them without damaging the nest.

However, people living in rural communities do not take advantage of these edible insects, at least not for nutritional purposes, but more as a tradition or an opportunity to earn money from their sale.

Previous studies have demonstrated that insects are a good source of macro and micronutrients making these insects a good option for the improvement of health and nutritional status of people living in rural and semi-arid zones [7-9]. The aim of this study was to assess the content of lipids and fat-soluble vitamins A and E in Escamoles ant eggs *(Liometopum apiculatum M.)* and to inform people living on areas where Escamoles are available (collectors and inhabitants of State of Hidalgo) by means of personal communication, of the benefits they could provide to their nutrition and health.

2. MATERIALS AND METHODS

2.1 Sampling

Conventional sampling [10] was performed during the second week of March, 2014, in the State of Hidalgo, which is considered one of the states with the most arid zones (39 % of the State) in Mexico. It is located at an altitude of 2100 meters above sea level (masl) with an arid and semiarid climate (BS kw) with xerophyte thicket vegetation (Fig. 1). The State of Hidalgo was visited to locate insect collection sites and to find people specialized in harvesting and handling Escamoles ("Escamoleros") to assist in conventional sample collection. Escamoles availability was determined while visiting the State of Hidalgo and monitoring different locations such as: El cardonal, Actopan, Santiago Anaya, Tulancalco, Huichapan, Apan, Tulancingo and Santuario. Insects were harvested in "El Cardonal", located northwest the State of Hidalgo, early in the morning from underground nests near maguey cacti *(Agave sp.)*.

Six nests were sampled and a 250 g sample was collected from each and pooled. Afterwards, Escamoles were whased and stored in glass containers on ice for further transportation by land to the laboratory for analysis. The time that passed between collection and analysis was about 24 hours.

Fig. 1. Location of escamoles (ant eggs). State of Hidalgo (left), at "el Cardonal", located northwest State of Hidalgo (right) with an altitude of 2100 masl (meters above sea level), BS kw climate (arid and semiarid climate)

2.2 Nutrient Content Analysis

2.2.1 Determination of moisture content

Moisture content of the sample was determined in triplicate using the direct drying method. Homogenized sample by weight of each organism (10 g) was dried in an oven at 60℃ for 24 hrs. The samples were powdered in a mortar, then passed through a 60 mesh. The fine powder obtained this way was used for further analysis.

2.2.2 Determination of lipid content

Lipid content determination was performed by the semicontinuous solvent extraction method [11] as follows: the sample (10 g) was extracted with 180 mL petroleum ether on a Soxhlet device (Sigma-Aldrich, México, México) for 10 h. Petroleum ether was removed by evaporation and the lipid residue was weighed. All samples were analyzed in triplicate and the results are expressed as g/100 g dry basis of sample.

2.2.3 Determination of Vitamins A and E

Quantification of lipid-soluble vitamins was performed in triplicate by weighing a homogenized sample (0.5 g±0.05 g) into a 50 mL centrifuge tube; then, 2 mL of ethanolic pyrogallol (2%) and 2 mL of ethanolic potassium hydroxide (10%) were added. After vigorous shaking, samples were incubated in the dark for 18 h. The sample was then shaken and 1 mL of the mixture was transferred into a 15 mL conical tube. 2 mL of petroleum ether and 1.5 mL of nanopure deionized water were added, the sample was mixed. After the two phases separated, the organic layer was transferred into another 15 mL conical tube which was then wrapped in aluminum foil to minimize light exposure. The extraction step was repeated twice more with 2 mL of hexane only and the extracted organic phases were pooled. If an emulsion occurred during the extraction, ethanol was added. The organic phase was then dried under nitrogen. The sample was redissolved in methanol and filtered through a Sri Titan PTFE filter (0.45 µm), the final volume was 400 µL. For HPLC analysis, 50 µL of both retinyl acetate (2 mg/L) and α-tocopherol acetate (150 mg/L) were added to correct variations in the injection volume. Retinol and α-tocopherol standards at three concentrations were treated as samples to determine recovery; mean recoveries were 85.4% and 39.5%, respectively. HPLC analysis was performed on a Supelcosil LC-18 (250 x 4.6 mm i.d., 5 µm) (Supelco, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) equipped with a Pelliguard LC-18 guard column (Supelco, Sigma-Aldrich Canada Ltd.) connected to a Gold HPLC system (Beckman Coulter, Mississauga, ON, Canada) with a programmable pump (Model 126), a diode array detector (Model 168) and a Gilson auto injector (model 231 XL). The sample (100 µL) was eluted with 100% methanol at a flow rate of 1 mL/min and monitored at 325 and 280 nm for vitamins A and E, respectively. Peak areas were determined by the HPLC system building software (Gold version 8.1 Beckman Coulter) and the concentrations were calculated with a 5-point calibration curve with external standards. The external standard concentrations ranged from 0.66 to 6.60 mg/L and 30.22 to 302.18 mg/L of all-trans-retinol and α-tocopherol, respectively [11,12].

