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Physicochemical, microbiological and sensory evaluation of spaghetti made from mealworm (*Tenebrio molitor* L) and corn (*Zea mays*)*

Evaluación fisicoquímica, microbiológica y sensorial de espaguetis elaborados a base de gusano de la harina (*Tenebrio molitor* L) y maíz (*Zea mays*)

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ABSTRACT

Tenebrio molitor L., also known as the mealworm, is used in the production of noodles as an alternative protein source, improving their nutritional value and providing a high content of essential amino acids. For this reason, the aim of this study was to evaluate the sensory, physicochemical, and microbiological characteristics of pastas made from Tenebrio molitor L. flour (HTM) and corn (Zea mays) flour (HM). A Completely Randomized Design (CRD) was used to evaluate sensory characteristics, physicochemical properties, cooking time, water absorption, and microbiological quality of the pastas. According to the results, sensory analysis showed that the inclusion of 71 % corn flour/7,5 % Tenebrio molitor L. flour (T2) had the highest acceptance, with means of 3,90±0,04 for taste, 4,03±0,03 for odor, 3,73±0,05 for color, and 3,70±0,04 for texture. Additionally, the physicochemical characterization showed values of acidity at 0,30±0,05 %, moisture at 36,30±0,04 %, protein at 9,40±0,05 %, and cholesterol at 54,30±0,02 mg/100g. The cooking time was similar between the Tenebrio molitor spaghetti (T2) and the control (T0), with both cooking in 25 minutes, although the T2 spaghetti had lower water absorption (72,32 %) compared to the T0 (120 %). Microbiological analysis showed the absence of Salmonella and low levels of molds and yeasts, meeting food safety standards. These results suggest that spaghetti with Tenebrio molitor has potential as a functional, nutritious, and acceptable food product for consumers, offering a sustainable alternative in the food industry.

KEY WORDS:
Cooking; Noodles; Protein source; Flour; Worms; Tenebrio molitor L; Zea mays

RESUMEN

El Tenebrio molitor L, también conocido como gusano de la harina, se utiliza en la elaboración de fideos como fuente de proteína alternativa, mejorando su valor nutricional y proporcionando un contenido elevado de aminoácidos esenciales Por esta razón, el objetivo de este trabajo fue evaluar las características sensoriales, fisicoquímicas, microbiológicas de pastas obtenidas a partir de harina de lombriz (Tenebrio molitor L) HTM y maíz (Zea mays) HM. Para el cual se utilizó un Diseño Completamente Aleatorizado (DCA) evaluando características sensoriales, fisicoquímicas, tiempo de cocción, absorción en agua y calidad microbiológica de las pastas. Según, los resultados sensoriales se determinaron que la inclusión de 71 % Corn flour/7,5 % Tenebrio molitor L flour (T2) presentó la mayor aceptación con medias de 3,90±0,04 en sabor, 4,03±0,03 en olor, 3,73±0,05 en color y 3,70±0,04 en textura. Así como también, en la caracterización fisicoquimcia presentó valores de acidez de 0,30±0,05 %, humedad de 36,30±0,04 %, proteínas de 9,40±0,05 % y colesterol de 54,30±0,02 mg/100 g. El tiempo de cocción fue similar entre los espaguetis con Tenebrio molitor (T2) y el control (T0), ambos cociéndose en 25 minutos, aunque los espaguetis T2 presentaron una menor absorción de agua (72,32 %) en comparación con los T0 (120 %). Los análisis microbiológicos mostraron ausencia de Salmonella y bajos niveles de mohos y levaduras, cumpliendo con los estándares de seguridad alimentaria. Estos resultados sugieren que el espagueti con Tenebrio molitor tiene potencial como un producto alimenticio funcional, nutritivo y aceptable para los consumidores, ofreciendo una alternativa sustentable en la industria alimentaria.

PALABRAS CLAVES:
Cocción; Fideos; Fuente proteica; Harina; Lombrices; Tenebrio molitor L; Zea mays

INTRODUCTION

Insects have been part of the diet of approximately 2 billion people worldwide (Van Huis *et al*., 2022), and their consumption is a common practice in various cultures in Africa and Asia. In the Western world, this activity has met with some resistance due to the eating habits of consumers, and there are also no regulations that guarantee the quality of insects intended for consumption (Djouadi *et al*., 2022).

