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Characteristics of Maize Flour Tortilla Supplemented with Ground *Tenebrio molitor* Larvae

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The larva of the *Tenebrio molitor*, known as the yellow meal worm, is a plague of wheat and flours. Consumption of the raw insects is not well accepted because of their appearance. The objective of the present work was to grow *T. molitor* larvae under standard conditions, to analyze the chemical composition of the larvae powder, and to prepare supplemented maize tortillas. Protein and fat contents were performed with standard methods. *Tenebrio* larvae powder had a 58.4% protein content; this protein was rich in essential amino acids such as phenylalanine, tyrosine, and tryptophan; the found values satisfied those recommended by the Food and Agriculture Organization. Fatty acid composition was determined by GC-MS showing high contents of oleic acid and linoleic acid (19.8 and 8.51%, respectively). A large proportion of unsaturated fatty acids of longer chains was detected. Long-chain fatty acids having two or three double bonds have been claimed as highly beneficial to health. Tortillas supplemented with larvae powder had excellent consumer acceptance, and tortilla protein content increased by 2% as well as the amount of essential amino acids. These results show new ways to consume insects and at the same time increase the nutritional value of the original food products.

KEYWORDS: ***Tenebrio molitor*; maize tortilla; fatty acids**

INTRODUCTION

Although insects have been eaten for millennia worldwide, they are generally consumed in very poor countries. In southern Mexico "escamoles" (black ant larva) and "ahuahutle" (eggs of water worms) are part of many ethnic dishes (*1*). In London it is possible to find canned fried bees, grasshoppers, and caterpillars (*2*). In the Occidental world, insects are consider as delicacies and the pleasure of eating insects is known as "entomophagia"; however, in the Western world they are considered as the last option to eat, but due to their accessibility, nutritional value, and flavor, insects are taking on an important role in the human diet (*3*, *4*). Very often insects are rich in proteins, fats, minerals, and vitamins; in addition, the chitin forming the exoskeleton may be a source of dietary fiber (*1*).

The adult beetle of *Tenebrio molitor* L. (Coleoptera:Tenebrionidae) is dark brown and is 12−20 mm in length. The larva ("worm") is 2.5−3.5 cm in length with a weight of ∼0.2 g; the optimum growth temperature is from 25 to 27 °C (*5*, *6*). *Tenebrio* is a wheat, flour, and cereals plague, causing nearly 50% loss of production in the Third world (*7*). Although insecticides are used to stop the contamination, these chemicals could be very dangerous to human health (*6*). There is little information about the nutritional characteristics of the insects (*8*, *9*), and for this reason we grew *T. molitor* larvae in clean cereal sources; the larvae powder was used as a supplement in the preparation of maize flour tortillas. The results obtained in this work give more information about *Tenebrio* characteristics and its possible uses in food preparation.

MATERIALS AND METHODS

The *T. molitor* beetle strain used was obtained from the insect collection of the Entomology Department located at INIFAP-Celaya, Guanajuato, Mexico. Massive growth of *T. molitor* larvae was carried out in a 40 × 30 × 25 cm plastic container with a screen cover. The larvae were grown on a sawdust bed, containing oat and corn flakes, dry bread, and pieces of vegetables as water supplement. The larvae were collected when they were 25−30 mm in length. After that, larvae were submerged in a boiling water bath for 3 min, dried in an oven at different temperatures (60, 70, and 100 °C), and milled (Tekmar A 10). The powder was kept at 4 °C until used.

Proximate Composition. Proximate analysis of larvae powder from *T. molitor* was determined according to AACC standard procedures

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(*10*). The protein factor used was 6.25. Microbiological analysis was carried for total count on agar standard and agar Tergitol 7, as selective medium for coliforms. Several dilutions were made, and 100 μL was plated in Petri dishes and incubated at 37 °C for 72 h (*11*).