3. RESULTS AND DISCUSSION

3.1 Sampling

Escamoles *(Liometopum apiculatum M.)* reproduce in arid zones (Fig. 1) during spring between February and May (Table 1); eggs can be found at an altitude range from 1800 to 3000 masl (meters above sea level) in nests built underground 1.00 to 1.50 m deep, for minimum humidity and a warm environment for eggs' reproduction and growth. Nests can be located at the intersection of two paths nearby maguey *(Agave sp.)* or nopal *(Opuntia sp.)* cacti, such plants act as sources of sugars and amino acids, foodstuff for insects [13] (Fig. 2).

Table 1. Escamoles availability, 2014 in the State of Hidalgo

Month	J	F	M	A	M	J	J	A	S	O	N	D
Availability		X	X	X	X							

**X= Month availability of Escamoles in the wild in the State of Hidalgo. Data obtained directely from local people, "Escamoleros" after monitoring the area*

Fig. 2. Escamoles nest and trabecula

Ants maintain a relationship with their habitat diversity and preserve the ecological equilibrium where they live [14], so eggs were carefully separated from the nest (trabecula) and the nest with some eggs was put back in the hole and covered with grass to ensure next season's production and to preserve the ecological equilibrium. Escamoles for analysis were washed and kept in a glass container on ice with some adult ants for taxonomy identification [15] (Table 2) and transported by land to the university (UAM-X) for lipid and vitamin A and E quantification.

Table 2. Escamoles (*Liometopum apiculatum M.)* taxonomy

Escamoles, ant eggs	
Class	Insecta
Order	Hymenoptera
Family	Formicidae
Genus	*Liometopum*
Specie	*apiculatum* M
Common name	Escamoles

3.2 Nutrient Content Analysis

3.2.1 Determination of moisture content

As it can be seen in Table 3, the moisture content analysis of Escamoles showed that almost half (51.12 ± 0.7%) of their weight is water.

3.2.2 Determination of lipid content

Mostly, lipid consumption is associated with obesity and other health problems; current recommendations regarding quantitative fat intake call for a reduction in total and saturated fat intake. The basis for such recommendations comes from epidemiological evidence indicating that body weight is positively correlated with quantitative fat intake and not with carbohydrate intake. Additionally, research has showed that increasing the fat content on a diet promotes fat storage in adipose tissues rather than fat oxidation. However, not all evidence establishes a link between fat intake and obesity. Thus, it may be more effective to focus on qualitative fat consumption (decrease saturated fat and increase unsaturated fat) combined with recommendations to restrict energy intakes. Such an approach would acknowledge the importance of lipids, which do play an important role in nutrition, as well as in metabolic actions and not only as a source of energy; for example, for an adequate absorption of fat-soluble vitamins (A, D, E, and K) it is necessary the formation of chylomicrons, lipoproteins that are essential for the absorption of vitamins in the intestine and further distribution, so the lack of lipids may lead to vitamin deficiency disorders [16].

Nevertheless, an excesive consumption of lipids will lead to health problems, such as coronary heart disease and stroke.

The lipid content of Escamoles is about 34.96 g /100 g dry sample (Table 3), thus, Escamoles have enough lipids to allow fat-soluble vitamins absorption through the intestine. Additionally, in a previous study performed on Escamoles by our research group [9], the main lipids found were oleic acid (67.66%), linoleic acid (2.61%), and arachidonic acid (0.16%), which are unsaturated fatty acids. These have an important role in different processes; oleic acid is a precurssor for synthesis of linoleic acid and has antioxidant properties that protect biological membranes from free radicals. Linoleic acid is an essential nutrient and must be included in the daily diet because it is part of the membranes from different tissues and it is a precursor for araquidonic acid, which is necessary for the strength of mitochondrias and for the synthesis of prostanglandins [17].

3.2.3 Determination of Vitamins A and E

Vitamins are a dispare group of compounds; they have little in common both chemically and in their metabolic funcions. Nutritionally, they form a cohesive group of organic compounds requiered in small amounts (milligrams or micrograms per day) to maintain a good health and metabolic integrity [2].