Interest in the use of worms in food production has increased because the breeding of these invertebrates involves less use of resources such as water and soil, as well as lower greenhouse gas emissions compared to the meat industry (Siddiqui *et al*., 2023; Peksever *et al*., 2024). Likewise, as Mwangi *et al*. (2018) point out, worms have high concentrations of proteins, which could be associated with the presence of elements such as zinc and iron.

A study by Omuse *et al*. (2024) revealed that around 2:205 species of insects are consumed in 128 countries, with Asia being the continent where these invertebrates are most in demand. In the case of Ecuador, this country is among the largest consumers of insects in America, ranking third with a total of 78 species usually consumed (Abril *et al*., 2022), with the Kichwa people standing out as the largest consumer of insects in the region (Guachamin-Rosero *et al*., 2022).

Tenebrio molitor, known as the mealworm, is a species that contains minerals such as zinc, magnesium and phosphorus, and whose fat and protein content is comparable to that of animal sources. Its application in food production has gained relevance, especially in the production of flour, due to the ease and low cost of obtaining this product, in addition to its versatility for the preparation of breads, pastas, among others (Costa *et al*., 2020; Kotsou *et al*., 2023). In fact, in products such as flour, the concentration of minerals or proteins could be increased without affecting the structure of the dough or its sensory qualities (Xie *et al*., 2022).

On the other hand, corn flour contains phenolic acids such as gallic, caffeic, chlorogenic, coumaric, syringic and trans-ferulic acid, which makes its use as a biofortifying additive in the preparation of foods such as pasta an interesting option (Hwan Hee *et al*., 2021). Since corn flour is naturally gluten-free, its incorporation into products such as pasta could be affected, so it is necessary to consider processes such as hydrothermalization of flour, which contribute to the formation of a starch network capable of resisting physical stress during cooking, avoiding the loss of firmness and elasticity of the final product (Dib *et al*., 2018).

The consumption of products made with gluten-free flours represents an alternative for people who suffer from intolerance to this protein, which is associated with disorders such as dermatitis herpetiformis and celiac disease, which affects more than 1% of the world's population (Vaiciulyte Funk *et al*., 2023; Bayrakci & Aktar, 2024). For this reason, the present research aimed to evaluate the sensory, physicochemical, and microbiological characteristics of pastas obtained from earthworm flour (*Tenebrio molitor* L) and corn (*Zea mays*).

METHOD

Obtaining flour

To prepare the spaghetti-type pasta, flour obtained from T. molitor L worms was used, which was acquired at the Malaga farm located in the city of Loja, Ecuador, while the yellow corn (Z. *mays*) was purchased at a food market in the city of Manta, Ecuador.

Spaghetti preparation

The dough preparation process was carried out following the methodology described by Martínez-Mora *et al*. (2017), starting with the sifting of the flour using a 100-micron sieve. Subsequently, all the ingredients were weighed, adjusting the quantities according to the formulations specified in Table 1. Then, the ingredients were mixed and the mixture was kneaded for 10 minutes, until a dough with a smooth and compact texture was obtained. Once the desired consistency was reached, the dough was pressed and stretched to a thickness of 7 mm, which allowed the preparation of the spaghetti pasta. Finally, the pasta was packaged in propylene bags and stored in order to carry out the corresponding analyses.

Sensory characterization of spaghetti pasta

A sensory panel consisting of 30 semi-trained criminal lawyers was selected, who evaluated the sensory categories (color, smell, taste and texture) using a 5-point hedonic scale, where 1 corresponded to "I don't like it" and 5 equals "I like it a lot" (Sánchez-Toledano *et al*., 2023).

Table 1. Treatment formulation.

Ingredients	Treatments		
	1	2	3
	%	%	%
H.M.	66	71	74,5
HTM	12,5	7,5	4
Xanthan Gum	1,5	1,5	1,5
Olive oil	5	5	5
Salt	2,5	2,5	2,5
Egg	10	10	10
Water	2,5	2,5	2,5
Total	100	100	100

Physicochemical characterization

Physicochemical analyses were performed on the noodle treatment that obtained the highest score in the sensory evaluation. Protein concentration was determined using the Kjeldahl method (AOAC 2001.11:2001), where total nitrogen was measured and multiplied by a conversion factor to calculate protein content. Ash concentration was assessed by incinerating the sample at 550 °C until a white residue was obtained, according to AOAC method 923.03:1990. Moisture content was measured by drying the sample at 105 °C to constant weight, following INEN NTE 518:2012. Titratable acidity was determined by titration with NaOH in the presence of phenolphthalein, according to INEN NTE 521:2013 procedure. To analyze the concentration of cholesterol in the treatments, it was carried out by gas chromatography coupled to a flame ionization detector (GC-FID), for which the AOAC 994.10 standard was considered, the same one that establishes the procedures to evaluate the cholesterol content in pasta.Finally, soluble solids were evaluated with a refractometer, according to AOAC method 932.12:2013, by measuring the refractive index of an aqueous solution of the sample.

