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Development and physicochemical evaluation of a snail protein-based worcestershire sauce (*Helix aspersa*)

Desarrollo y evaluación fisicoquímica de una salsa inglesa con proteína de caracol (*Helix aspersa*)

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Abstract

The garden snail, *Helix aspersa*, is a common species and its flesh is rich in high biological value proteins that provide all the essential amino acids for nutrition. Based on this, the aim of this study was to utilize snail protein in the development of an English-style sauce to add value. The formulation was developed and the physicochemical and microbiological characteristics of the resulting product were evaluated, ensuring the quality and safety of the process. The obtained results indicated that, once the English-style sauce with snail protein was standardized, the physicochemical parameters of pH, ºBx, and acidity did not show significant differences compared to the control sauce. This suggests that consumers accustomed to consuming commercial English-style sauce will not perceive differences when trying the sauce made with garden snail. In conclusion, the development of an English-style sauce with snail protein has the potential to add value to the product, taking advantage of the high protein quality of the garden snail. This may be of interest to the food industry in product diversification and the promotion of unconventional food consumption.

***Helix*, Physicochemical parameters, microbiological evaluation**

Resumen

El caracol de jardín, *Helix aspersa*, es una especie común y su carne es rica en proteínas de alto valor biológico, que aportan todos los aminoácidos esenciales para la alimentación. Con base en esto, el presente estudio tuvo como objetivo aprovechar la proteína de caracol en el desarrollo de una salsa tipo inglesa para agregarle valor. Se desarrolló la formulación y se evaluaron las características fisicoquímicas y microbiológicas del producto obtenido, con lo que se aseguró la calidad e inocuidad del proceso. Los resultados obtenidos indicaron que, una vez que la salsa tipo inglesa con proteína de caracol fue estandarizada, los parámetros fisicoquímicos de pH, ºBx y acidez no mostraron diferencias significativas en comparación con la salsa control o testigo (SIC). Esto sugiere que los consumidores habituados al consumo de salsa inglesa comercial no percibirán diferencias al probar la salsa elaborada con caracol de jardín. En conclusión, el desarrollo de una salsa tipo inglesa con proteína de caracol presenta un potencial para añadir un valor agregado al producto, aprovechando la alta calidad proteica del caracol de jardín, lo que puede ser de interés en la industria alimentaria en la diversificación de productos y la promoción del consumo de alimentos poco convencionales.

***Helix*, Parámetros fisicoquímicos, evaluación microbiológica**

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Introduction

The worldwide consumption of land snails is widespread in the world, particularly in Europe, where France is the main consumer of snails in the world. Within the denomination of snails are included a great diversity of species that present some very evident morphological differences. The most common snail, known as garden snail or common land snail is the species *Helix aspersa*. Other species are the Roman snail (*Helix pomatia*), the Turkish snail (*Helix lucorum*) and the Christian snail (*Otala punctata*). This mollusk is considered a delicacy and is a must in most famous restaurants. There are several ways of commercialization for the land snail, among which are: live, frozen, and packaged. The main producing countries are located in the northern hemisphere in areas close to France, with Greece and Turkey standing out as the main suppliers of the French market.

In this research it is assumed that the garden snail is a valuable food resource due to its nutritional properties and potential in the food industry. While its production and processing can be difficult, there has been promising research on its use in processed food production and as an ingredient in food production (Krzeminska, *et al*, 2017; Cofrades, *et al*, 2016). Due to the above and with the aim of contributing to the food industry, the present research has been developed, whose objective is to formulate an innovative Worcestershire sauce that incorporates snail protein (*Helix aspersa*) as a functional ingredient, in order to improve the nutritional value of the final product and contribute to the sustainable use of the snail as a raw material. This is justified given that more research is needed to explore the potential of snails in the food industry.

On insects in the development of new products

In recent decades, food production has been the subject of increasing concern regarding its sustainability. The search for alternative protein sources to meat has led to the exploration of new sources, such as edible insects. According to FAO, insects are a food source rich in protein, vitamins and minerals, and are more sustainable than traditional protein sources due to their low environmental impact (García-Gómez *et al*., 2020).

Within the animal group, minor species that have potential, such as mollusks that have potential, such as mollusks and insects have been used for centuries in human food in various parts of the world, however, only recently have their nutritional properties and their potential in the food industry been further investigated (Rumpold and Schlüter, 2013). Insects are rich in protein, healthy fats, vitamins and minerals, and many of them contain a significant amount of essential fatty acids and amino acids that our body cannot synthesize Garcia (idem). In addition, insects have a lower environmental impact than traditional farm animals, as they require less land, water and feed to produce the same amount of protein (Van Huis *et al*., 2013). Despite the nutritional benefits of insects, there is still some cultural rejection of their consumption; the development of new innovative and attractive food products for consumers could help overcome this barrier and take advantage of their nutritional benefits.

