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Sensory Acceptance, Microbiological, and Nutritional Properties of A Sausage-Like Meat Product Produced with Partial Inclusion of *Rhynchophorus Palmarum* Larvae

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Abstract

Rhynchoporus palmarum is widely considered to be a harmful pest of many crops. Nevertheless, it is highly valued as a food source in some countries, providing valuable components, mainly protein and fatty acids. The aim of this study was to develop a sausage with partial incorporation of *R. palmarum* larvae and to explore its potential as an alternative ingredient, taking into account the sensory acceptance of the final product, as well as nutritional and microbiological aspects. Experimental formulations containing 30, 20, and 10% larvae (F1, F2, F3, respectively) partially replaced pork meat and vegetable fat, and a control (F0) without *R. palmarum* larvae. One hundred and fifty semi-trained panelists rated the taste, aroma, color, texture, and overall acceptability of the four formulations using a 5-point hedonic scale. F2 showed favorable sensory acceptance for taste, color, texture, and overall acceptability, with no significant statistical difference from F0, but aroma showed a significantly better score than F0. Nutritional data showed increasing patterns for energy, fat, and sodium, while decreasing values for cholesterol, protein, and total carbohydrate for F1, F2, and F3, respectively. The microbiological characteristics of the experimental formulations for aerobic plate count, *E. coli* count, *S. aureus*, and *Salmonella* spp. met the requirements of the Ecuadorian standard NTE 1338:2012 for cooked meat products. This research highlights the potential

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of *Rhynchophorus palmarum* larvae as a viable and sustainable option for partial meat substitution in sausage production, offering an innovative approach to improve nutritional value and diversify product offerings in the food industry.

Introduction

The consumption of meat and meat products is increasing globally due to the growth of the population, leading to concerns about sustainability, animal welfare, and public health. Problems, such as the expansion of the agricultural frontier causing biodiversity loss and deforestation, soil degradation, and increasing irrigation water demands, as well as others, are well documented.[1]

As a result, there is a growing interest in alternative protein sources, such as insects, as a more sustainable and eco-friendly option.[2] One such insect is the *Rhynchophorus palmarum*, commonly known as the palm weevil, consumed in many parts of the world as a delicacy and a protein-rich food source.3 In some regions, larvae of this genus are considered pests affecting the production of edible products for the population.[4, 5] However, insects in the larval stage have a high nutritional value because they provide a high percentage of nutrients.[6]

Currently, producing biomass from insects represents an economical alternative to protein supply in some countries.[7] Especially in countries lacking protein in people's daily diets. The output of insect-based proteins will help to increase protein sources in food in the coming years. Moreover, it has a low environmental impact and is food for direct use in diet.[8]

The palm weevil is widely distributed in tropical regions, and its larvae are considered a nutritious food source rich in protein, fat, and fiber. The larvae are also a rich source of minerals, including calcium, iron, and zinc. Moreover, the larvae have a high feed conversion rate, making them a highly efficient source of protein.[9] In addition, it has been reported the presence of essential amounts of unsaturated and saturated fatty acids, the most abundant the oleic and palmitic acids;[10] also, due to its fatty composition, is attributed to some antimicrobial and anti-inflammatory properties in traditional Peruvian,[11] and Brazilian medicine.[12]

The larvae of *R. palmarum* are traded in markets and tourist centers in the Amazon region, and these larvae are highly valued for their characteristic flavor.[13] Other palm weevil species, such as *R. phoenicis* and *R. ferrugineus*, are delicacies in several communities worldwide, especially in Asia and Africa.[14] On the other hand, the effective technological potential of chitosan production from the remains of *R. palmarum*, a polysaccharide with antimicrobial activity and wide application in the food industry, has been investigated.[15]

Recent research presented an alternative to fresh meat products, including insect proteins in a mixed form to produce textured intermediates with high protein content.[16] However, more research is needed to compare insects and conventional proteins, regardless of the processing method.[17]

Despite the potential of palm weevil larvae as a protein source and the promising results of some industrial applications, to our knowledge, there are no previous attempts to incorporate this species as the main or partial ingredient in meat products or analogs. Therefore, this study aims to evaluate the partial incorporation of *Rhynchophorus palmarum* larvae in elaborating a meat product, specifically sausages, focusing on its sensory acceptance, nutritional, and microbiological properties.

