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Assessment of the addition of *Rhynchophorus palmarum* L. biomass in bread formulations

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Abstract

The goal of this research work was to propose formulations of bread products including South American palm weevil (*Rhynchophorus palmarum*) larvae harvested in the Ecuadorian Amazon region. Three bread formulations (treatments) were proposed to make bread pieces; replacement percentages with biomass of South American palm weevil larvae of each treatment were 5% (T_1), 10% (T_2), and 15% (T_3). A standard treatment, T_0, with 0% replacement was also part of the experiment. Fat was characterized in bread pieces obtained. Contents of saturated, mono, and polyunsaturated fatty acids were measured by gas chromatography. Firmness was determined by texturometry by a simple cycle compression. As the South American palm weevil larvae biomass addition percentage increased in the proposed formulations, mono-unsaturated fatty acids amount also increased from 3.37% (T_1) to 3.51% (T_3), in the same subject, the percentage of polyunsaturated acids were in the range from 1.28% (T_1) to 1.55% (in T_3). Firmness increased as the percentage of palm weevil larvae biomass were higher (5,348 gf in T_1 and 6,925 gf in T_3). The inclusion of palm weevil larvae biomass in bread making contributed to increasing the levels of mono and polyunsaturated fatty acids, resulting in a product with functional properties.

Palabras Clave: bakery products; Chontacuro; entomophagy; fat; micro-livestock.

Evaluación de la inclusión de biomasa de *Rhynchophorus palmarum* L. en formulaciones de pan

Resumen

El objetivo del presente trabajo es proponer formulaciones de productos panificables con inclusión de biomasa de larvas de chontacuro (gorgojo cigarrón, *Rhynchophorus palmarum* L.) obtenidas de la región amazónica ecuatoriana. Se propusieron 3 formulaciones para la elaboración de piezas de pan. Los porcentajes de sustitución con biomasa de larvas de gorgojo cigarrón para cada tratamiento fueron 5 % (T_1), 10 % (T_2) y 15 % (T_3). Como parte de la experimentación, también se planteó la inclusión de un tratamiento de referencia, T0, con 0% de sustitución. Se caracterizó la grasa de las piezas de pan obtenidos. Se determinaron los contenidos de ácidos grasos saturados, mono y poliinsaturados por cromatografía de gases. La firmeza fue determinada por texturometría con comprensión de ciclo simple. Las cantidades de ácidos monoinsaturados se incrementaron desde 3,37 % (T_1) hasta 3,51 % (T_3), que es directamente proporcional a la cantidad formulada por añadirse de biomasa de larvas de gorgojo cigarrón. En este sentido, el porcentaje de ácidos poliinsaturados estuvo en un rango entre 1,28 % (T_1) a 1,55 % (T_3). La firmeza se incrementó en función directamente proporcional del porcentaje de biomasa de larvas de gorgojo cigarrón (5348 gf en T_1 y 6925 gf en T_3). La inclusión de biomasa de larvas de gorgojo cigarrón en la fabricación de pan contribuyó con el incrementó de cantidades presentes de ácidos grasos mono y poliinsaturados; esto resultó en la obtención de productos con propiedades funcionales.

Keywords: productos de panificación; Chontacuro; entomofagia; grasa; micro-ganado.

