Memories of the Agricultural and Livestock Area of the VI CCIUTM, Ecuador

Extraction of Cocoa Powder for the Preparation of a Drink by Adding Mucilage and Guava

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Abstract | The objective of this research was to prepare a drink based on mucilage and cocoa powder infected with 25% of *Moniliophthora roreri* (monilla), adding a tropical fruit such as Guava (*Psidium guajava* L.). A completely randomized design was applied, with five treatments and four repetitions. giving a total of 20 study objects. To determine differences between treatments, Tukey's multiple range test was used at 5% probability, the treatments were prepared from five experimental clones (T1-Testigo EET-103, T2-CCAT-46-57, T3-CCAT-46-75, T4-CCAT-46-88, T5-CCAT-49-98) said varieties were obtained from the Experimental Farm " "The Dam." For the evaluation of the physical-chemical parameters, the following variables were analyzed (acidity, °Brix, pH, potassium, fat, protein, fiber and energy), sensory variables (flavor, color, taste, smell and texture). In the analysis of variance (ANDEVA) it is concluded that in the bromatological analyzes T2 contains the greatest amount of energy with a value of 403.57kcal/100g, in T1 a value of 0.46% protein was determined and in T4 8.51% mg/ potassium. 100 g were obtained; These variables are important indicators for an energy drink. In the sensory evaluation of the drink, the non-parametric Kruskal-Wallis test was applied, showing that the best treatment was T1 (CCAT-46-57), where the intensity of each of the attributes established for this organoleptic test was evaluated.

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Introduction

The origin dates back to around 2000 years where it appeared in Meso America (Mexico, Guatemala and Honduras), current evaluations show that 5000 years ago a variety of cocoa existed in the upper Amazonia, currently the existing variety has been modified until find clones and genotypes and

hybrids of *Theobroma Cacao* L. Enhancing the fine aroma quality and its resistance to microorganisms or pests in addition to improving climatic resistance (Valdez and Zarrillo, 2016).

The cacao paste goes through a pressing process where the cocoa butter is obtained and finally the cocoa powder remains. Cocoa powder is used as a flavoring and coloring component of preparations intended for family consumption (drinks, puddings, sweets), or for industrial use (chocolate coatings) in addition to color and in the eventuality, reduction of bacterial load (Erazo *et al.*, 2023).

Cocoa mucilage is a fresh pulp that surrounds the cocoa seeds, and that is necessary for the production of alcohol and acetic acid in the fermentation of almonds, but it follows that there is a waste of 70 liters per ton of this mucilaginous material, which has benefits that were known through a scientific study in the INIAP Laboratories, resulting in cocoa mucilage having high levels of sugars, proteins, and nutrients (Intriago *et al.*, 2023; Vásquez *et al.*, 2023).

The objective was to develop a new product for the market taking advantage of the raw material infested with the monilla (*Moniliophthora roreri*) obtaining from this the cocoa powder, using the cocoa mucilage and giving an added value to a tropical fruit such as guava (*Psidium guajava* L). It is expected to obtain beneficial results for the cocoa population that discards this raw material, generating monetary increases within the country.

Materials and Methods

Localization

The raw material was obtained from the experimental farm "La Represa" of the State Technical University of Quevedo, located at Km 7.5, "Fayta" Campus of the Quevedo San Carlos road, province of Los Ríos.

Variables studied

Titratable acidity: To determine the total acidity, the sample was prepared by placing 50 ml of water in a flask and 10 ml of sample in a test tube; then mix the two quantities in an Erlenmeyer flask. 5 drops of phenolphthalein solution were added, then the NaOH solution (0.1N) is added little by little until a pale pink color is obtained, which remains for approximately 30 seconds and the volume of the NaOH solution (0.1N) spent is recorded (INEN 341, 1978).

pH: It was determined by the potentiometric method, with a digital pH-meter, in duplicate (INEN 381, 1985), placing 50 ml of the sample in a precipitation vessel, immersing the pH meter electrode in the sample and waiting for the digitized data on the screen of the equipment to stabilize.

Degrees brix: This variable established the total quotient of sucrose dissolved in the drink, it was determined using a refractometer, for which one to two drops of the drink sample were placed to take its reading through the eyepiece of the refractometer (Vásquez *et al.*, 2023).

The analysis of fat, protein, fiber, energy: It was determined based on the technical standard of the Chemistry Laboratory of the UTE.