Cooking time

Cooking time is determined using the methodology described by Granito *et al*. (2014). To do this, 25 g of pasta were weighed and immersed in 300 mL of boiling water. Samples were taken every 5 minutes and the colour of the centre of the pasta was checked visually. When the colour of the centre was no longer white, it seemed that the spaghetti had reached the optimum cooking point.

Water absorption (Aa)

It was evaluated considering the methodology used by Lux *et al*. (2023), with certain modifications, for which 25 g of each pasta was taken and immersed in cooking water. To calculate this index, the following equation was considered:

$$Aa: \frac{peso\ de\ fideos\ cocidos - peso\ de\ fideos\ crudos}{peso\ de\ fideos\ crudos} * 100 \qquad (Eq.1)$$

Microbiological evaluation

A Salmonella spp. analysis was carried out using the qualitative reference method specified in the INEN NTE 1529-15 standard. In addition, moulds and yeasts will be determined by plate counting, according to the procedure described in the INEN NTE 1529-10 standard.

Experimental design

A completely randomized design (CRD) was used, for which the normality of the data was assessed using a Shapiro-Wilks test, then a Friedman test was used to identify differences between treatments with a significance level ($p<0.05$). The description of the treatments is presented in Table 2.

Table 2. Treatments under study.

Treatments	Description
T1	66 % Corn flour/ 12,5 % Tenebrio molitor L flour
T2	71 % Corn flour / 7,5 % Tenebrio molitor L flour
T3	4,5 % Corn flour/ 4 % Tenebrio molitor L flour

RESULTS

Sensory characterization of spaghetti pasta

Figure 1 presents the sensory results, where a significant difference ($p<0,05$) is observed in the categories of smell and texture between the treatment means. However, no significant variability is evident in the categories of flavour and colour. In this context, it is worth highlighting that treatment T2 was the one that obtained the greatest acceptance by the sensory panel, with means of 3,90±0,04 in flavour, 4,03±0,03 in smell, 3,73±0,05 in colour and 3,70±0,04 in texture. These results suggest that the inclusion of up to 7,5 % of Tenebrio molitor L. favours the obtaining of a product that is pleasant for the consumer.

Previous research has shown that the addition of dehydrated chive flour in pasta production can improve the sensory characteristics of the product, especially when 3 % of the powder is incorporated (Marioxy & Navas, 2009). Similarly, the concentrations of worm meal in the treatments did not significantly affect the tasters' sensory perception. It is relevant to highlight that the organoleptic characteristics of the pasta depend both on the composition of the raw material and the processes used during its production, as well as on the sensory characteristics and the age of the tasters (Betz *et al*., 2020; Skotnicka *et al*., 2023).

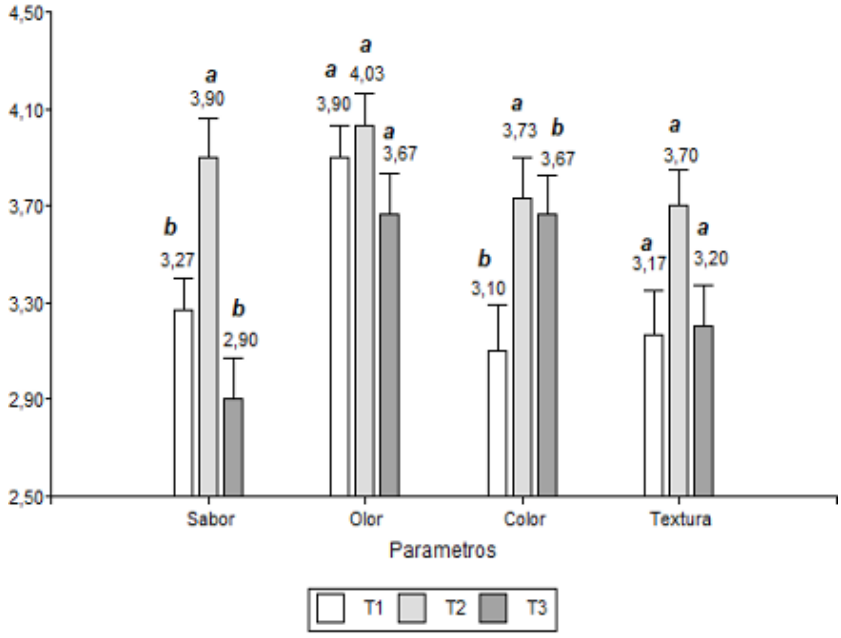

Figure 1. Sensory analysis of spaghettis. Different letters represent significant differences between treatments (Tukey p<0.05).