About the garden snail (Helix aspersa)

The garden snail (*Helix aspersa*) is a species of gastropod commonly used in food processing, such as French cuisine. This snail is prized in many places for its edible meat and is therefore considered an important food resource. In addition to its gastronomic value, the garden snail is also valued for its nutritional properties and its potential in the food industry has been investigated (Adegoke, *et al*.,2016).

Krzeminska (ibidem) asserts that the garden snail is a good source of protein and essential fatty acids, such as linoleic acid and oleic acid. In addition, it contains a wide variety of vitamins and minerals, such as iron, zinc, selenium and vitamin B12. However, its use in the food industry is limited due to the difficulty of its production and processing. Despite this, studies have been conducted on the use of snail in the production of processed foods, such as hamburgers and sausages, with promising results as mentioned by Krzeminska (ibidem). In addition, the use of snail meal in food production has also been investigated. A study by Cofrades (ibidem) found that the addition of snail flour to empanada dough improved its nutritional and sensory value, with a higher amount of protein and minerals. On the other hand, Montowska *et al*. (2018) found that garden snail meat has a high nutritional quality, similar to that of chicken meat.

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The above research, shows the potential of the snail to be used as another ingredient in the development of new food products, such as the one that concerns us in this research.

About Worcestershire sauce

Worcestershire sauce, commonly called Worcestershire, is a popular and widely used condiment in international cuisine. Its distinctive umami flavor and culinary versatility have led to an increasing demand and exploration of new formulations and improvements in its composition (Jones *et al*., 2021).

In analyzing recent research, several trends and advances in the formulation and application of Worcestershire sauce were found. A study conducted by Montowska (ibidem) focused on the reduction of sodium content; while Jones (ibidem) explored the use of natural and fermented ingredients in the production of Worcestershire sauce, with the aim of improving its nutritional profile and increasing its added value. Lee *et al*., (2023) focused on the development of Worcestershire sauces with specific flavors, such as citrus or smoked notes, using natural extracts and flavoring techniques. These findings support the importance of continued research and innovation in the condiment industry in order to meet changing consumer demands and promote healthier culinary choices, as a Worcestershire sauce with hydrolyzed Helix aspersa protein results.

Materials and methods

Experimental research was carried out for the development of a new product in which a main ingredient is the addition of snail in order to add more nutritional value to the product. Consequently, the research was developed in the following stages.

Collection and preparation of the snail (*Helix aspersa*): The snail used in the preparation of the Worcestershire sauce was collected during the months of September-October in plots in the municipality of Ixmiquilpan Hidalgo. Once the snails were in the UTVM laboratories, they were dehydrated. With a solution of 75% water at a temperature of 23º C and 25% calcium hydroxide, leaving it to stand for 24 hours, so that the snail would expel the fecal feces and eliminate the mucus or slime of the snail.

Then the snail meat was washed with purified water to remove impurities. The soybeans were then cooked in a kettle at 95-97°C for one hour, and then homogenized and mixed (50% snail and 50% soy) for 30 minutes. A dough was obtained, which was laminated until obtaining a thickness of 1.5 cm, to be later sectioned into 2 X 2 cm cubes. The sectioned cubes were left to rest in an incubator for 8 days at a temperature of 36° C. After incubation, a 2% brine was prepared, introducing the cubes until they were completely covered. It was left to ferment for 30 days at a temperature of 25°C and with intermittent mixing every day at 15-20 rpm. for 5 minutes. This first stage is concluded by filtering the insoluble solids, recovering the miscible liquid for the final mixture.

Formulation and standardization of the production process:

Worcestershire sauce was made by adding traditional ingredients, to the liquid recovered in the previous stage, in the following proportions: Pepper (0.5%), Onion (0.1%), Garlic (0.28%), Salt (0.5%), Ginger (0.28%), Mustard (0.1%), Cinnamon (0.64%), Sugar (0.3%), 5% acetic acid was added to hydrolyze the miscible liquid to incorporate the soy protein and mainly snail protein into the sauce. Subsequently, it was filtered to separate the larger particles and thus obtain a liquid free of sediment. The sauce was pasteurized for 30 minutes at 75ºC, and potassium sorbate (0.1%) and sodium benzoate (0.1%) were added as preservatives. Finally, the product was packaged at a temperature of 85º C to generate vacuum in an amber-colored container.

