Universidad de Guayaquil
Facultad de Ingeniería Química
Ingeniería Química y Desarrollo
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Evaluation of the application of essential oils as preservatives in the preparation of chorizo from Cuenca

Evaluación de la aplicación de aceites esenciales como conservantes en la elaboración de chorizo cuencano

Edgar Fernando Landines Vera [1] * ; Dayanna Narcisa Soledispa Chancay [2]

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*Autor para la correspondencia.

Abstract
The present work seeks to evaluate the application of essential oils as preservatives in the preparation of chorizo from Cuenca. Sausages, being processed meats seasoned with various aromatic herbs and spices introduced in a natural animal casing, have a high probability of germinating several pathogenic microorganisms in their manufacturing processes, so the application of rosemary and feijoa essential oils is justified since these are obtained from vegetable matter and have characteristics that originate aromas, light and very volatile that are obtained directly from plants, flowers, leaves, trees, roots, among others; that is why they have a very important role in the food industry to be applied for its antimicrobial activity that is why a study will be managed and / or conducted in a laboratory to the sample with greater acceptance where its microbiological and physicochemical progress is checked to demonstrate that it is suitable for consumption, in addition to obtaining the results shelf life which are 15 days according to the analysis that were conducted in the laboratory PROTAL.

key words
Rosemary, Feijoa, Chorizo cuencano, Essential oils, preservative, microbial

Resumen
El presente trabajo busca evaluar la aplicación de aceites esenciales como conservantes en la elaboración de chorizo cuencano. Los embutidos al ser carnes procesadas y condimentadas con diversas hierbas aromáticas y especies introducidas en una tripa natural de originen animal tienen una alta probabilidad de germinar varios microorganismos patógenos en sus procesos de elaboración, por lo que se justifica la aplicación de los aceites esenciales de romero y feijoa ya que estos se obtienen de materia vegetal y poseen características que originan aromas, ligeros y muy volátiles que se obtienen directamente de las plantas, flores, hojas, arboles, raíces, entre otros; es por eso que tienen un papel muy importante en la industria alimenticia al ser aplicados por su actividad antimicrobiana por eso se gestionara y/o realizara un estudio en un laboratorio a la muestra con mayor aceptación donde se comprueba su avance microbiológico y fisicoquímico para demostrar que es apta para el consumo; además de obtener los resultados tiempo de vida útil que son 15 días según los análisis que se realizaron en el laboratorio PROTAL.

Palabras clave
Romero, Feijoa, Chorizo cuencano, Aceites esenciales, conservante, microbiana

1. Introduction

The main objective of this study is to evaluate the application of essential oils as preservatives in the production of Cuenca-style chorizo.

Processed meats, known as sausages, are typically seasoned with various aromatic herbs and spices and encased in natural animal casings. Their origin dates back to ancient times when humans sought ways to preserve their food. It was discovered that salt could be applied to food around 2670 B.C. Historical records indicate that the Egyptians obtained salt from the desert and used it to preserve meat through the salting method, allowing them to store and consume it later [1], [2].

It is also worth mentioning that essential oils are derived from plant material and have characteristics that produce light, highly volatile aromas directly extracted from plants, flowers, leaves, trees, roots, and more [3]. This product can be utilized for its various properties, including home remedies, cosmetics, and perfumery. Essential oils are obtained through extraction or distillation by steam or direct extraction methods. [4]

1.1 Origin of Sausages

The origin of sausages is closely linked to the salting process, with salt making its appearance around 3000 B.C., enabling the trade of salted fish and meat [5]. As a result, the development of sausages evolved, becoming popular in ancient Greece, where references to ham, bacon, and other products were already made [6]. In Homer's Odyssey, there is mention of casings filled with blood and fat, cooked over fire, which is known today as blood sausage [7].

1.2 Types of Sausages

[1] Universidad de Guayaquil; edgar.landinesv@ug.edu.ec .
[2] Universidad de Guayaquil; dayanna.soledispac@ug.edu.ec.