Materials and Methods

Rhynchophorus Palmarum Larvae

The larvae of *Rhynchophorus palmarum* were purchased at the open-air market "Los Plátanos" in the city of Puyo, located in the Ecuadorian Amazon Rainforest. 5 kg of live larvae were purchased, placed in polyethylene terephthalate (PET) plastic bags with perforations of 1.5 cm in diameter, sealed and placed in a container that maintained the ambient temperature (25°C) during transport and until processing, which was performed twelve hours later. The development process of the meat products was carried out in the Meat Products Laboratory. In contrast, the nutritional and microbiological analyses were carried out in the Food Chemistry Laboratory of the Faculty of Agricultural Sciences of the Agrarian University of Ecuador, Guayaquil Campus.

Product Formulation

Table 1 shows the composition of the treatments of the developed product, starting from a standard formulation that included a percentage of Tenebrio molitor meal in the preparation of chicken breast sausages.[18] On the other hand, three experimental formulations with different larvae values were designed to compare their sensory acceptability and a control treatment with 0% *R. palmarum* larvae.

Table 1: Formulations and components used in elaborating the meat product

Component	F0 (%)	F1 (%)	F2 (%)	F3 (%)
R palmarum larvae	0	30	20	10
Lean beef meat	60	60	60	60
Frozen pork meat	10	0	0	0
Vegetable fat	20	0	10	20
Plantain flour	4	4	4	4
Seasonings and spices*	2	2	2	2
Curing salts	3	3	3	3
Ice cubes	1	1	1	1
Total	100	100	100	100

*The seasonings were equal parts of cumin powder, hot pepper powder, and powdered bay leaves.

Product Development Process

First, the *Rhynchophorus palmarum* larvae were cleaned of foreign material using abundant potable water, and then the heads and internal organs were removed and discarded using stainless steel knives. The disinfection process consisted of spraying a 2% V/V solution of lactic acid on the surface of the larval carcasses for 15 s with a dwell time of 5 min.[19] The larvae were blanched (100 °C x 10 min) to inactivate the enzymes and reduce the microbial load.[20] The larval carcasses, lean beef, and pork were ground in a Vevor AL-22 stainless steel grinder (Vevor, USA) while mixing with plantain flour, curing salts (sodium nitrate) and spices, and incorporating half of the ice. Finally, the vegetable fat and part of the remaining ice were added to the mixture and emulsified for about 4 min, carefully controlling the mixture's temperature from 12 to 14 °C by adding or removing ice cubes where needed. The emulsified mixture was stuffed into 20 mm synthetic cellulose casings; the sausages were immediately boiled in water to an internal temperature of 80±2°C for 20 minutes, then cooled by thermal shock to room temperature (20±2°C), the sausages were packed into PET casings and vacuum sealed using a Techtongda DZ 500 stainless steel vacuum packer (Techtongda, China). Finally, the casings were stored under refrigeration (2°C) until further use.

Sensory Evaluation

The evaluation was carried out in the Sensory Analysis Laboratory of the Department of Agroindustrial Engineering of the Agrarian University of Ecuador on the same day, and the samples were served consecutively. 25 g of each formulation, covered with aluminum foil to maintain its temperature (65±2°C,[21]), identified with a random number, and enough water to rinse the mouth were given to each evaluator. One hundred and fifty semi-trained panelists divided into five groups (equal numbers of men and women, students of the last semesters of the program mentioned above and who have received basic training in sensory analysis, terminology, evaluation methods, and sensory attributes) evaluated, within the same day, five sensory parameters: flavor, aroma, color, texture, and overall acceptability using a 5-point hedonic scale, where level 5 is equivalent to "I like it very much," while level 1 means "I dislike it very much."