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INTRODUCTION

Consumption of insects as food is an alternative source of nutrients for a number of developing countries. Entomophagy, i.e., the consumption of insects as food, is practiced traditionally in some countries in Asia, Africa, and Latin America. The intake of insects complements the dietary needs of about 2 billion people in the globe and has been a perennial habit in the human-being eating behavior. On average, insects feature 35-61% protein, 15-40% fat, and 3-10% minerals (1), therefore, they embody an interesting alternative to enrich other food types or as a source to produce functional food products (2, 3). Among some examples, Mexican insects called *chapulines* had become a highly appreciated delicatessen; consumers had catalogued *chapulines* as a food with nutritive and, even, aphrodisiac notes; some even consider it as a gourmet dish. *Chontacuro*, i.e., South American palm weevil (*Rhynchophorus palmarum* L.), larvae, consumed in Ecuador, are harvested from some species of palm trees; larvae are commonly eaten directly or after some degree of thermal treatment. The development of the husbandry (some specialists may refer to this type of animal production as "micro-livestock") of South American palm weevil may be a source of profits for small-scale producers in the region (4, 5), allowing local communities to widen market offers, thus reducing poverty levels. There is then the need to develop processing, harvesting, and post-harvesting technologies that may yield efficiently considering also microbiological aspects (6). Insects may provide an alternative food source for commonly consumed foods, especially wheat-based products. Wheat-based foods are highly consumed elsewhere; pasta and bread are staple food to most of the population everywhere. Bread has a salient role in food intake trends in Latin America due, but not limited to, its low cost, availability, and as a rather cheap energy source. The supply of bakery products is diverse in order to satisfy the requirements of consumers. Moreover, some bread products include beneficial ingredients, such as dietary fiber, minerals, and vitamins. The use of functional ingredients in bread formulations has effects over the technological and nutritional properties (7, 8). Depending on its nature, the addition of fat in bread formulations provide desirable characteristics to the final product concerning flavor, color, texture, and nutritional value. The addition of functional components to baking products provides health benefits and may prevent the occurrence of diseases (9). Nonetheless, the frequent consumption of bread has been linked negatively to a number of clinical conditions such as increased blood pressure, cardiovascular diseases development, heart failure, acute myocardial infarction, and kidney failure, as well as to non-cardiovascular effects, such as the development of nephrolithiasis, gastric cancer, obesity, asthma, and osteoporosis (10, 11). Palm weevil larvae may have potential as a food source of high nutritional value concerning protein, fat, vitamins, etc. and might be used as replacement of common ingredients or as an additive. Insects, as food source, fulfill two primary characteristics of sustainability: nutritional quality and abundance; these characteristics might provide innovative approaches about their use and application in a number of productive systems, e.g., replacement of commonly used food sources by low-cost alternatives (12). The bakery industry has already a wide range of products destined to certain consumers demanding special dietary requirements, such as "vitamin-enriched," "mineral-fortified," "high-protein," "low-sugar," "high-fiber," "light," "gluten-free," etc. (13). The consumption of functional bread may be enhanced with the addition of innovative ingredients resulting in the development of healthy food products that would satisfy the increasing demand for them. South American palm weevil larvae feature, on average, 3.4 mm length after egg hatching, a ventral-curve shape, and a creamy white color (14). The larvae stage lasts between 42 and 62 days (15). A size of 5-6 cm is reached in further developing stages; larvae feature darker yellowish tonalities after pupating (16); on this stage, larvae are optimal for its harvesting and consumption. The skins of South American palm weevil larvae feature a number of fatty acids and oils, such as palmitic, stearic, myristic, linoleic, linolenic, and palmitoleic. Table 1 shows fatty acids featured in the skin and in the digestive tract content of South American palm weevil (17).

Tabla 1. Fatty acids composition in oils present in the South American palm weevil larvae skin and digestive tract content (DTC)

Fatty acid	Skin (%)	DTC (%)
Myristic	1.91	2.27
Palmitic	41.78	43.65
Palmitoleic	0.75	1.01
Stearic	9.41	8.52
Oleic	43.10	41.57
Linoleic	2.00	1.93
Linolenic	1.05	1.05

The objective of the present research work was to propose formulations to manufacture bread including biomass extracted from South American palm weevil (*Rhynchophorus palmarum* L.) larvae, i.e., Chontacuro, thus contributing to diversifying the use of this food source applied in highly demanded products; a source of profits in rural areas where the larvae is currently harvested can be seen as means to an end should a sustainable production process might be bolstered.

MATERIAL AND METHODS

The present study was carried out at the laboratory of Innovative Products of the Faculty of Chemical and Health Sciences, Universidad Técnica de Machala, Ecuador. The South American palm weevil (*Rhynchophorus palmarum* L.) larvae were purchased from local farmers in El Puyo, Pastaza, Ecuadorian Amazon Region. As one of the main components for the bread formulations to be designed, 2 types of flour were used: refined flour of wheat (*Triticum vulgare*) and whole-grain flour of wheat (*Triticum aestivum*). In addition, and according to Ecuadorian standards, yeast (*Saccharomyces cerevisiae*) for baking purposes, salt, oil, sugar, and tap water were part of the formulation (18). The experimental part of the present work consisted of 3 treatments, T_1, T_2, and T_3. Treatments are showed in Table 2. Each treatment consisted in a percentage of replacement of flour with larvae biomass, these being 5, 10, and 15%, respectively. A T_0 standard treatment was also used, 0% of larvae biomass inclusion, as means of comparison.