Cooked Sausages
These are sausages where the meat is cooked, and in most cases, pressed, such as York ham, butifarra, mortadella, and Frankfurt sausages. [8]

Fig. 1. Sausage
Source: [9]

Raw Sausages
These are sausages where the meat is preserved by seasoning with spices or paprika and then dried in cold, dry air until it reaches a hard consistency, such as longaniza, fuet, chorizo, loin, bacon, and serrano ham [9].

Table 1
Composition of Pork Meat

Per 100 g of pork meat		
Name	**Amount**	**Unit**
Water	76.21	g
Energy	118	kcal
Energy	494	kj
Protein	17.27	g
Total lipids (fats)	4.36	g
Ash	0.84	g
Carbohydrates by difference	1.33	g
Total dietary fiber	0	g

Source: [10]

Table 2
Composition of Pork Fat

Per 100 g of pork fat		
Name	**Cantidad**	**Unidad**
Water	0	g
Energy	897	kcal
Energy	3753	kj
Protein	0	g
Total lipids (fats)	99.5	g
Ash	0.5	g
Carbohydrates by difference	0	g
Total dietary fiber	0	g

Source: [11]

Fresh Sausages
These are sausages where the meat is seasoned and encased, such as sausages or chistorra. Chistorra, for example, is aired for three days, and chorizo can be cured or fresh [12].

Blood Sausages
These sausages contain meat, offal, lard, bacon, and other ingredients, in addition to blood sausages and cold cuts. They are similar to cooked sausages but of lower quality [12].

1.3 Food Additives Applied in Sausages

Colorants
These are additives currently used in the food industry with the primary goal of altering the color, which is one of the attributes when choosing a type of food. Generally, natural colorants are recommended, but artificial colorants are applied in the food industry. These products need to be colored to be visually appealing to consumers, with red being the predominant color added to sausages and hot dogs. [13]

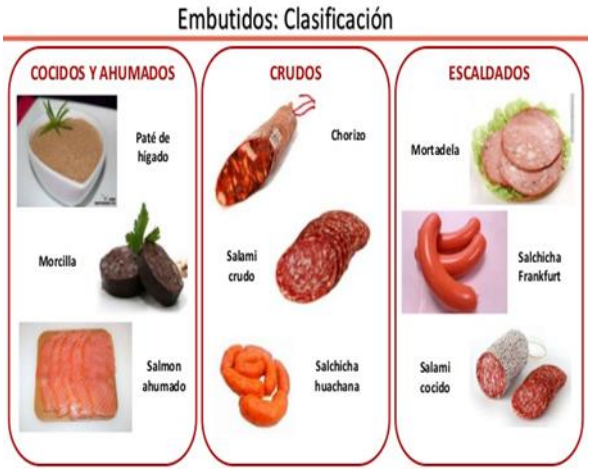

Fig. 2. Types of Sausages
Source: [14]

Preservatives

Preservatives are chemical products that benefit food by extending its shelf life and preventing the product from undergoing any alterations in appearance. There is a wide variety of microorganisms, such as bacteria, yeasts, and molds, that are ready to attack and rapidly deteriorate food, especially sausages and dairy products. This type of additive must be applied with caution because excessive use can be toxic to the digestive process. Among preservatives, sorbic acid and sulfites are commonly used. [15]

An example of a preservative is nitrite, which is an additive used as a preservative in a wide range of cured

meat products, such as salami, chorizo, ham, and cooked sausages. It is also found in sterilized meat products like cooked sausages. Additionally, some marinated meat preparations like marinated pork loin, kebabs, marinated pork head, and marinated pork ribs contain nitrites. Nitrates naturally occur in soil, water, vegetables, and animals, and their levels increase in food crops due to the use of nitrogen-based fertilizers. [16]

Antioxidants

These are additives added to food to prevent rancidity. Generally, food in contact with oxygen in the air deteriorates as it loses its nutritional properties, specifically vitamins A and C. It is also known that fats deteriorate, accelerating the rancidity process, which results in an unpleasant taste for the consumer and can even harm health. Antioxidants can be found naturally and synthetically [17].

