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Morphological, physicochemical and microbiological characterization of Macambo cocoa (*Theobroma bicolor* Humb & Bonpl.) in Ecuador

Caracterización morfológica, fisicoquímica y microbiológica de cacao Macambo (*Theobroma bicolor* Humb & Bonpl.) en Ecuador

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ABSTRACT

Macambo cocoa (*Theobroma bicolor* Humb & Bonpl.), a lesser known variety compared to Criollo or Forastero cocoa, is a valuable resource in Ecuador for its distinctive qualities. The present research sought to characterize the main physical, chemical and microbiological parameters of Macambo cocoa grown on the lands of the State Technical University of Quevedo. The experimental design consisted of a two-factor randomized design with 9 treatments and 3 replications. The treatments varied according to the division of the pod and the levels of fermentation, using Rohan boxes, polyethylene covers and jute bags. A fermentation time of 72 hours was applied, followed by 6 days of sun drying to reduce humidity, the results showed that the fermentation of the almonds from the upper part of the pods in jute bags resulted in the lowest humidity recorded (7.41%). Although the treatments showed similar results in ash, a higher pH was observed in the T3 treatment (6.12). In addition, the measurement of the fermentative degree was conclusive and the cadmium level was within the acceptable limits according to the regulations. No microbiological growth was detected in the dried almonds.

Keywords: cadmium; fermentation; moisture; microbiology; physical tests

RESUMEN

El cacao Macambo (*Theobroma bicolor* Humb & Bonpl.), una variedad menos conocida en comparación con el cacao criollo o forastero, es un recurso valioso en el Ecuador por sus cualidades distintivas. La presente investigación buscó caracterizar los principales parámetros físicos, químicos y microbiológicos del cacao Macambo cultivado en los terrenos de la Universidad Técnica Estatal de Quevedo. El diseño experimental consistió en un diseño al azar bifactorial con 9 tratamientos y 3 repeticiones. Los tratamientos variaron según la división de la mazorca y los niveles de fermentación, utilizando cajas Rohan, fundas de polietileno y sacos de yute. Se aplicó un tiempo de fermentación de 72 horas, seguido de 6 días de secado al sol para reducir la humedad, los resultados mostraron que la fermentación de las almendras de la parte alta de las mazorcas en sacos de yute resultó en la menor humedad registrada (7,41%). Aunque los tratamientos exhibieron resultados similares en cenizas, se observó un pH más alto en el tratamiento T3 (6,12). Además, la medición del grado fermentativo fue concluyente y el nivel de cadmio se encontró dentro de los límites aceptables según las normativas. No se detectó crecimiento microbiológico en las almendras secas.

Palabras clave: cadmio; fermentación; humedad; microbiología; pruebas físicas

1. INTRODUCTION

The cocoa bean is of great importance to both producing and consuming countries, due to its significant impact on marketing and consumption, which generates substantial economic revenue (Vásquez et al., 2023). This bean is an essential ingredient in a variety of industries, including confectionery, food and beverage, as well as pharmaceutical and cosmetics. Sustainability in its production is crucial. Although cocoa is native to the Amazon, it is world-renowned and adapts to a wide variety of soils. Its use dates back to approximately 3500 years ago in Zamora Chinchipe, where it was part of the daily staple diet (Gómez et al. 2019).

The cocoa tree is a species of the genus *Theobroma*, from the *Malvaceae* family, which includes more than 22 species. Among them is *Theobroma bicolor*, known as Macambo or pataxe, pataxe cacao, pataste patashe, cimarron cacao, and in Ecuador, it is also called white cacao. This type of cacao is native to the tropical zone of America, has a wide distribution, and is found in the Colombian, Peruvian, Brazilian Amazon, as well as in Ecuador, Venezuela, and Mexico, where four varieties are found: Nacional, Trinitario, Criollo, and Forastero, which differ in shape, color, and flavor (Vera et al. 2022).