Protein Fractionation Procedures. Fractionation of larvae protein powder was carried out according to the method of Osborne (*12*), with some modifications. Suspensions of larvae powder/water (1:10 w/v) were stirred for 3−4 h at room temperature and centrifuged at 13000*g* for 15 min at 18 °C. The supernatant was called albumin fraction. The pellet was resuspended with a solution of 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and stirred as before. The resulting supernatant was designated globulin, and the pellet was resuspended with 70% aqueous 2-propanol (2PrOH) and extracted under stirring. After centrifugation, the resulting supernatant was designated prolamin fraction and the pellet was resuspended in a solution of 0.1 M NaOH. After centrifugation, the supernatant was designated the glutelin fraction and the pellet was called a residue (*13*, *14*).

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (*15*) with reduction of the protein with 2-mercaptoethanol (2ME) in 15% polyacrylamide gels. Protein samples (1 mg/mL) were dissolved in 0.1 M Tris-HCl (pH 6.8) and 2% (w/v) SDS. Electrophoresis was conducted at a constant current of 20 mA for 2−3 h. The gel was stained with an ethanol/acetic acid/water (45:45:1 v/v/v) solution containing Coomassie Brilliant Blue R250 at a final concentration of 0.25% (w/v). Destaining was achieved by washing the gel during 2 h with the same solution but without the dye. Molecular weights of protein subunits were calculated using a 10 kDa protein ladder (Gibco-BRL, Life Technologies, Gaithersburg, MD).

Amino Acid Analysis. Amino acid analysis was performed by reversed-phase high-performance liquid chromatography (RP-HPLC). Protein samples (100 mg) were hydrolyzed with 6 N HCl at 150 °C for 24 h. After filtration, 25 μL was used for derivatization with phenyl isothiocyanate, and derivatized samples were analyzed in a C_{18} Pico-Tag column (*16*). Data were integrated using a model 19-740 integrator (Waters Chromatography, Millipore Corp.). Duplicated runs were carried out, and mean values are reported.

Fatty Acid Analysis. Total fat was extracted following the in situ procedure reported by Park and Goins (*17*). Methanol/dichloromethane (2:1) was used as solvent, and 0.01 mg of heptadecanoic acid was used as internal standard. After 30 min of sample sonication, an aliquot of 100 μL was derivatized with a mixture of NaOH/MeOH (0.5 M) at 90 °C for 30 min. The samples were cooled at room temperature, and a 14% solution of BF_3/MeOH was added, followed by further heating at 90° for another 30 min. Fatty acid methyl esters were extracted with hexane, taken to dryness, and resuspended again with 50 μL of isooctane. One microliter of each sample was analyzed by gas chromatography coupled to mass spectrophotometry (GC-MS) using a 50 m capillary column SP-2330 and helium gas as the carrier.

Maize Tortilla Preparation. Maize tortillas were made using 14 g of commercial maize flour (Maseca) each with the addition of 1 g of larvae powder. Sensory evaluation of flavor and texture was conducted by applying the triangular test of differences (*18*), with 18 trained judges. Two replications of the test were run. Data were analyzed by using the exact probability tables (*19*, *20*). An opinion survey with the judges after the triangular test was conducted to have an indication trend to the acceptance of the product.

RESULTS

The best conditions to grow *T. molitor* larvae included pieces of vegetables such as carrots and lettuce added to a water source at 28 °C. The larvae were collected when they were 2.5−3 cm long (around 3−4 months). The production of larvae was ∼150 g for each plastic container; it has been reported that under very careful conditions, the production can reach up to 5250 larvae/weak (*9*). Growing *T. molitor* larvae at home ensures that they are clean and free from pesticides or any other chemicals that could have harmful effects on human health (*21*). After the larvae had been boiled, less color was developed by using a temperature for drying them of 60 °C; at higher temperatures larvae take on a dark-brown color. In Table 1 is shown the proximal composition of *T. molitor* larvae powder compared with those of maize flour and meat. The total protein content of larvae powder was 58.5% (w/w dry basis); a similar value has been reported for meat (*22*), but larvae powder protein content was very high compared with that of maize flour (10.2% w/w dry basis). *Tenebrio* larvae powder presented a medium protein content value as compared with other insects such as honey-ants (94.5%) and black bees (81.7%). For "jumiles" (a type of small, smelly, brown Mexican beetle known vulgarly as "bedbug of the mountains") and grasshoppers values of 36.1 and 31.9% have been reported, respectively (*4*, *21*). According to Osborne's solubility, it was found that the main protein fraction of *Tenebrio* larvae powder corresponded to glutelins. The main band observed in the glutelins fraction was located at ∼97 kDa. Similar findings have been reported (*4*), but more studies should be done to characterize *Tenebrio*'s main protein.