Flavor Enhancers

These are substances or concentrations generally used in foods. As the name suggests, they do not provide any flavor themselves but instead enhance the flavor of other components. This type of additive creates a sensation on the palate and increases the viscosity, for example, in soups and sauces, although it can be used in various products. Examples of flavor enhancers include L-glutamic acid, monosodium glutamate, potassium glutamate, calcium glutamate, ammonium glutamate, and magnesium glutamate. [18]

1.4 Permitted Additive Limits According to Ecuadorian Legislation

The current INEN STANDARD establishes the maximum dosage of additives that can be applied to sausages.

Table 3.
Sausage

Sausage			
Additive	**Year**	**Maximum Dosage**	**Unit**
Sunset Yellow FCF	2008	300	mg/Kg
Carmines	2005	500	mg/Kg
Carotenoids	2011	100	mg/Kg
Beta-Carotenes, Vegetable	2005	5000	mg/Kg
Ascorbyl Esters	2001	5000	mg/Kg
Grape Skin Extract	2009	5000	mg/Kg
Phosphates	2010	1100	mg/Kg
Hydroxybenzoates	2010	36	mg/Kg
Iron Oxides	2005	1000	mg/Kg
Polysorbates	2007	1500	mg/Kg
Ponceau	2008	500	mg/Kg
Riboflavins	2008	1000	mg/Kg
Allura Red	2009	300	mg/Kg
Green S	2009	100	mg/Kg

1.5 Permitted Additive Limits According to Spanish Legislation

The current SPANISH COMMISSION REGULATION establishes the maximum dosage of additives that can be applied to sausages

Table 4
Casings

Casings		
Additive	**Maximum Dosage**	**Unit**
Colorants	Adequate amount	mg/Kg
Food Colorants with Combined Maximum Limits	500	mg/Kg
Food Colorants with Combined Maximum Limits	Adequate amount	mg/Kg
Curcumins	Adequate amount	mg/Kg
Riboflavin	Adequate amount	mg/Kg
Cochineal, Carmine Acid, Carmines	Adequate amount	mg/Kg
Annatto, Bixin, Norbixin	20	mg/Kg
Lycopene	500	mg/Kg
Lycopene	30	mg/Kg
Sorbic Acid and Sorbates	Adequate amount	mg/Kg
Sorbic Acid and Sorbates, P-Hydroxybenzoates	1000	mg/Kg
Phosphoric Acid, Phosphates, Di-, Tri-, and Polyphosphates	4000	mg/Kg
Nitrites	125	mg/Kg

Source: [19]

1.6 Permitted Additive Limits According to U.S. Legislation

The current CODEX ALIMENTARIUS establishes the maximum dosage of additives that can be applied to sausages.

Table 5
Sausage

Sausage	
Additive	**Maximum Dosage**

Sunset Yellow FCF	300 mg/Kg
Brilliant Blue FCF	100 mg/Kg
Carotenoids	100 mg/Kg
Caramel III Ammonia	BPF
Caramel IV Sulphite Ammonia	BPF
Carmines	500 mg/Kg
Beta-Carotenes, Vegetable	5000 mg/Kg
Grape Skin Extract	5000 mg/Kg
Phosphates	1100 mg/Kg
Hydroxybenzoates	36 mg/Kg
Nisin	7 mg/Kg
Nitrite	80 mg/Kg
Polysorbates	1500 mg/Kg
Ponceau	500 mg/Kg
Riboflavins	1000 mg/Kg
Allura Red AC	300 mg/Kg
Sorbates	10000 mg/Kg
Tartrates	2000 mg/Kg
Tocopherols	5000 mg/Kg
Green S FCF	100 mg/Kg
Ascorbyl Esters	5000 mg/Kg
Interesterified Polyglycerol Esters of Ricinoleic Acid	5000 mg/Kg
Polyglycerol Esters of Fatty Acids	5000 mg/Kg
Iron Oxides	1000 mg/Kg

Source: [20]

1.5 Essential Oils

Essential oils are aromatic liquids of varying colors depending on the flowers or vegetables from which they are extracted. Examples include flowers (lavender, ylang-ylang), wood (Atlas cedar, white sandalwood), roots (ginger, valerian, vetiver), or seeds (coriander, green anise, carrot). Essential oils are obtained through steam distillation, and this type of oil can be extracted from aromatic plants by rubbing them until the oil droplets are released into the atmosphere and reach the nose. [21]

Fig. 3. Essential Oils

2. Materials and Methods

Sensory Analysis

Sensory analysis involves evaluating food using the senses. It requires specific and standardized techniques. In this thesis, sensory analysis is applied to ensure proper quality control by evaluating the use of essential oils as preservatives in "chorizo cuencano" during development or processing. According to the sensory analysis division, this study will use hedonic tests, paired preference tests, and acceptance tests.