Sensory analysis

It is the scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of food and other substances, which are perceived by the senses of sight, smell, taste, touch. A four-point interval scale was used for the measurement of the respective attributes (Tables 1 and 2).

To perform the sensory analysis of the drink, a descriptive test was used, where the organoleptic characteristics (taste, taste, smell, color and texture) perceived through the senses of sight, smell, taste, touch will be determined; and non-parametric statistics were applied, the Kruscal Wallis test ($p \leq 0.05$).

Procedure

A panel of 10 semi-trained tasters was chosen, who tasted each sample with approximately 20 ml of the drink, in identical plastic containers coded with random numbers.

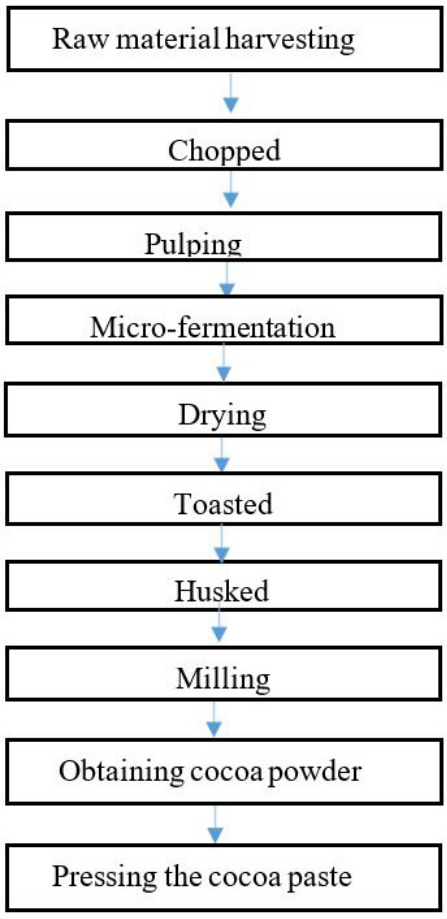
Cocoa powder extraction process

The procedures for extracting cocoa powder are presented schematized in the Flowchart 1.

Harvest: The raw material was obtained in the experimental farm "La Represa", the harvest was made from the five experimental clones (T1-EET-103, T2-CCAT-46-57, T3-CCAT-46-75, T4-CCAT-46-88,

T5-CCAT-49-98).

Chopped: The ears were cut transversely and longitudinally. The cobs were cut transversely and longitudinally with the help of a machete.



Flowchart 1: *Obtaining cocoa powder from almonds infested with monilla.*

Pulped: The healthy and infested ears were opened to extract the cocoa almonds, then the placeta was manually removed. Foreign materials were removed.

Micro fermentation: The almonds were placed in the micro-fermentation box that had small holes in the side that allowed the cocoa slime or mucilage to come out. Once the box was filled, it was covered with banana leaf or banana, this helped to lose moisture to the cocoa, for its subsequent fermentation in two to three days.

Dried: After the grains have been fermented, they have a certain amount of humidity that must be reduced, they were transported to a flat surface, where solar energy was used, during the drying in the sun the grains moved constantly to achieve a uniform drying.

Roasting: The temperature of the roasting reached 100 °C, the time and the degree of humidity involved in roasting, depend on the type of moisture of the almond.

Shelling: The husking was done manually after roasting. Shelled cocoa undergoes alkalization, usually with potassium carbonate, to enhance flavor and color.

Milling: The nibs (pieces of crushed cocoa) are ground to obtain the cocoa paste. The temperature and degree of grinding varies according to the type of grain used and the product required.

Pressing cocoa paste: The cocoa cocoa paste is pressed to extract the cocoa butter which represents 50% of the total weight.

Obtaining cocoa powder: The process now takes two different paths. Cocoa butter is used to make chocolate. On the other hand, the cocoa powder is crumbled into small granules that are then pulverized to obtain the cocoa powder.

Cocoa mucilage extraction process

The procedures for obtaining cocoa mucilage are presented schematized in Flowchart 2.

Reception of cocoa cobs: The raw material was obtained in the experimental farm "La Represa" of the cocoa harvest, this was carried out hours before processing.

Washing and disinfection: The cocoa fruits were subjected to a rinsing process.

Court: The cutting process was carried out through the use of a machete, the cutting of the cob was transversely and longitudinally so as to facilitate the extraction of mucilaginous almonds.