Vitamin A has four metabolic roles; it acts as a prostetic group of the visual pigments; as a nuclear modulator of gene expression; as a carrier of mannosyl units in the synthesis of hydrophobic glycoproteins, and in the retinoylation of proteins. On the other hand, vitamin E plays a role in the modulation of gene expression and regulation of cell proliferation, suggesting that the potential beneficial effects of vitamin E against heart disease and cancer may not be due to its antioxidant action [2]. In addition, the functions of fat-soluble vitamins are related to immune processes for normal functioning of phagocytes and activation of T- and B-lymphocytes and with cell walls protection from damaging reactive molecules associated with the response of the innate immune system [18]. In this context, vitamin A is a lipid-soluble micronutrient whose deficiency impairs immune functions and decreases host resistance to infections. For example, vitamin A deficiency impairs both the innate and adaptive immune responses to infection. In particular, mucosal integrity is significantly impaired, so its deficiency compromises the mucosal epithelial barriers found in the conjunctiva of the eye, as well as in the respiratory, gastrointestinal, and urogenital tracts. This loss of the protective mucus diminishes resistance to infection by pathogens that would ordinarily be trapped in the mucus and swept away by the cleansing flow of mucus out of the body. On the other hand, Th2-mediated responses are compromised. These alterations in host defense increase the risk of death from common infections in children and pregnant women. Correcting vitamin A deficiency decreases mortality from diarrhea and other infections [18-20]. Thus, vitamin A content in Escamoles may help to diminish the incidence and severity of infectous diseases.

Regarding the lipid-soluble vitamin E, it has been demonstrated that free radicals and lipid peroxidation suppress immune responses; Vitamin E is the major antioxidant in the body and it is requiered for the protection of membrane lipids from peroxidation. Additionally, α-tocopherol is the most active form of vitamin E and has affinity for phospholipids of the mitochondria, endoplasmic reticulum, and plasma membrane, and it constitutes the first line of defense against the peroxidation of polyunsaturated fatty acids contained in these phospholipid membranes. When interacting with the cellular membranes, the reactive oxygen species (ROS) break the polyunsaturated fatty acids that compose the membrane. The tocopherols can neutralize these reactions via the donation of phenol hydrogen to the peroxil free radical of the polyunsaturated fatty acid [18,21], thus, vitamin E is considered an element that optimizes and enhances inmune system response.

Vitamins intake recommendations for adults are: for vitamin A, 0.6 mg/day and for vitamin E, 200 mg/day [18].

Vitamin analysis showed that Escamoles have vitamin A (0.3024 mg retinol/100 g) and vitamin E (3.29 mg/100 g) (Table 3), so they are a good source of these vitamins when compared with the main components of the diet of people living in rural semi-arid areas, such as black beans (0.00 mg retinol /100 g), potatoes (0.00 mg retinol /100 g), zucchini (0.025 mg retinol /100 g), carrots (0.835 mg retinol/100 g), bananas (0.018 mg retinol/100 g and 0.001 mg tocopherol/100 g), oranges (0.012 mg retinol/100 g and 0.00017 mg tocopherol/100 g) and edible cactus (0.130

mg retinol/100 g) [22]. They are an option to fulfill the nutritional requirements in people who live in rural and semi-arid zones to maintain a good health.

Table 3. Escamoles (*Liometopum apiculatum M.)* nutrient content

Nutrient	Content
Water	51.12 ± 0.7%
Dry sample	48.88%
Lipids	34.96 g/100 g dry sample
Vitamin A	0.3024 mg /100 g
Vitamin E	3.29 mg/100 g

On the other hand, even though inhabitants of rural communities consume Escamoles, they do it mainly for tradition but not as a part of their daily diet because they do not know of the benefits they could obtain from them. Most of the time people collect the insects in order to sell them due to the high demand of Escamoles in restaurants.

In addition, it is important to consider that the serving size of Escamoles in restaurants is around 10-15 g. This means that the amount of consumed nutrients is small; the reason for this is that Escamoles are usually eaten as an entree and not as the main dish. Thus, in order to obtain the real benefits from Escamoles, it would be necessary to include them as part of the regular diet in combination with the rest of the available foods, then Escamoles may improve the regular diet of people living in rural and arid areas.

Nevertheless, there is not enough information regarding the nutritional benefits that could be obtained from these edible insects; subsequently, by spreading this information and teaching locals how to preserve and care for these animals, people could include them as part of their diet, improving their health and maybe, it might create new job opportunities.

4. CONCLUSION

Escamoles are an additional source of essential fat-soluble vitamins A and E, as well as lipids that will help with the absorption of these micronutrients.

Escamoles consumption could help increase human health contributing to a balanced diet.

Due to their micronutrient contents, which play an important role on immune system functions, Escamoles are a good option for individuals in rural areas, where proteic-caloric malnutrition has a high impact, making them susceptible to infectious diseases due to the lack of essential nutrients requiered for an appropiate immune response.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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