Hedonic Tests

Hedonic tests are used to gauge how much a consumer enjoys a product—in this case, "chorizo cuencano" with the application of rosemary and feijoa essential oils—by using a scale provided by the analyst. Two different tests will be conducted to measure how much the designated consumers, who are students from the University of Guayaquil, like or dislike this product. These tests use categorized scales, which can have a different number of categories, typically ranging from "like very much" through "neither like nor dislike" to "dislike very much."

Paired Preference Tests

The paired preference test is used in this case to compare two types of samples. Essential oils of rosemary and feijoa will be applied to "chorizo cuencano," with different three-digit codes to differentiate them. This test will be performed with general consumers, who may have little knowledge of the product; the next test will be conducted with students from the University of Guayaquil's Gastronomy degree program, and will answer a single question: "Which do you prefer?"—that is, evaluate the two product samples.

Acceptance Test

The acceptance test aims to understand the sensory criteria of the sample that consumers will taste in order to gauge their opinions. These tests do not require experts in the product, so students from the University of Guayaquil's Gastronomy degree program were selected. Those conducting the test should not know why the study

is being carried out, only the procedure of the test, and should simply respond. A minimum of 80 people will be subjected to this type of test. The data will be processed based on the number of students who accept the sample versus those who reject the coarse paste sausage with the application of rosemary and feijoa essential oils. This will be analyzed in a significance estimation table to determine if the acceptance is statistically significant.

Experimentation

Rosemary

According to Estévez, Ventanas & Cava (2005), in an experimental study with sausages, rosemary essential oil was applied. The main ingredients for the product were as follows: 50 g of meat, 10 g of animal fat, 37 g of distilled water, 2 g of sodium caseinate, and 1 g of starch. Additionally, additives such as sodium chloride (2%), sodium triphosphate (0.5%), sodium ascorbate (0.05%), and sodium nitrite (0.03%) were applied. Three types of rosemary essential oil additions were made: 150 ppm, 300 ppm, and 600 ppm, along with four groups without essential oil for comparison.

The tests were maintained at a temperature of 4ºC for 60 days, with sampling conducted on three different occasions (0, 20, 40, and 60 days), observing color and texture. According to the results of protein oxidation with the addition of rosemary essential oil, the samples with 300 ppm (0.3) and 600 ppm (0.6) significantly reduced the release of iron [22]

Feijoa

As Nyström Angelica (2013) states in her study conducted on the skin, seeds, and pulp of feijoa (Acca sellowiana), essential oil was distilled, which served as a reference for this experimentation. For the experimentation with feijoa and rosemary essential oils, it was crucial to follow the proper process to utilize the antibacterial properties that could benefit "chorizo cuencano." Steam distillation and decantation were applied for approximately 7 days, extracting 2 g of feijoa essential oil.

To obtain the essential oil, 4.338 kg of feijoa was blended with 2.5 liters of ethyl alcohol (96%), resulting in a homogeneous mixture of 4.154 kg. The mixture was refrigerated for 4 days at 4ºC to achieve proper maceration, followed by filtration and, as a final step, direct distillation. The following materials were required for this procedure:

- Steam distillation equipment
- Hygiene and cleaning materials
- Macerated feijoa juice

Illustration 1 Distillation Equipment
Source: [23]

To obtain the essential oils, the equipment was set up, including a thermometer to control the temperature, a burner, and a water valve to regulate the water's temperature. The distillation process began at 90ºC and lasted for 5 hours, yielding 400 ml of alcohol with a layer of essential oil, which was left to rest for 24 hours for decantation. This process was repeated over 7 days at the Institute of Technological Research.