Tabla 2. Experimental treatments of blends consisting in refined and whole-grain flour/larvae biomass

Treatment	Refined flour (%)	Whole-grain flour (%)	Larvae biomass (%)
T0	40	60	0
T1	40	55	5
T2	30	60	10
T3	25	65	15

Larvae biomass sourcing. The slaughtering of the South American palm weevil larvae consisted in a two-step process: letting them stand in cold water at 5°C for 30 minutes and a decapitation afterwards. Larvae were then skinned; larvae guts were taken apart from the carcass. The fleshy part and the skins were cut into small pieces. These pieces were then dried in a laboratory oven (INB 500, Memmert GmbH + Co. KG, Schwabach, Germany) at 54°C for 72 hours. After this, the dried pieces were ground and thus obtaining the larvae biomass to be added as the replacement component.

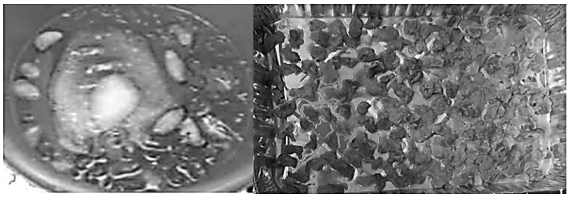

Figure 1. Larvae slaughtering and dried biomass

Bread making. Bread pieces were manufactured after the Ecuadorian Technical Standard (19). The formulation for standard whole-grain bread included 600 g water, 500 g flour, 70 g oil, 80 g sugar, 20 g yeast, 15 g salt, 100 g natural yeast dough, and 500 g whole-grain flour. The replacement of flour with South American palm weevil larvae biomass was performed according to the proposed treatment. For example, a 10% replacement was performed with the following formulation: 100 g larvae biomass, 400 g flour, 450 g whole-grain flour, 80 g sugar, 15 g salt, 100 g natural yeast dough, 20 g yeast, 600 g tap water, 70 g oil. The resulting dough was processed for 8 minutes in a kneader (Whirlpool, Benton Harbor, United States). The fermentation process in the kneaded dough lasted 60 minutes. The dough was cut into small pieces of about 50 g and were rounded manually. The rounded pieces were put onto stainless steel trays. After 15 minutes, trays were located in a convection oven (Andino, Quito, Ecuador) at 180 º C for 25 minutes.

Profiling of lipids. Total fat in the obtained functional bread was measured after the technique acquainted in AOAC 920.39; the lipidic profile was determined by gas chromatography techniques based on the AOAC 991.39 technique (20). Derivatization reagents were used as extraction medium. Two levels were set for the following conditions: derivatization reagent, solvent type, reaction time, and sample amount. Volumes used in derivatization were 250 and 500 µL sodium methoxide (Sigma Aldrich, Missouri, United States) 0.5 M, while for the boron trifluoride method, 700 µL of the reagent (Sigma Aldrich, Missouri, United States) were used. Reaction times for sodium methoxide and boron trifluoride were 45 and 15 minutes, respectively.

Firmness measurements. Trials for firmness measurements were performed with the Perten TVT 6700 texture analyzer (PerkinElmer, Waltham, United States). The profile used was 01-02.01 AIB “White Bread Firmness.” Bread pieces underwent the simple cycle compression. Samples were located on the base dish where the probe was inserted to measure firmness.

Statistical analysis. The statistical analysis was carried out by the statistical programming language R (R Foundation, Vienna, Austria). Results declared were the average of 3 repetitions ± standard deviation. The least significant difference (LSD) Fisher test with a significance level of 5 % ($p < 0.05$) was used to determine whether differences among experimental treatments were to be found.

RESULTS AND DISCUSSION

Characterization of fat and measurements of fat amounts occurred in functional bread including South American palm weevil biomass were determined after 48 hours of storage at local room temperature (20 °C) and the environment relative humidity (80%). Figure 2 describes the chromatogram for the fatty acids present in functional bread with 15% palm weevil biomass.

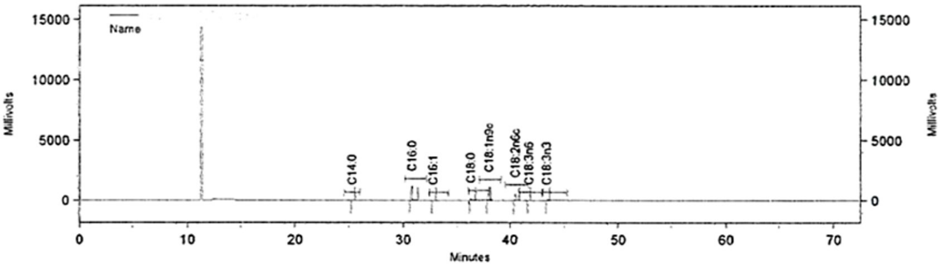

Figure 2. Chromatogram for fatty acids present in functional bread with 15% palm weevil

Areas, considering height and width in the baseline, were determined in the spikes obtained for fatty acids in chromatograms. Table 3 shows the areas measured for each spike.