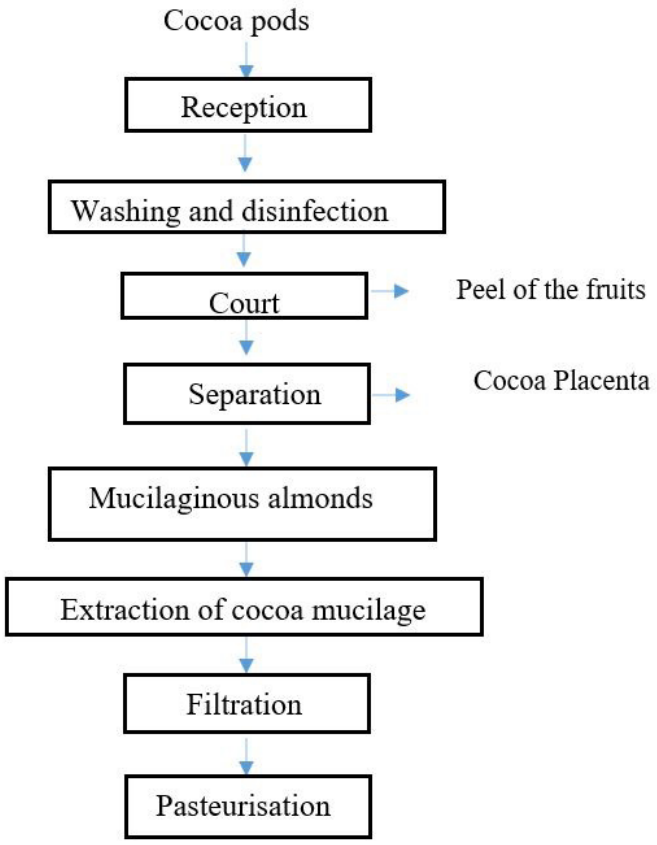
Separation of almonds and placenta: The seeds were extracted and the placenta was manually separated from the mucilage almonds.

Cocoa mucilage extraction: This research is oriented towards the use of the residual cocoa, techniques that affect the fermentation process of cocoa almonds can not be used, in this context, a methodology was applied, from which only the liquid excess of the pulp

was obtained without removing the protective cover of the beans.

Filtration: The removal of suspended particles found in the mucilage was separated through a filtrate using a cloth canvas.

Pasteurisation: Once the cocoa mucilage was obtained, it was carried out to rapid pasteurization in a stainless-steel container, at a temperature of 70 to 75 °C for a period of 12 to 15 minutes, to inactivate the enzymes present in the mucilage in order to avoid enzymatic browning and the elimination of pathogenic microorganisms.



Flowchart 2: *Diagram of the cocoa mucilage extraction process.*

Guava juice extraction process

The procedures for obtaining guava juice are presented schematized in Flowchart 3.

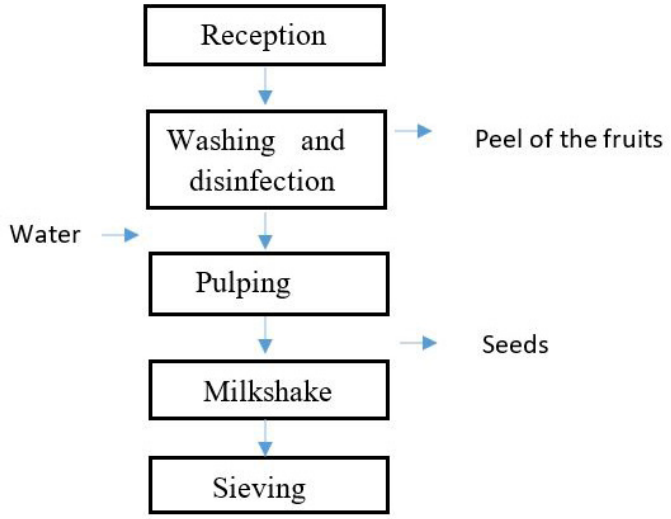
Reception of guavas: The objective of the selection is to choose only the fully ripe fruit that does not have microbial damage, separating those that do not have the required quality such as units on ripe, putrefyed, bruised, burned by cold, with fungi, spots or with wounds where microorganisms may have entered since this affects the deterioration of the pulp.