Illustration 2 Decanter
Source: [23]

To carry out the experimental procedures, one or more independent variables were manipulated to examine their

effects on one or more dependent variables, allowing the researcher to control the experiment.

3. Results

his section describes the application of essential oils (rosemary and feijoa) as preservatives in the production of "Chorizo Cuencano."

Process of Obtaining "Chorizo Cuencano"

For the "Chorizo Cuencano" experimentations, a preliminary process was conducted, including sanitization with approved disinfectants applied to the equipment and utensils, such as the sausage stuffer, mixer, stainless steel table, stainless steel pan, knife, cutting board, and bowls..

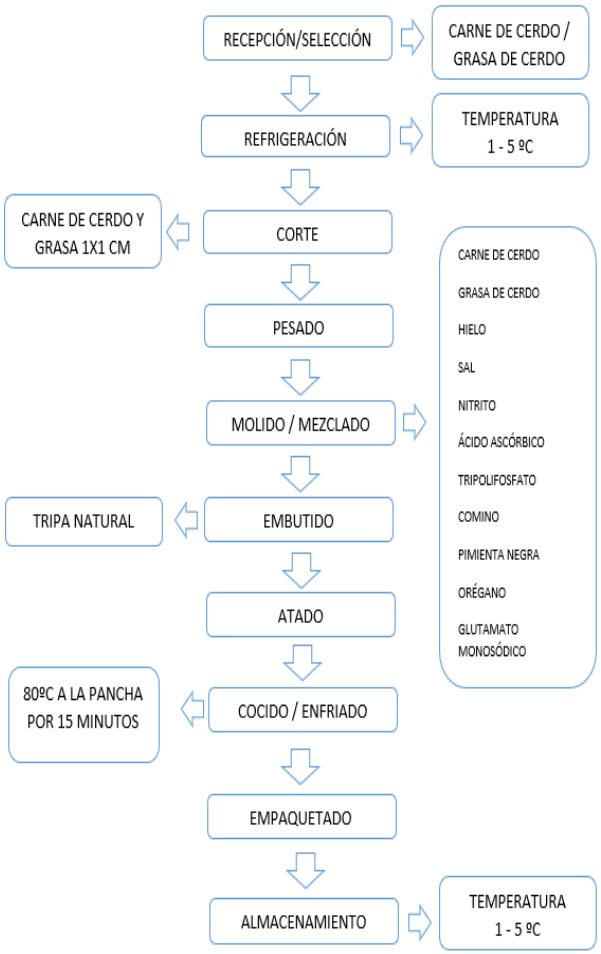

Fig. 4. Flow Diagram of "Chorizo Cuencano"

Description of the Production Process

Reception and Selection: The selection of suitable pork and pork fat is performed with proper handling.
Refrigeration: The pork must be kept in a cold chain between 1 to 5 ºC.

Cutting: The meat and fat are cut into small pieces approximately 1 x 1 cm for grinding.

Weighing: In the mise en place, all condiments and additives are weighed using a scale and a milligram scale.

Grinding and Mixing: The pork is ground using a 3mm disc, and the pork fat is cut into small pieces. In mixing: 1) salt and nitrite are combined for one minute, 2) the meat and salt with nitrite are added to the mixer, followed by the fat, triphosphate, and ice, 3) condiments and the other half of the ice are added, and 4) ascorbic acid is added last. The entire process must be completed in 10 minutes at 10 ºC.

Stuffing: Natural pork casing is used, placed on the funnel, and filled with the previously mixed mass, removing any air that may enter the casing.

Tying: The "Chorizo Cuencano" is tied with string to approximately 8 cm.

Cooking and Cooling: The sausages are scalded at 80 ºC in herb and salt-flavored water, scalding times vary depending on the diameter, followed by baking for 40 minutes at 200ºF. Cooling is achieved through thermal shock to stop the cooking process.

Packaging and Storage: The sausages are vacuum-packed and stored between 1 to 5 ºC.

Results of the First Hedonic Test

For the first hedonic test, 80 general consumers evaluated three samples with different amounts of essential oil:

First sample 842: contained 300mg/kg of rosemary essential oil and 100mg/kg of feijoa essential oil.
Second sample 649: contained 300mg/kg of rosemary essential oil and 200mg/kg of feijoa essential oil.
Third sample 584: contained 300mg/kg of rosemary essential oil and 400mg/kg of feijoa essential oil.