Tabla 3. Fatty acids composition according to chromatographic spikes

Spike	Fatty acid	Area (%)
1	C14:0	0.52
2	C16:0	40.54
3	C16:1	0.12
4	C18:0	4.54
5	C18:1n9c	37.57
6	C18:2n6c	15.9
7	C18:3n6	0.25
8	C18:3n3	0.56
Total		100

Total fat content

Percentage of fat increased significantly ($p \leq 0.05$) in the treatments studied as the amount of South American palm weevil larvae biomass added to the formulation increased, following an expected pattern, as the studied raw material featured a higher percentage of fat content than the conventional ingredients in bread loaves made with Inca peanut (Plukenetia volubilis) (21). On the other hand, lower fat content in functional bread were reported against traditional bread with compounded flours with lower fat content than in wheat flour (22). Control treatment T0 yielded 4.39% saturated fatty acids. Table 4 shows results concerning saturated, mono, and polyunsaturated fats in the functional bread obtained. Saturated fats decreased significantly upon 4.16% in T3. Total unsaturated fat content in functional bread yielded values closer to those reported in whole-grain bread; unsaturated fat values in whole-grain bread should be around 3-3.5% (23).

Tabla 4. Content of fat and saturated, mono, and polyunsaturated fatty acids in functional bread including South American palm weevil biomass

Analysis	Treatments			
	T_0	T_1	T_2	T_3
Total fat (%)	8.94a±(0.06)	8.95aa±(0.06)	9.15b±(0.04)	9.22b±(0.03)
Saturated fatty acids (%)	4.39a±(0.008)	4.30b±(0.01)	4.25c±(0.01)	4.16d±(0.009)
Monounsaturated fatty acids (%)	3.31a±(0.02)	3.37ab±(0.01)	3.42ab±(0.01)	3.51c±(0.04)
Polyunsaturated fatty acids (%)	1.24a±(0.02)	1.28a±(0.01)	1.48a±(0.02)	1.55a±(0.02)
Trans fat (%)	0	0	0	0

Average results n=3 ± standard deviation. Different superscripts in the same row show significant differences ($p \leq 0.05$).

According to the quantity of South American palm weevil biomass added, the amount of monounsaturated fatty acids increased significantly; the amount of polyunsaturated fatty acids also increased; however, no significant differences were found.

Figure 3. Dough of functional bread including palm weevil biomass and resulting bread pieces

Firmness analysis

The addition of palm weevil biomass, between 5 and 15% of the formulation, resulted in a firmness range between 5,348 and 6,925 gf. Fig. 4 shows firmness values for the functional bread pieces obtained. Values found in the present research work were higher to those reported previously in bread pieces made including insect biomass, such as 10% replacement with mealworm (*Tenebrio molitor*) and with darkling beetle (*Alphitobius diaperinus*); texture values for these types of bread were 1.216 and 1.037 gf, respectively (24). In addition, comparing the reached firmness values with the ones reported for bread made with tuber meals (1,613.3 and 1,889.1 gf), this property is higher for the bread obtained in the present work (25). This difference may be attributed to the higher protein content in palm weevil biomass. Firmness was also higher to those values reported for conventional whole-grain bread (T_0, 5,218 gf) (26).

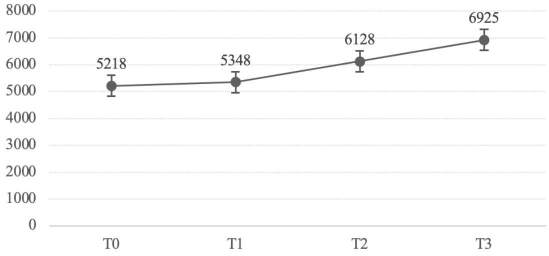

Figure 4. Firmness values for the functional bread pieces made with biomass of palm weevil

CONCLUSIONS

As the replacement percentage with biomass of palm weevil in whole-grain bread increased, the level of mono and polyunsaturated fatty acids also reached a higher level of quantity, obtaining a solid alternative in the supply of functional food products. However, firmness values were higher than those for traditional bread and bakery products. The results of this research work are intended to be one of the support points to diversify the consumption options of palm weevil larvae through its application in high consumption food products, thus enhancing the local production of food goods and the establishment of food industries, thus improving their economic horizons by offering a functional food product that contributes to the control of public health problems.

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