Table 1. Proximal Composition of *Tenebrio* Larvae Powder, Maseca Flour, and Meat (Percent, Dry Basis)[a]

component	larva flour	maseca flour	meat[b]
protein	58.4 ± 0.3	10.2 ± 0.1	68.5
fat	32.4 ± 0.2	4.5 ± 0.2	30.2
fiber	6.3 ± 0.2	1.2 ± 0.1	
ash	3.0 ± 0.1	1.4 ± 0.0	
carbohydrates[c]		82.7	

[a] Means of triplicates ± standard deviation. [b] Paul and Southgate (*22*). [c] Determined by difference.

Table 2. Amino Acid Composition of *Tenebrio* Larvae Powder, Maize, and Meat (Grams per 100 g of Protein)

				FAO/WHO/UNO (*23*)	
amino acid	larvae[a]	maize[a]	meat[b]	child	adult
isoleucine	2.6 ± 0.1	3.7 ± 0.2	4.8	2.8	1.3
leucine	4.6 ± 0.1	12.5 ± 0.1	7.4	6.6	1.9
lysine	1.6 ± 0.3	2.7 ± 0.2	8.5	5.8	1.6
methionine + cysteine	1.6 ± 0.2	3.5 ± 0.2	3.8	2.5[c]	1.7[c]
phenylalanine + tyrosine	7.5 ± 0.2	8.8 ± 0.2	7.8	6.3[d]	1.9[d]
threonine	2.7 ± 0.2	3.7 ± 0.1	4.3	3.4	0.9
valine	3.8 ± 0.1	4.1 ± 0.2	5.1	3.5	1.3
histidine	2.1 ± 0.2	2.7 ± 0.3	3.2	1.9	1.6
tryptophan	1.8 ± 0.1	0.6 ± 0.1	1.1	1.1	0.5

[a] Mean of duplicates ± standard deviation. [b] Paul and Southgate (*22*). [c] Requirements for methionine + cysteine. [d] Requirements for phenylalanine + tyrosine.

Amino Acid Composition. The amino acid content of *Tenebrio* larvae powder showed the requirements of essential amino acids (Table 2) as reported by FAO/WHO/UNU (*23*). Larvae powder had high phenylalanine + tyrosine (7.7 g/100 g of protein) and tryptophan contents (1.8 g/100 g of protein). It has been reported that the maguey worm is rich in leucine, isoleucine, and valine and that jumiles are rich in tryptophan (1.5 g/100 g of protein). Grasshoppers are rich in threonine with values of 4.9 g/100 g of protein (*4*).

Fatty Acid Composition. The fatty acid analysis (Table 3) showed an extraordinary composition of fatty acids of long chain (C_{18}−C_{24}), oleic acid ($C_{18:1}$) being the main component followed by linolenic acid ($C_{18:2}$) and palmitic acid (C_{16}) with values of 19.8, 8.5, and 6.8%, respectively. Similar results have been reported (*24*). Small but significant amounts of fatty acids such as linolenic ($C_{18:3}$) (known as fatty acid ω3) were found. Omega