Few studies are available that determine the characterization of the main physical parameters of Macambo cacao and its post-harvest process in Ecuador. Consequently, this research aims to generate results that allow evaluating its benefits and thus encourage its conservation. In the Ecuadorian context, *Theobroma bicolor* cacao is known as white cacao; in the Kichwa communities of the Amazon, it is called patas tree and its seeds patas muyo, these being consumed for the pulp of the fruit and toasted seeds, which are marketed in markets through roasted tasting in tourist places (Peñuela & Zurita, 2020).

1.1. Macambo taxonomy

Theobroma bicolor, a wild species, reaches heights of 25 to 30 meters with a trunk diameter of 20 to 30 centimeters. Its crown is oblong and irregular, with wine-colored flowering. Unlike *Theobroma cacao*, its fruits develop from the trunk in pendulous verticils, being the largest in the genus. The shell is woody and the fruit falls to the ground when ripe (Alvarado et al. 2024).

Theobroma bicolor, commonly known as Macambo, is a botanical species belonging to the class Magnoliopsida, subclass Caryophyllidae, order Malvales, and family Sterculiaceae. This tree, which is part of the genus *Theobroma*, stands out for its importance in various regions of Latin America. In Ecuador, it is known as Macambo Patasmuyo or simply Patas, while in Peru it is called majambo, and in Brazil, it is known as cacau de Perú. In Colombia, it is known as bacau or maraco. The English name for this species is patashe. *Theobroma* bicolor is valued for its edible seeds and its potential in the food and medicinal industry, standing out for its nutritional characteristics and adaptability to different climatic conditions and soil types in the Amazon región (Paradas et al. 2019).

The nutritional composition of Macambo cacao (*Theobroma bicolor* Humb & Bonp.) shows that unlike other types of cacao, it contains a high content of protein, fiber, and fat. The study presents the nutritional and physicochemical composition of the fruit and seeds of *Theobroma bicolor* (Macambo). The proximate composition of the dry fruit, based on 100 g, includes 87.90% moisture, 1.66% protein, 0.48% oil, 7.44% carbohydrates, 1.44% fiber, and 1.08% ash, totaling 100% (Sanclemente & Tigrero, 2018),

Regarding the dry cacao seeds of Macambo, the literature reports a moisture content of 3.55%, a pH of 6.03, an acidity of 0.452%, an ash content of 3.529%, a fat content of 50.354%, carbohydrates of 49.245%, and protein of 21.30%. These characteristics highlight the diversified and nutrient-rich composition of the fruit and seeds of Macambo, underscoring its nutritional and functional potential (Quinteros et al. 2018).

Theobroma bicolor seeds are rich in proteins, healthy fats, and micronutrients such as magnesium and iron. This nutritional profile makes them a valuable ingredient for foods designed to offer specific nutritional benefits. Similar to cacao, Macambo seeds can be used in the manufacture of chocolates, beverages, bakery, and confectionery products, providing a basis for innovation in flavors and textures.

The cultivation of *Theobroma bicolor* can contribute to sustainability and the conservation of biodiversity in the Amazon regions where it originates. Promoting its cultivation and use can help preserve local ecosystems and provide additional income to farming communities.

The main objective of this research is to conduct a comprehensive characterization of Macambo cacao (*Theobroma bicolor* Humb & Bonpl.) in Ecuador, covering morphological, physicochemical, and microbiological aspects, in order to establish a scientific basis for its valorization and potential commercial exploitation. The study focuses on characterizing the main physical, chemical, and microbiological parameters of Macambo cacao, including the phenotypic characterization of the pod and the physical characteristics of fermented and dry almonds, the estimation of the fermentation degree under aerobic and anaerobic conditions, as well as the analysis of the microbiological quality of dry almonds.

2. MATERIALS AND METHODS

2.1. Research location

This research was conducted in the laboratory of the Universidad Técnica Estatal de Quevedo, located at the La María Experimental Farm, at 79° 28'29'24" S latitude, 85 meters above sea level. The area is characterized by an average precipitation of 2442.6 mm, a temperature of 25°C, and a relative humidity of 85.15%. Additionally, the research involved the participation of the laboratory of the Instituto Nacional de Investigaciones Agropecuarias de Ecuador (INIAP) (Vásquez et al. 2023).