Nutritional and Microbiological Analysis

The parameters under study were taken from the Ecuadorian Technical Standard NTE INEN 1334-3 referring to "Labeling of Food Products for Human Consumption".[22] Microbiological quality requirements were taken from Ecuadorian Technical Standard NTE INEN 1338 for Cooked Meat Products.[23]

For the analyses, the official procedures of AOAC International (2012)[24] described below were used: AOAC 928.08 for protein content, AOAC 991.36 for fat, AOAC 976.[26] for cholesterol, AOAC 983.04 for sodium, AOAC 2020.07 for carbohydrates, energy by calculation.

The microbiological analysis procedures used were: AOAC 996.[23] for aerobic plate count, AOAC 966.[23] for mesophilic aerobic count, AOAC 967.[26] for *Salmonella* spp, AOAC 2003.07 for *Staphylococcus aureus* and AOAC 991.14 for *Escherichia coli* count.

Statistical Analysis

The data obtained were tabulated using the statistical software InfoStat version 2020.[25] One-way analysis of variance (ANOVA) was used to compare the means between groups. Tukey's test ($p<0.05$) determined significant differences between treatments. All nutritional and microbiological analyses were performed in triplicate.

Results and Discussion

Results of Sensory Acceptance

Table 2 shows the sensory acceptability results of the five parameters evaluated. In the sensory attribute flavor, formula F2 presented the highest acceptance with a mean of 3.70, but it did not show significant differences concerning the control ($p>0.05$). On the other hand, F3, containing 10% of *R. palmarum* larvae, displayed the lowest score with a mean of 2.97. In the sensory attribute aroma results, formula F2 presented the highest acceptance, with a mean of 4, and it showed significant differences from the control (F0), which gave the lowest score, with a mean of 3.13.

For the sensory attribute color, F0 had the highest acceptance with a mean of 3.67, closely followed by F2 with a mean of 3.60, with no significant differences between the four formulations.

Similarly, for the sensory attribute texture, the F0 formulation showed the most remarkable acceptance with a mean of 3.47, followed by F2 with a mean of 3.43, and there were no significant differences between the two.

Finally, in terms of overall acceptability, the highest score was for F2 with an average of 3.75, closely followed by the control formulation F0 with 3.70, then F1, and at last, F3 with an average of 3.1, which showed a statistically significant difference compared to the previous three formulations.

The results of our study of the sensory attributes of formulations containing different percentages of *R. palmarum* larvae in a meat product, particularly their flavors, aromas, colors, textures, and overall acceptability, provide valuable insights into its potential use as a food ingredient. Formula F2 emerged as the most preferred option among the tested formulations regarding flavor, aroma, and overall acceptability. The high acceptance of F2 suggests that incorporating *R. palmarum* larvae in specific proportions could effectively enhance the sensory attributes of food products.

Interestingly, despite F2 displaying the highest accepta acceptance for flavor, it did not exhibit significant differences compared to the control formulation (F0). This implies that adding *R. palmarum* larvae in F2 did not compromise the overall flavor profile of the product, making it a promising option for potential commercial use.

Moreover, the significant differences observed in the aroma attribute between F2 and the control formulation (F0) indicate that *R. palmarum* larvae may contribute positively to the aroma profile, adding an enticing sensory element to the product. This finding agrees with the report in wheat-based buns, where up to 20% thermite flour inclusion did not modify the sensory acceptability of aroma.[26] It has been suggested that the cooking technique in the insect preparation may directly impact their sensory acceptance because of aromatic compounds' formation.[27]

Regarding color and texture, the lack of significant differences between formulations F0 and F2 suggests that including *R. palmarum* larvae did not adversely affect these attributes. This finding is critical because consumers often value sensory characteristics such as the appearance and texture of food products.[28] The similarity in scores between the control and F2 formulations for these attributes indicates that *R. palmarum* larvae can be incorporated into food products without compromising their appearance and texture.

However, formula F3, containing the lowest proportion of *R. palmarum* larvae (10%), displayed the lowest acceptance scores for flavor and overall acceptability. This result could be attributed to a potential "acquired taste" effect, where consumers might not be accustomed to the distinct flavor profile associated with higher insect content. It also underscores the importance of finding an optimal balance in the formulation to achieve better consumer acceptance.