Physicochemical characterization

In Table 3, the physicochemical results of treatment T2 are presented. This treatment showed an acidity of 0,30 %, which is within the limits established by the Ecuadorian Technical Standard INEN NTE 1375, which sets a maximum acidity of 0,45 % for pasta. It is important to note that although Tenebrio molitor L. may have a minor direct impact on the acidity of the noodles, maize, due to its alkaline nature, likely has a more significant effect in reducing the acidity of the final product (Moruzzo *et al*., 2021).

Regarding the ash content, a value of 2,50 % was obtained, exceeding the maximum limit of 1,50 % established by the aforementioned reference standard. This value was also higher than those reported by Hamed *et al*. (2015), who analyzed the effect of incorporating Darién (Erythrina edulis) and pumpkin (*Cucurbita maxima*) flours at concentrations of 25 % and 50 % in spaghetti production, finding ash content values ranging from 1,03 % to 1,38 %. Similarly, in a study on pasta made with apple flour and 50 % oat bran, Espinosa-Solis *et al*. (2019) reported ash content values between 1,90 % and 2,00 %.

The spaghetti from treatment T2 showed a moisture content of 36,3 %. Temperature is a key factor in the drying process, and in this case, the spaghetti was dried at room temperature (20-25 °C), which explains the relatively high moisture level. In contrast, previous studies, such as that by Rumbos *et al*. (2020), used pre-drying and drying at higher temperatures (75 and 120°C, respectively). Additionally, Vedia-Quispe *et al*. (2016), when making pasta with whole amaranth flour, found moisture values ranging from 13,59 % to 13,69 %, while Zhang *et al*. (2020), who studied the effect of drying temperature on Chinese noodles, reported a moisture content of 10 %.

The protein content of T2 was 9,4 %. In a study by Roncolini *et al*. (2019), the inclusion of Tenebrio molitor L. powder increased the protein content in experimental breads, with the highest values (11,53 %) found in breads containing 10 % mealworm flour. In another study, Xie *et al*. (2022) found that the protein content in cookies gradually increased from 9,13 mg/100 g in the control to 16,0 mg/100 g when 20 % mealworm powder was added.

The cholesterol analysis in the T2 pasta, which showed a content of 54.30 mg/100 g, indicates that this value is significantly lower than the limit set by the Ecuadorian Technical Standard INEN NTE 1375, which allows up to 350 mg/kg. This result is also considerably lower than the value reported by Kolarič *et al*. (2024) for conventional pasta, which was 329.2 mg/kg. These data suggest that T2 pasta not only meets regulatory standards but also contains much less cholesterol than conventional pasta, which could be an advantage in terms of health and in the market for healthier food products.

Finally, the soluble solids content in treatment T2 was 9,54 %, which is within the minimum limit required by the Ecuadorian Technical Standard INEN NTE 1375 (3 %). This result is comparable to that reported by Muthupandian *et al*. (2011), who analyzed the physicochemical characteristics of noodles enriched with skim milk powder and whey protein concentrate, finding soluble solids content values ranging from 8,07 % to 13,21 %.

Table 3. Physicochemical characterization

Parameters	Results
Acidity (%)	0,30±0,05
Ash (%)	2,50±0,04
Humidity (%)	36,30±0,04
Proteins (%)	9,40±0,05
Cholesterol (mg/100 g)	54,30±0,02
Total solids (%)	9,54±0,03

Cooking time and water absorption

According to the data presented in Table 4, the cooking time for the commercial pasta (T0) and the pasta made with a mixture of corn flour and mealworm (T2) was 25 minutes in both cases. This result indicates that the composition of the pasta did not significantly affect this parameter, as no differences in cooking time were observed between the two types of pasta. Cooking duration is an important factor in the final quality of pasta, as it influences its texture, firmness, and nutrient retention, such as carotenoids. A study by Oduro-Obeng *et al*. (2021) mentions that cooking times longer than 8 minutes may increase the presence of carotenoids in noodles, which could be beneficial from a nutritional standpoint. However, according to Sobota *et al*. (2013), prolonged cooking times can also negatively affect the firmness and texture of pasta, making it softer and less desirable in terms of sensory quality.