Table 3. Fatty Acid Composition of *Tenebrio* Larvae Powder[a]

fatty acid	mg of fatty acid/ 100 mg of sample	fatty acid	mg of fatty acid/ 100 mg of sample
C_{12}	0.16 ± 0.013	$C_{18:1}$	19.77 ± 0.988
C_{13}	0.10 ± 0.005	$C_{18:2}$	8.51 ± 0.223
C_{14}	1.77 ± 0.081	$C_{18:3}$	0.11 ± 0.010
C_{15}	0.09 ± 0.006	C_{20}	0.08 ± 0.008
C_{16}	6.76 ± 0.024	$C_{20:1}$	0.03 ± 0.002
$C_{16:1}$	1.51 ± 0.013	$C_{20:2}$	0.02 ± 0.000
$C_{16:2}$	0.13 ± 0.009	C_{22}	0.01 ± 0.000
C_{18}	1.46 ± 0.060	C_{24}	0.01 ± 0.003

[a] Composition of fatty acid methyl esters was obtained by GC-MS as indicated under Materials and Methods. Mean of triplicates ± standard deviation

Table 4. Amino Acid Composition of Maize Tortilla and Maize Tortilla Supplemented with *T. molitor* Larvae Powder (Grams per 100 g of Protein)

amino acid	tortilla	
	control	supplemented
isoleucine	3.1 ± 0.1	5.8 ± 0.1
leucine	10.2 ± 0.1	11.1 ± 0.2
lysine	2.9 ± 0.2	4.4 ± 0.1
methionine + cysteine	4.1 ± 0.3	7.1 ± 0.2
phenylalanine + tyrosine	5.5 ± 0.1	8.9 ± 0.1
threonine	2.7 ± 0.1	4.5 ± 0.1
valine	3.5 ± 0.1	5.0 ± 0.1

Table 5. Results from Triangular Test[a]

test	judgment		signif level[b] (p)
	total	correct	
texture	36	21	0.002
flavor	36	21	0.002

[a] Sensory evaluation was conducted with 18 trained judges in duplicate. Correct judgments mean the assessors perceived differences between samples. [b] Significance level according to the tables from Roessler et al. (*19*).

acids are found mainly in sea products, and currently they are considered as nutraceutical components, having a wide range of beneficial effects in human health (*25*). It is interesting to note that insects containing essential fatty acids are generally located in sea species.

Microbiology Analysis. Microbiology results showed very low colony-forming units (cfu), and there was no detection of coliforms in the selective agar. These results were considered to be satisfactory because they have the microbiology quality required (*26*).

Tortilla Preparation. The resulting tortilla obtained from the addition of larvae powder to maize flour, although a little darker than the control, had good acceptance. In addition, the supplemented tortilla had a 2% increase in total protein and a 1% increase in fat content. Also, a considerable increase in essential amino acids was detected (Table 4). The triangular test showed a significant difference ($p = 0.002$) between the larvae−maize tortilla and the control for flavor and texture attributes (Table 5) (*19*). An opinion survey of the product showed acceptance of it. The assessors perceived better functional characteristics for taco rolling, exceptional mouth feel sensation, and better taste for the supplemented tortilla. Additional tests should be done to identify the components responsible for this sensory sensation. Also, formal consumer testing should be carried out to predict acceptability of and willingness to purchase this supplemented tortilla.

DISCUSSION

Growing larvae of *T. molitor* free of pesticides can be easily done at home. Boiling the larvae and drying them at 60 °C had the best characteristics with respect to the final color (lighter) and flavor of the powder. The larvae powder protein had levels of the essential amino acids recommended for adults by FAO/WHO/UNU (*23*). In addition, $\omega 3$ fatty acids were detected as a component of larvae powder fat. More studies should be done in protein larvae characterization. The supplemented tortilla had very good taste, color, and texture acceptability. The overall results of this work gave more insights on the properties of insects as food and presents a new way to consume them. Further work has to be done on nutritional aspects such as the protein digestibility of tortillas containing larvae powder. Much work will be necessary to learn more about other kinds of potential edible insects.

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