Considering these findings, future research could investigate methods to mitigate potential "acquired taste" challenges associated with higher insect content in formulations. Additionally, studying the nutritional profile and potential health benefits of *R. palmarum* larvae could further support their incorporation into food products.

Table 2: Results of sensory evaluation

Formulations	Flavor	Aroma	Color	Texture	Overall acceptability
F0	3.10±0.12[ab]	3.13±0.13[b]	3.67±0.32[a]	3.47±0.16[a]	3.70±0.27[a]
F1	3.33±0.21[ab]	3.63±0.22[ab]	3.40±0.17[a]	3.00±0.23[ab]	3.45±0.16[a]
F2	3.70±0.16[a]	4.00±0.19[a]	3.60±0.21[a]	3.43±0.14[a]	3.75±0.18[a]
F3	2.97±0.23[b]	3.43±0.19[ab]	3.47±0.11[a]	2.47±0.11[b]	3.1±0.18[b]

Mean values±standard deviation of the columns with the same letter did not show any statistically significant differences (p>0.05).

In conclusion, our study highlights the potential of *R. palmarum* larvae as a viable ingredient in food formulations. Formula F2, with a balanced proportion of larvae, demonstrated the highest overall acceptability and positive effects on flavor and aroma attributes. These results contribute to the growing body of literature supporting the use of edible insects as sustainable and nutritious alternatives in the food industry.[29, 30] By understanding the implications of our findings and building upon this research, we can pave the way for innovative and eco-friendly food products that cater to consumer preferences while addressing global sustainability challenges.

Results of Nutritional Assays

The nutritional analysis presented in Table 3 provides valuable insights into the composition of the experimental treatments, specifically their energy content, fat, cholesterol, protein, sodium, and total carbohydrate values. The variations observed in these nutritional parameters among the different formulations (F1, F2, and F3) are noteworthy and may have significant implications for the potential use of *R. palmarum* larvae in food products.

The variation in energy content observed across the formulations (F1 to F3) in our study is a crucial outcome that warrants further discussion. The energy content ranged from 275.23 to 309.5 Kcal, with F3, the formulation with the lowest inclusion of *R. palmarum* larvae, exhibiting the highest energy content at 309.5 Kcal. In contrast, F1, with the highest inclusion of larvae, had the lowest energy content at 275.23 Kcal. These results have significant implications for the potential use of these larvae as an ingredient in food products and can shed light on the formulation's nutritional characteristics and market appeal.

The unexpected pattern of F3, the formulation with the lowest inclusion of larvae, having the highest energy content, contrasts with what has been previously reported, as fat is the second most abundant component (after protein) of insects.[31, 32] However, other ingredients in F3, apart from the larvae, might have contributed to its higher energy content. Further analysis of the nutritional composition and interactions between different components in the formulations could provide valuable insights into this unexpected pattern. Relating our findings to existing literature, the energy content variation aligns with studies showing how different ingredients can influence the nutritional profile of food products. For example, formulations with varying amounts of fats, carbohydrates, and proteins can result in variations in nutritional composition.[33] The unique nutritional composition of *R. palmarum* larvae, rich in proteins and fats, might have interacted with other components in the formulations, leading to the observed differences in energy content.

Fat content increased from 19.03% for F1, 21.07% for F2, and 22.65% for F3. This increasing trend suggests that the inclusion of *R. palmarum* larvae in the formulations contributes to the overall fat content of the products, as insects are considered a valuable source of lipids.[34] In addition, possible explanations for this pattern involve interactions between *R. palmarum* larvae and other ingredients in the formulations. The specific formulation of F3, including additional vegetable fats, contributed to the observed peak in fat content.