Physical-chemical analysis:

Physicochemical analyses were performed on the final product according to the methods recommended by the Association of Official and Analytical Chemists (AOAC, 1984). These included the determination of acidity, pH, carbohydrate content, fat, protein, moisture and minerals. The results obtained were expressed as mean ± standard deviation. The results obtained were compared with those of a commercial Worcestershire sauce (SIC), the intention of which was to match the flavor and/or mask the flavor of the snail so that the consumer would not detect the differences in flavor, odor and texture of the new product.

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To determine the differences between the Worcestershire sauce with Helix (SIH) and the SCI, the results were analyzed with a randomized block design with a significance level of α=0.5, with six replications; the SCI was considered as a control, for which a Dunnet's test of contrasts was performed with α=0.5. Minitab ver 21.1 was used for statistical analysis.

Microbiological analysis:

Microbiological analyses were performed according to the Mexican Official Standard NOM-130-SSA1-1995.Tests were carried out for the detection and enumeration of pathogenic microorganisms, such as coliform bacteria, Salmonella spp. and Staphylococcus aureus, as well as mesophilic aerobic counts and fungi and yeasts. Seeding techniques were followed in appropriate culture media and viable counts were performed for each target microorganism.

Shelf-life evaluation:

A shelf life study was conducted to determine the stability of the developed sauce. The stability of the sauce was evaluated at 30, 60 and 90 days keeping the temperature under control (25ºC) pH. These were the response variables to evaluate changes in the quality of the product over time. Periodic measurements were taken and statistically analyzed to determine the evolution of the variables as a function of time and storage temperature.

Results and discussion

Standardization of the production process

To obtain the final formulation of Worcestershire sauce with snail (*Helix aspersa*), it was necessary to make different formulations until achieving one that would match the sensory characteristics of commercial Worcestershire sauce (SIC). Table 1 shows this formulation; the use of the garden snail, which increased the protein value of the product, stands out.

Ingredient	Quantity (%)
Snail meat	50
Wheat flour	68
Apple vinegar	4
Apple juice	3
Pepper	0.5
Onion	0.1
Garlic	0.28
Salt	2
Ginger	0.28
Mustard	0.1
Cinnamon	0.64
Water	0.3

Table 1 Formulation of Worcestershire sauce with *Helix aspersa.*
Source: Own elaboration (2022).

Once the formulation was similar to the SIC, the process was standardized, which is described below (Figure 1). It is important to detail that during the process it was observed that the control points that directly impact the quality of the final product were fermentation and hydrolysis due to the separation of the amino acids that make up the mollusk protein.

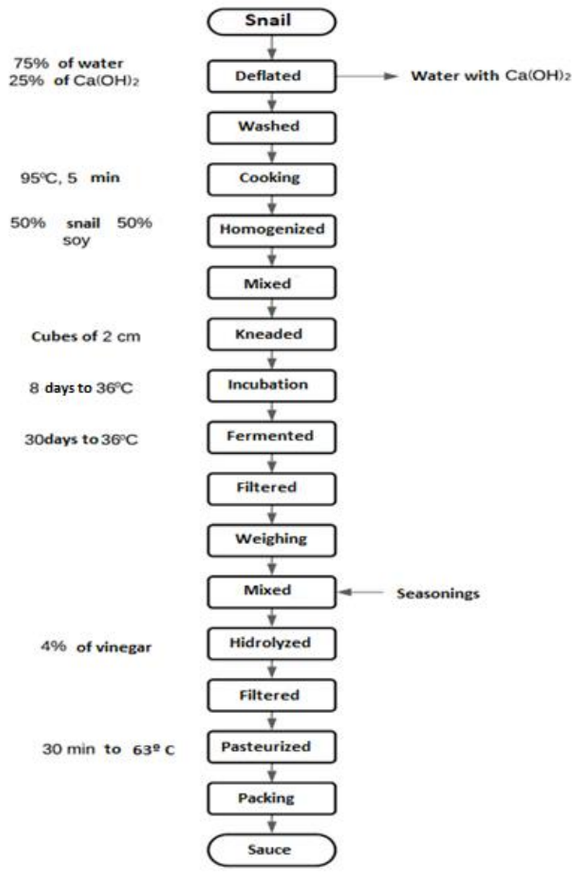

Figure 1 Standardization of the process of elaboration of Worcestershire sauce with *Helix aspersa.*
Source: Own elaboration, (2022).

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Physicochemical analysis

Once the SIH was standardized, its pH, ºBx and acidity expressed in percentage of acetic acid were evaluated (Table 2). It was found that these parameters did not show a significant difference in relation to the control or control treatment (SIC) with an α=0.05. This suggests that the consumer who is accustomed to the consumption of commercial Worcestershire sauce will not find a difference with the sauce made with garden snail. This is important because Van Huis et al. (2013) asserts that despite knowing the benefits of consuming some terrestrial insects and mollusks, the population is reluctant to consume them and even to try them.