The results were as follows:

Table 6
First Hedonic Test Data

	842	649	584
Average	5,36	3,36	6,24
Variance	2,54	2,87	1,27
Std. Dev.	1,59	1,69	1,13
Sample No.	80	80	80

Sum	429	269	499

Using *Analysis of Variance (ANOVA),* it was demonstrated that the sample with the highest acceptance among consumers was 584 ***(mean: 6.24),*** statistically proven by the consumer's preference difference with a 95% confidence leve.

Table 7 ANOVA

ANALYSIS OF VARIANCE						
Origin of variations	**Sum of Squares**	**Degrees of Freedom**	**Mean Squares**	**F**	**Probability**	**Critical Value for F**
Rows	157,6	79	1,99493671	0,9304797	0,62520247	1,45115232
Columns	330,625	1	330,625	154,210332	2,9451E-20	3,96189204
Error	169,375	79	2,14398734			
Total	657,6	159				

Hypothesis: The critical F is less than 0.05, indicating significant differences between the groups or the three samples, concluding that there is a statistically significant difference/preference between the means of Test 1 (5.36), Test 2 (3.36), and Test 3 (6.24) at the 5% level (95% confidence), as the calculated value (154.21) is greater than the critical value (3.96).

Results of Physical-Chemical and Microbiological Analyses

The following presents the results of the microbiological and physical-chemical analyses conducted on "Chorizo Cuencano" according to INEN 1338: 2012 standards: Meat and Meat Products.- Cooked Meat Products.

In the first hedonic test, it was shown that sample 584 had the highest acceptance among the general public. Therefore, a physical-chemical and bromatological analysis was conducted on the preparation of this formulation, prepared with a weight of 250 g and packaged.

The requirements applied for the analysis and obtaining these results were as follows:

Sausage Name: Chorizo Cuencano
Reference: Meat Products
Packaging: Plastic Containers
Sample Storage: Refrigeration 0°C - 4°C
Declared Net Content: 250 g
Date of Manufacture: 07/02/2020
Expiration Date: 22/02/2020
Shelf Life: 15 days

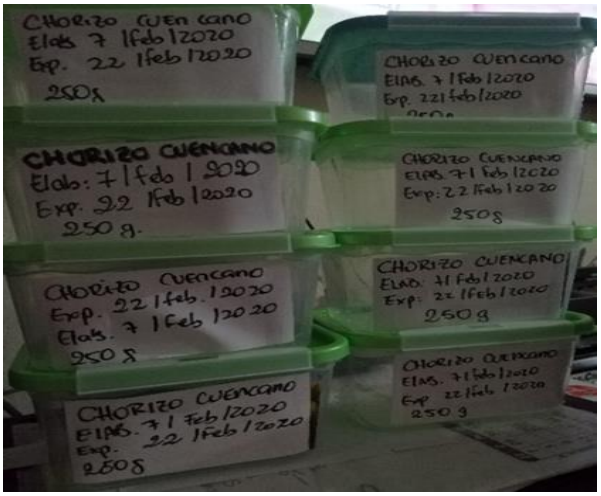

Illustration 3 Chorizo Cuencano" Packaging
Source: [23]

Table 8 Microbiological Analysis

Microbiological Analysis				
Test Conducted	**Unit**	**Result**	**Requirements**	**Methods/Ref.**
Mesophilic Aerobes	UFC/g	2.0 x 10^2	Max: 1.0 x 10^5	AOAC 21st 966.23 (ME03-PG20-PO02-7.2 M)
Escherichia coli *	UFC/g	<10	<10	AOAC 21st 991.14 (ME04-PG20-PO02-7.2 M) *
Staphylococcus aureus	UFC/g	<10	Max: 1.0 x10^2	AOAC 21st 2003:11 (ME12-PG20-PO02-7.2 M)
Salmonella	Absence/Presence	Absence	0	AOAC 21st 967.26 (ME20-PG20-PO02-7.2 M)