Cholesterol levels were in decline, 0.45% for F1, 0.30% for F2, and the lowest value for F3 was 0.29%. The decrease in cholesterol levels in all formulations is consistent with the known dietary composition of insects, which are generally low in cholesterol,[35] particularly *R. palmarum* larvae.[36]

Similarly, the protein content presented a decreasing pattern coinciding with the treatment with the highest inclusion of *R. palmarum*, F1 with 12.1%, 10.66% for F2, and 10.45% for F3. The decreasing pattern in protein content with higher inclusion of larvae aligns with prior knowledge of protein-rich insects.[37] Likewise, the sodium content presented decreasing values concerning the formulations, the lowest value being 14.5% for F1, 13.22% for F2, and 12.90% for F3, suggesting that *R. palmarum* larvae might possess attributes that influence sodium levels in the formulations.

Finally, the total carbohydrate values also showed a decreasing pattern, with the highest being 14.5% for F1, 13.5% for F2, and the lowest being 12.9% for F3. The similar decreasing trend in total carbohydrate values across the formulations is consistent with the expectation that including protein-rich ingredients such as *R. palmarum* larvae would reduce the carbohydrate content. This observation is relevant in the context of carbohydrate-conscious diets.

Table 3: Results of nutritional composition

Parameter	F1	F2	F3
Energy (Kcal)	275.23±1.2[a]	290.82±0.98[ab]	309.5±1.37[b]
Fat (%)	19.03±1.33[a]	21.70±1.14[a]	22.65±0.76[a]
Cholesterol (%)	0.45±0.19[a]	0.30±0.10[ab]	0.29±0.11[b]
Protein (%)	12.1±0.95[a]	10.66±0.78[ab]	10.45±0.97[b]
Sodium (%)	3.22±0.90[a]	3.51±0.65[a]	3.87±0.77[a]
Total carbohydrates (%)	14.5±1.02[a]	13.22±0.89[a]	12.90±1.23[a]

Mean values±standard deviation of the rows with the same letter did not show any statistically significant differences (p>0.05)

Results of Microbiological Assays

Table 4 shows the microbiological results of the three experimental formulations. The results of all formulations for the aerobic plate count, *E. coli* count, S. aureus, and *Salmonella* spp. have complied with the requirements established in the Ecuadorian Standard for Meat and Meat Products. These findings demonstrate that the formulations meet the microbiological standards necessary to ensure the safety of food consumers.[38]

Adherence to microbiological standards is paramount in the food industry as it directly impacts public health.[39] This suggests that the experimental meat products' manufacturing process, handling, and storage conditions were adequately controlled and executed. It also reflects the effectiveness of any safety protocols implemented during production.[40]

Conclusion

This study aimed to evaluate the sensory acceptance, nutritional, and microbiological properties of sausages containing 30, 20, and 10% of *Rhynchophorus palmarum* larvae in their composition. Formulation F2 with 20% larvae showed a satisfactory sensory acceptance, as it was not statistically significantly different for four parameters: taste, color, texture, and overall acceptability, except for aroma, which was significantly better than a conventional meat product without *R. palmarum* larvae. The experimental formulations showed a respectable performance in terms of nutritional composition with moderate levels of protein, energy, fat, total carbohydrate levels, and particularly low cholesterol and sodium levels. In addition, the microbiological requirements for aerobic plate and *E. coli* counts, S. aureus and *Salmonella* spp., complied with the Ecuadorian Standard NTE INEN 1338:2012 for Meat Products, which guarantees food safety and quality. This paper is a valuable contribution to support the use of *R. palmarum* larvae as a promising ingredient, either as a partial or main component for meat or as part of other food products. Further research on instrumental textural properties and color analysis is recommended to complement its technological use.

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Conflict of Interest

The authors declare no conflict of interest.

Author´s contribution

Luis Zuniga-Moreno: Conceptualization, Data Curation, Software, Writing – Original Draf preparation, Writing – Review and Editing, Supervision. Ahmed El-Salous: Conceptualization, Formal Analysis, Investigation, Methodology. Jesús Melendez: Software, Investigation, Project Administration. Evelyn Castro: Formal Analysis, Investigation, Methodology. Winston Espinoza: Supervision, Visualization, Writing – Original Draft preparation. Daniel Mancero-Castillo: Supervision, Visualization, Writing – Original Draft preparation. Pablo Nuñez-Rodriguez: Resources, Validation. Freddy Arcos: Resources, Validation.

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