Parameter	SIH	SIC
pH	3.5±0.183[a]	3.6±0.152[a]
ºBx	10.3±0.130[a]	10.2±0.113[a]
Acidity (% of acetic acid)	4.11±0.121[a]	4.15±0.118[a]
Note: Different letters, for each parameter evaluated, indicate significant difference (p<0.05).		

Table 2 Comparative evaluation of physicochemical parameters of Worcestershire sauce with *Helix aspersa* vs. commercial Worcestershire sauce.
Source: Own elaboration, (2022).

According to the results of the proximate chemical analysis presented in Table 3, it can be observed that the developed sauce presents a higher content of moisture, fat, protein and minerals, with percentages of 91.5%, 0.8%, 1.0% and 1.5%, respectively. These differences are statistically significant (α=0.5) compared to the control (SIC). This is explained by the fact that the elaborated sauce presents an increase in protein content of 243.9 % with respect to the control sample. It is important to note that the commercial sauce shows a higher carbohydrate content, mainly because it contains elements rich in carbohydrates such as molasses and corn syrup, and in some cases piloncillo.

Parameter	SIH (100g)	SIC (100g)
Moisture (%)	91.5±1.109[a]	82.09±0.987[b]
Fat (%)	0.8±0.012[a]	0.2±0.009[b]
Protein (%)	1.0±0.123[a]	0.41±0.087[b]
Carbohydrate (%)	5.2±0.143[a]	16.1±1.89[b]
Minerals (%)	1.5±0.120[a]	1.2±0.113[a]
Note: Different letters, for each parameter evaluated, indicate significant difference (p<0.05).		

Table 3 Proximal chemical analysis of Worcestershire sauce with *Helix aspersa* vs. commercial Worcestershire sauce.
Source: Own elaboration, (2022).

Microbiological analysis

After performing the microbiological analyses corresponding to the sauce developed, it was found that all the parameters established in NOM-130-SSA1-1995 are within the maximum limits allowed by current regulations (Table 6). This means that the garden snail Worcestershire sauce complies with the quality and safety standards required for its commercialization and consumption.

Determination	SIH	Maximum Permissible Limits (CFU/g)
Fungi	7	20
Yeasts	20	50
Total and fecal coliforms	0	0

Table 4 Microbiological analysis of Worcestershire sauce with *Helix aspersa* vs. commercial Worcestershire sauce.
Source: own elaboration (2022).

Shelf-life tests (product stability)

Table 5 shows the stability of Worcestershire sauce with snail at different storage times (30, 60 and 90 days) at a controlled temperature of 25ºC. It is observed that the Brix degrees (°Bx) decrease with time, which is explained by the possible fermentation of the sugars present in the sauce, leading to a reduction in sugar content and, therefore, in soluble solids. According to Lee (ibidem) this phenomenon can affect the texture and flavor of the sauce, so it is important to constantly monitor the soluble solids content during storage to ensure that quality standards are met. On the other hand, pH tends to decrease over time due to microbial activity and the production of acetic acid and other organic acids as a result of ingredient fermentation. However, the decrease in pH is observed to be slow due to storage temperature and the specific composition of Worcestershire sauce. However, as suggested by Jones (ibidem), periodic pH monitoring during storage is crucial to ensure product quality and safety.

Days	pH
30	3.217±0.183
60	3.182±0.093
90	3.417±0.141

Table 5 Stability of Worcestershire sauce with *Helix aspersa* at 25ºC.
Source: Own elaboration, (2022).

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Conclusions

In conclusion, the results of this research demonstrate that it is technically feasible to develop an English-type sauce using snail protein. The physicochemical analyses performed are within the parameters established for sterilized products with a pH ≤ 4.5, in accordance with NOM-130-SSA1-1995. From the microbiological point of view, it was determined that the product is innocuous, which guarantees its safety for consumption and provides reliability.

One of the main advantages of this development is its high protein content compared to the commercial product, as well as the reduction of carbohydrates since it does not contain added sugars. This can be a positive aspect for consumers seeking healthier and more nutritious food options.

To continue advancing the study, immediate future research involving product acceptance tests with a group of experts is suggested, with the objective of confirming its viability in the market and evaluating its potential for purchase. These evaluations will allow obtaining valuable information on the acceptability and consumer perception of snail sauce, which will be fundamental for its subsequent commercialization and positioning in the sauce market.

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