Washing and disinfection: Once all the fruit has been selected, it undergoes washing and disinfection by contacting the guava with drinking water. The retention time of fruits is about 5 minutes.

Pulped: In this operation the edible part of the fruits, pulp is separated; of the inedible, shell and seed.

Milkshake: The pulp was liquefied and water was added. The liquefaction process lasted 4 minutes until a homogeneous mixture was obtained.

Sieving: A white canvas was used in which the guava juice was placed and pressure was exerted, in order to extract the liquid and eliminate the seeds, which was collected in a plastic container, for the realization of this process gloves were used to take care of the hygiene and safety of the final product.



Flowchart 3: *Outline of the guava juice extraction process.*

Process of making the drink

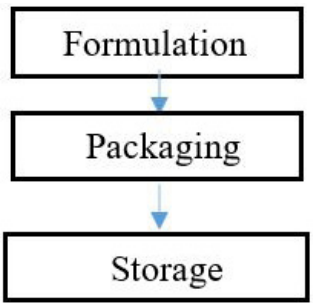
The procedures for obtaining the drink are presented schematized in Flowchart 4.

Formulation: The addition of the other components of the drink such as mucilage, cocoa powder and guava, were added according to the proportion established in the formulation, the process was carried out at an ambient temperature, to facilitate the process of dissolution of the added components.

Packaging: For the packaging of the beverage, previously sterilized glass containers were used for a better conservation of the product and to avoid the development of sporulated thermophilic

microorganisms.

Storage: The glass jars were stored in refrigeration at 4 °C.



Flowchart 4: *Diagram of the preparation of the drink.*

Table 1: *Intensity scale used in sensory analysis.*

Scale	Intensity
1	Light
2	Moderate
3	Pretty much
4	A lot

Table 2: *Sensory attributes evaluated.*

Acidity	Color	Sweetness	Taste	Odor	Thickness	Texture
Acid	Beige	Cocoa	Cocoa	Thick		granulosa
	Café	Guava	Guava			

Drink formulation

The process of obtaining the drink was carried out based on the following formulation, which can be seen in Table 3.

Table 3: *Formulation for 880 g of drink.*

Raw material	Formulation	
	%	Quantity
Mucilago de Cacao	48.42	430g
Guava	45.27	402g
Cocoa Powder	1.80	16g
Sugar	4.51	40g

Table 4: *Treatments, experimental clones.*

Treatments	Experimental clones	Genotypes	N° plants
T0	WITNESS (TSE 103)	National type	10
T1	CCAT-46-57	National type	10
T2	CCAT-46-75	National type	10
T3	CCAT-46-88	National type	10
T4	CCAT-49-98	National type	10

Data processing

For the determination of physical, microbiological and sensory parameters, the treatments shown in Table 4 were used.

Results and Discussion

Acidity

The analysis of variance showed statistically significant differences between treatments according to Tukey's test ($p \leq 0.05$) (Table 5), with a coefficient of variation of 3.11%, treatments (0 and 1) presented the highest mean value of 0.91, while treatment 2 obtained a value of 0.86, followed by treatments (3 and 4) with 0.84 that presented the lowest value. Within the Ecuadorian technical regulations INEN 2337 (2008) the mentioned physical parameter is not found.

The values of acidity observed exhibited a proportional relationship with the results obtained with the research carried out by Escobar (2014), in the elaboration of this nutritious drink from whey and concentrate of native fruits values of 0.92 are obtained in the control treatment made only with whey and when using 15% of pitahaya concentrate determined a value of 1.02 (Escobar, 2014).

Degrees brix

In Table 5, for the variable Brix degrees, according to the analysis of variance, no significant difference was found between the treatments, according to Tukey's test ($P \leq 0.05$). The highest value of 19.73 was recorded in T0, while in T1 the lowest value was 18.52. The overall coefficient of variation for treatments was 3.06%. A comparison was made with the requirements of INEN 2337 (2008) and indicates that the general average obtained is within the permitted ranges corresponding to min. and max. 20° Brix.

Hydrogen potential (pH)

According to the analysis of variance, the treatments did not register statistically significant differences (Table 5). Tukey's analysis (0.05%) established that T4 had an average value of 3.75, while in T0 it obtained a mean of 3.66 respectively. The treatments (1.2 and 3) registered values of 3.67, 3.68 and 3.72. A coefficient of variation of 1.15% was also determined. While when making a comparison with the requirements of the (INEN 389, 1985), it must have a pH less than 4.5 it is indicated that the values obtained are within the allowed ranges that correspond to min. and max.