Source: [24]
Prepared by: Author

Analysis: The data presented in this table indicates that "Chorizo Cuencano" with the application of rosemary essential oil (0.3) and feijoa (0.4) is a safe product for consumption. This is because during its preparation, proper food handling was ensured, work was conducted in a sanitized area, and high-quality raw materials were used, with appropriate temperature control, cooking of

the sausage, and storage. It is determined that in microbiology, values such as <1.0, <1.1, <1.8, <2, <3, and <10 estimate the absence of microorganisms depending on the method used. Based on the values presented in this microbiological analysis, it is confirmed that the requirements of INEN 1338:2012 standards for Meat and Meat Products.- Cooked Meat Products have been met.

Table 9
Physical-Chemical Analysis

Physical-Chemical Analysis				
Test Conducted	**Unit**	**Result**	**Requirements**	**Methods/Ref.**
Moisture	%	50.00 ± 0.60	-	AOAC 21st 950.46 B (ME14-PG20-PO02-7.2 FQ)
Protein	%	16.90 ± 0.7	-	AOAC 21st 981.10 (ME22-PG20-PO02-7.2 FQ)
Ash	%	1.84 ± 0.06	5	AOAC 21st 920.153 (ME05-PG20-PO02-7.2 FQ)
Fat	%	31.26 ± 1.28	30	AOAC 21st 960.39 (ME17-PG20-PO02-7.2 FQ)

Source: [24]
Prepared by: Author

Analysis: Based on the data obtained from the Physical-Chemical analysis, it is determined that "Chorizo Cuencano" is high in fat, has high moisture content due to the cooking process it undergoes, is high in protein due to the amount of pork and pork fat in its formulation, and is low in ash with the limit permitted by INEN 1338:2012 standards for Meat and Meat Products.- Cooked Meat Products. It is important to note that this sausage is high in protein and exceeded the INEN standards by 1%.

4. Conclusions

- The method used to obtain feijoa essential oil was direct distillation, yielding 2 g of essential oil per 4.338 kg of fruit. Additionally, procedures developed by other authors were followed to obtain the necessary quantities for experimentation in this thesis work.
- Essential oils contain an antimicrobial agent that stops or inhibits the growth of microorganisms, meaning that they combat resistant pathogens found in processed products, such as sausages like "Chorizo Cuencano," by applying rosemary essential oil at 300 ppm (0.3) and feijoa essential oil at 400 ppm (0.4).
- The antioxidant and bactericidal properties of rosemary and feijoa essential oils have beneficial effects by inhibiting microbiological growth, preventing oxidation, and extending shelf life. These properties of essential oils were confirmed through shelf-life studies and microbiological analyses conducted at the PROTAL laboratory.
- Through various laboratory experiments, the shelf life of "Chorizo Cuencano" with the application of rosemary essential oil (0.3) and feijoa essential oil (0.4) was determined to be 15 days, as verified by PROTAL laboratory analyses.
- Sensory tests conducted with general consumers using preference testing and ANOVA statistical analysis demonstrated a statistical difference between Test 1 (5.36), Test 2 (3.36), and Test 3 (6.24) at a 5% level (95% confidence), as the calculated value (154.21) was greater than the critical value (3.96). The second sensory test showed a paired comparison between samples 350 and 676, where the result was less than 50, indicating no strong preference, and the acceptance test where sample 676 obtained 79.57%, meeting the minimum 70% required for market approval.
- The physical-chemical and microbiological analyses conducted on "Chorizo Cuencano" according to INEN 1338:2012 standards for Meat and Meat Products.- Cooked Meat Products determined that "Chorizo Cuencano" met the necessary requirements, including microbiological parameters such as Mesophilic Aerobes (2.0x10^2), Escherichia coli (<10), Staphylococcus aureus (<10), Salmonella (Absence), and physical-chemical parameters such as moisture (50.00 ± 0.60), protein (16.90 ± 0.7), ash (1.84 ± 0.06), and fat (31.26 ± 1.28). However, the fat content needs to be reduced by 2% to fall within the permitted range according to the standards.

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5. Appendices

Appendix 1. Experimentations

Appendix 2. Distillation