In the case of gluten-free pastas, such as those made from legume or insect flours, cooking time can also depend on the flour concentration used. Giuberti *et al*. (2016) found that increasing the concentration of legume flour in the mixture also increased the cooking time, suggesting that the proportion of flour in the recipe can alter the thermal behavior during preparation. Similarly, in a study by Pasini *et al*. (2022), it was observed that when insects like Acheta domesticus and Tenebrio molitor were incorporated into the pasta flour, the cooking times varied between 11,5 and 13 minutes, depending on the insect concentration (12 %), reinforcing the idea that the composition of the dough influences cooking duration.

Regarding water absorption, it was observed that the spaghetti made with the corn flour and mealworm mixture (T2) exhibited a lower water absorption rate, with a value of 72,32 %, compared to the commercial pasta, which had a value of 120 %. The lower water absorption in the experimental pasta could be related to the specific properties of the corn and insect flours, which have a lower capacity to retain water than the traditional flours used in commercial pasta. Water absorption is closely linked to the protein content in pasta, as proteins interfere with the pasta's ability to retain water during cooking. According to Sozer and Kaya (2008), pastas with higher protein content tend to absorb less water, and this trend becomes more pronounced when cooking times exceed twelve minutes. In this study, the results show that the water absorption in T2 was lower than in the control (commercial pasta), which aligns with the findings of Gwekwe *et al*. (2024), who observed that water absorption in pastas made from bean, sorghum, and potato flours was lower, particularly when cooking times ranged from 5 to 7 minutes.

On the other hand, the values obtained in this study are lower than those reported by Gwekwe *et al*. (2024) in their research on pastas made from alternative flours. In contrast, the results were higher than those presented by Xie *et al*. (2022), who evaluated water absorption in cookies made with 20 % T. molitor flour, observing a higher water absorption rate. This may be explained by differences in food matrices and the high fiber and protein content in insect flour, which influences water retention differently in pastas and cookies. Variations between studies may also be due to the proportion of insect flour used, the type of base flour, and specific cooking conditions in each case.

Table 4. Cooking time and water absorption in pasta

Variables	T0	T2
Cooking time (min)	25	25 min
Water absorption (%)	120 %	72,32

Microbiological evaluation

Table 4 presents the results obtained in the microbiological characterization at T2, there was an absence of Salmonella spp, however, a high presence of yeasts was observed (62x103 CFU/g), while in the case of molds found <10 UPC/g).

During food preparation, pathogenic microorganisms can generate toxins, which cannot be eliminated during thermal processes such as cooking (Pop, 2017). As shown in Table 5, the presence of molds was <1x10, lower than that established in the INEN NTE Standard 1529:10 (3X102), Which complies with the requirements of the regulations. However, in the work carried out by Özyurt *et al*. (2015), it was observed that in the pasta enriched with spirulina in concentrations of 5 to 15 %, there was an absence of molds and yeasts. The results obtained were in accordance with those reported by Ricci *et al*. (2007) who evaluated the microbiological characteristics of 85 different brands of commercial pasta from Italy. In the case of yeasts, a value of 62x10 3 was obtained, exceeding what is permissible in the 3x102.

Table 5. Microbiological characterization of spaghetti pasta

Parameters	Result	Rule
Molds (UPC/g)	<10	3x102
Yeasts (CFU/g)	62x103	3x102
Salmonella (Absence/presence)	Absence	Absence

CONCLUSIONS

In conclusion, the physicochemical, microbiological, and sensory analysis of spaghetti made from Tenebrio molitor L. and corn (Zea mays) shows promising results for the development of an alternative and functional product. From a sensory perspective, the inclusion of up to 7,5 % Tenebrio molitor L. (T2) improves consumer acceptance, particularly in the categories of smell and texture, while flavor and color show no significant differences. In terms of physicochemical properties, the spaghetti has a balanced composition, with a protein content of 9,4 %, low acidity, and moderate moisture (36,3 %), making it suitable from a nutritional standpoint. Furthermore, the spaghetti exhibits an appropriate cooking time (25 minutes) and more efficient water absorption compared to the control (T0), which could improve its cooking quality. Microbiologically, the results are satisfactory, with the absence of salmonella and mold and yeast levels within established limits, ensuring the product's safety. Overall, the Tenebrio molitor L. and corn spaghetti demonstrates favorable sensory, nutritional, and microbiological characteristics, suggesting its viability as a nutritious and sustainable alternative in the food industry.

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