Table 5: *Average values of bromatological analysis of the drink.*

Treatments	Physical chemical parameters							
	Acidity (%)	° Brix (%)	pH (%)	Potassium (mg/100g)	Grease (%)	Protein (%)	Fiber (%)	Energy (kcal/100g)
T0 (Witness ETT-103)	0.91a	19.73 a	3.66 a	5.70 c	1.64 a	0.25 b	1.36 b	399.77 b
T1 (CCAT-46-57)	0.91a	18.52 a	3.67 a	6.92 b	1.47 a	0.46 a	1.18 b	399.34 b
T2 (CCAT-46-75)	0.86 b	19.28 a	3.68 a	7.03 b	1.47 a	0.43 a	0.17 c	403.57 a
T3 (CCAT-46-88)	0.84 b	19.00 a	3.72 a	8.25 a	1.47 a	0.43 a	6.48 a	378.31 c
T4 (CCAT-49-98)	0.84 b	19.11 a	3.75 a	8.51 a	1.37 b	0.41 a	0.15 c	403.29 a
Average	0.87	19.13	3.70	7.28	1.48	0.40	1.87	396.86
C.V (%)	3.11	3.06	1.15	6.47	6.94	8.40	9.87	0.20
p-value	0.0021	0.1131	0.0521	<0.0001	0.0283	<0.0001	<0.0001	<0.0001
s.e.	*	n.s.	n.s.	**	*	**	**	**

*Means with a letter in common are not significantly different (p≤0.05). C.V. (%)= Coefficient of variation. p - value= Probability associated with values greater than or equal to the 5% points for the distribution F. s.e.= Statistical significance (n.s.= not significant, *=significant and **=very significant).*

The pH values made in this research are in the range according to Hours *et al.* (2005), the pH level in pasteurized juices and reconstituted concentrate has a obtained value of 3.84 This data can be compared directly with other juices due to the uncertain origin with respect to the variety of orange used in its preparation.

Grease

Table 5, shows the percentages of fat present in the drink with mucilage, cocoa powder and guava for each of the treatments. That is, there was a significant difference between T0 with a value of 1.64% and in T4 with 1.37%. While in treatments 1, 2 and 3 there was a general average of 1.47%. According to Tukey's test, 5% probability established a coefficient of variation of 6.94%.

Results that differ from the research carried out by Salamanca *et al.* (2010), in the elaboration of a functional drink of high biological value based on borojo which obtained a fat percentage of 0.77%, however, in this research project reached values of 1.47–1.64% for the content of cocoa powder in its formulation.

Protein

Table 5, details the analysis of variance performed on the protein contained in the beverage, finding statistical differences at (p ≤ 0.05) with T1 being the highest value with 0.46% and with a lower value T0 with 0.25%, establishing a coefficient of variation of 8.40% in all treatments.

Determines the value of the protein in the Araneda *et al.* (2014) protein analysis that was 0.40% with sugar and 0.19% without sugar in the preparation and evaluation of maqui juice.

Fibre

In relation to the ANDEVA analysis of the variable fiber of the drink (Table 5), it was determined that there are statistical significances between the treatments, but the T0 stands out for presenting a higher fiber content of 1.36% compared to T4 that has a lower content of 0.15%, also indicating a general coefficient of variation of 9.87% for all treatments.

Contreras *et al.* (2011), in the increase of the protein content of a drink based on amaranth (*Amaranthus hypochondriacus*), these mixtures of amaranth for the drink presented the highest percentage of fiber (3.18 and 2.4 %, respectivly); due to the vegetable origin of the protein source.

Energy (kcal/100g)

Based on the ANDEVA analysis of the energy variable of the drink (Table 6), it is observed that there is statistical significance between the treatments that correspond to T0 (ETT-103 witness), T1 (CCAT-46-57), T2 (CCAT-46-75), T3 (CCAT-46-88) and T4 (CCAT-46-98) But T2 stands out by presenting the highest energy content of 403.57 kcal/100 g and T3 when obtaining the lowest value of 378.31 kcal/100 g, where the general variation coefficient corresponds to 0.20%.

Table 6: *Average values of the sensory analysis of the drink.*

Treatments	Sensory attributes									
	Taste/ Acid	Taste/ Sweet	Color/ Beige	Color/ Café	Taste/ Cocoa	Taste/ Guava	Smell/ Cocoa	Smell/ Guava	Texture/ Thick	Texture/ Granulosa
T0 (Witness ETT-103)	1.90 a	2.08 b	2.03 a	1.48 a	1.48 a	2.58 a	1.33 a	2.58 c	1.98 a	1.78 a
T1 (CCAT-46-57)	2.07 a	1.77 a	2.00 a	1.33 a	1.57 a	2.07 a	1.57 a	1.63 a	1.53 a	1.67 a
T2 (CCAT-46-75)	2.03 a	1.97 a	2.13 a	1.23 a	1.60 a	2.27 a	1.23 a	2.00 abc	1.83 a	1.53 a
T3 (CCAT-46-88)	1.67 a	2.60 b	2.03 a	1.30 a	1.40 a	2.30 a	1.33 a	2.07 abc	1.83 a	1.53 a
T4 (CCAT-49-98)	2.53 a	1.83 a	1.90 a	1.20 a	1.30 a	2.57 a	1.30 a	2.23 bc	1.53 a	1.67 a
Average	2.04	2.05	2.02	1.31	1.47	2.36	1.35	2.10	1.74	1.64
K - W (H)	7.03	8.77	1.07	1.97	1.27	5.04	3.38	14.37	5.92	3.48
p-value	0.0889	0.0380	0.8782	0.4362	0.7167	0.2374	0.2218	0.0035	0.1364	0.3745
s.e.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.

*Means with a letter in common are not significantly different (p≤0.05). K – W (H) = Kruskal – Wallis statistic. p - value = Probability associated with values greater than or equal to the Kruskal – Wallis statistic observed (corrected for ties). s.e.= Statistical significance (n.s.= not significant, *=significant and **=very significant).*

Sensory analysis

The averages obtained from the organoleptic assessments considered, acid and sweet taste, beige and coffee color, cocoa and guava flavor, cocoa and guava smell, thick and granular texture by treatments, according to the Kruskal Wallis test are shown in Table 1.

Taste/Acid

According to the nonparametric statistical analysis applied to determine the effect of the treatments on the taste/acid variable, it was determined that there is no significance between treatments with an H value of 7.03 and with a confidence level of 95%. The highest rating was issued by T4 with an average of 2.53 and T3 with the lowest value of 1.67, with the rest of treatments being found between both values.

The values obtained in the treatments are shown in Table 6. From the analysis of the medians it was determined that in treatments 0, 1.2 and 4 mark on the interval scale an intensity of 2 (moderate), while in T3 a score of 1 (light) was observed.

Taste/Sweet

According to the Kruskal –Wallis test for the sweet attribute, significant differences were generated between the means of the treatments. T3 had a higher value of 2.60, while in T1 it was observed in Table 6, a lower value with an average of 1.77. It was established that T1, T2 and T4 did not present significant differences, however, T1 was significantly different with a confidence level of 95%.

Based on the scale established to measure intensity, T1, T2 and T4 presented values of 1 (light), while T0 obtained a value of 2 (moderate). In the case of T3 the intensity observed was 3 that was found on the scale of quite.

Color/Beige

For the beige attribute according to the Kruskal Wallis Test (Table 6) it was demonstrated that there is no significance between treatments with an H value of 1.07. However, T2 obtained the highest value of 2.13, while T4 had the lowest value of 1.90, with a general median of 2, which corresponds to the moderate scale.

Color/Coffee

It was observed that there were no significant differences between the means of the treatments, a confidence level of 95% was estimated for this variable, Table 6 shows the values established by the Kruskal–Wallis nonparametric analysis. The OT achieved the highest score of 1.48, registering a median of 1 (light), while the T4 obtained a value of 1.20, with a median of 1 (light) according to the scale of established intervals.

Flavor/Cocoa

According to the Kruskal Wallis nonparametric test (p≤0.05), no significant differences were observed between the means of the treatments in the variable cocoa flavor. T2 achieved the highest score (Table 6), while the lowest score was obtained by T4.

From the results obtained by the panelists based on the scale previously established to evaluate the

intensity of the flavor of the drink, the treatments (0, 1, 2, 3.4) presented a value of 1 (light).

Flavor/Guava

According to the Kruskal – Wallis Test for the guava flavor attribute (Table 6), it was found that there is no statistical significance between treatments with an H value of 5.04. Where T0 stood out with the highest value of 2.58 and T1 with the lowest value of 2.07.

The score obtained from the medians for the attribute to measure intensity corresponds to scale 2 (Moderate) for treatments (1 and 2). While for scale 3 (Quite) for treatments (0, 3.4).

Smell/Cocoa

In the attribute to cocoa according to the Kruskal – Wallis Test (Table 6), it was determined that there is no statistical significance between treatments with an H value of 3.38, but T1 presented the highest value of 1.57 and T2 the lowest value of 1.23, with a general median of 1 being this rating corresponding to the scale of 1 (Slightly).

Smell/Guava

According to the Kruskal Wallis test ($p \leq 0.05$), significant differences were observed between the treatments in the odor/guava variable. In T4 a higher value of 2.23 was obtained, while the lowest value was determined in T1 of 1.63. In T2, T3 and T4 there were medians that fluctuated from 2 that according to the interval scale would be equivalent to a moderate intensity. On the other hand, T0 marked a value of 3 that corresponds to a fairly intense intensity. While T1 reached the median value of which is equivalent to a slight intensity.

Texture/ Thickness

According to the means of the texture/thickness attribute according to the Kruskal – Wallis Test (Table 6), it did not present statistical significance among the treatments with an H value of 5.92. Highlighting the highest value for T0 and in T1, T4 the lowest value of 1.53 was obtained, with a general median for both treatments of 1 corresponding to the scale of 1 (Light). While for treatments (0, 2, 3) the value of the median of 2 belonging to the scale (Moderate) was determined.

Texture/ Granulosa

Based on the Kruskal–Wallis Test in the texture/grain attribute (Table 6), it was observed that there is no statistical significance between treatments with an H value of 3.48. The T0 presented the highest value of 1.78 and in the T2, T3 obtained the lowest value of 1.53, indicating a median of 1 for the treatments (1, 2 and 3); the values that were recorded corresponded to scale 1 (Light) and 2 (Moderate) respectively for treatments (0 and 4).

Conclusions and Recommendations

According to the objectives set out in this research project, the following conclusions are established.

It was determined that in the sensory assessment made to the drink showed that the best treatment was T1 (CCAT-46-57), where taste/ acid, color/ beige, taste/ guava, smell/ guava and texture / thick prevailed.

It is concluded that in the bromatological analyzes the T2 contains more energy with a value of 403.57kcal/100g, in the T1 a value of 0.46% of protein was determined and in the T4 8.51% of potassium mg/ 100g was obtained; These variables are important indicators for an energy drink.

Acknowledgments

We want to express our gratitude to Díos and the State Technical University of Quevedo, as well as each of the authors of this research.

Novelty Statement

Currently, the cultivation of (*Theobroma cacao* L.) in Ecuador is facing serious problems mainly due to pest and disease attacks, with Moniliasis (*Moniliophthora roreri* Cif & Par) being the most severe disease. This disease causes estimated losses ranging from 50% to 80% of the total annual production, depending on environmental conditions, crop management, control measures applied, and the cultivated varieties. Therefore, a proposed solution involves conducting experimental research to enhance the use of cocoa powder in the production of a beverage that meets the needs of consumers. The formulation will also include cocoa mucilage and a tropical fruit such as guava. This proposal aims to add value to cocoa mucilage, considering an estimated waste of 70 liters per ton. This mucilaginous material contains high levels of sugars, proteins, and nutrients. Hence, the research serves as

a viable alternative for future investigations regarding the use of raw materials infested with moniliasis, contributing to the development of different foods and the industrial progress of the country.

Author's Contribution

Jaime Vera Chang: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript, conducted the research, prepared and drafted the initial manuscript. Conceptualization, methodology, visualization, resources, and writing— original draft preparation.
Arianna Torres Coronel: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript.
Luis Vásquez Cortez: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript. Conducted the research, prepared and drafted the initial manuscript.
Kerly Alvarado Vásquez: Conceptualization, data curation, and writing—review & editing.
Frank Intriago Flor: Designed the study, analyzed the data and drafted the manuscript. Conceptualization, data curation, and writing—review & editing.

All the authors read and approved the final manuscript.

Conflict of interests
The authors have declared no conflict of interest.

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