2.2. Research design

For the characterization and fermentation of Macambo cacao (*Theobroma bicolor* Humb & Bonpl.), a completely randomized design (CRD) with a bifactorial arrangement was implemented, including 9 treatments distributed in 3 repetitions, as detailed in Table 1. This descriptive study used analysis of variance (ANOVA) for evaluating the results. Tukey's test was applied for the comparison of means between treatments, with a significance level set at $p<0.005$.

2.3. Arrangement of study treatments

Table 1 shows the treatment arrangements with respect to the study treatments and their respective codes, with specific descriptions.

Table 1.

Treatment arrangements

N°	Code	Description
1	Aoro	Cocoa beans from the upper part of the pod, fermented in Rohan boxes.
2	Aos1	Cocoa beans from the upper part of the pod, fermented in polyethylene bags.
3	Aoif2	Cocoa beans from the upper part of the pod, fermented in jute sacks.
4	M1ro	Cocoa beans from the middle part of the pod, fermented in Rohan boxes.
5	M1s2	Cocoa beans from the middle part of the pod, fermented in polyethylene bags.
6	M1if3	Cocoa beans from the middle part of the pod, fermented in jute sacks.
7	B2r1	Cocoa beans from the lower part of the pod, fermented in Rohan boxes.
8	B2s2	Cocoa beans from the lower part of the pod, fermented in polyethylene bags.
9	B2if3	Cocoa beans from the lower part of the pod, fermented in jute sacks.

2.4. Sample Collection and Morphological Characterization of the Fruit

At the experimental farm La María of the Technical State University of Quevedo, 50 pods from *Theobroma bicolor* trees were selected. Their morphological characterization was carried out following the descriptive parameters of Intriago et al. (2023), focusing on 10 characteristics: total pod weight, length, number of ridges, circumference, and pod width.

2.5. Morphological Variables of the Pods

Pod Division

The pod was placed vertically on an A1-sized technical drawing sheet with a numbering system. The total length of the pod was measured, and this measurement was divided by 3 to separate the pod into upper, middle, and lower parts. The division into three parts was done using a previously sterilized machete.

Width

After dividng the pods into three parts, the width of each section (lowe, middle, and upper) was measured in centimeters (cm) using a manual caliper.

Number of beans

Once the pods were Split into lower, middle. And upper parts, the number of beans in each section was counted.

Weight of Beans

The weight of the beans from each section measured in grams (g) using a CAMRY electronic scale.

Thickness

For the determination of the thickness was made with a "vernier or caliper", the reading was measured in centimeters (cm) of each of the parts of the cob.

Weight of Empty Pod

The empty weight of each part of the pod (lower, middle, and upper) was measured in grams (g) using a CAMRY electronic scale.

2.6. Cocoa Fermentation

Within the experimental design framework, the fermentation process was individually conducted for the different sections of the cocoa pod (upper, middle, and lower). Each section underwent three different fermentation methods: Rohan boxes, polyethylene bags, and jute sacks, using a capacity of 2 kg of fresh Macambo beans per treatment. In total, 27 kg of fresh cocoa mass were used for the experiment.

The seeds were manually extracted from the pods. Then, the seeds from the upper part of the pod were fermented in Rohan boxes, those from the middle part in polyethylene bags, and those from the lower part in jute sacks. All samples were covered with banana leaves to initiate the first stage of fermentation, which is anaerobic. During the three-day (72-hour) fermentation period, three key variables were monitored: temperature, Brix degrees, and pH. These measurements were taken before starting anaerobic fermentation and repeated every 12 hours until the process was completed (Erazo et al. 2021).

2.7. Cocoa Drying

After fermentation, the cocoa was dried traditionally in the sun for six consecutive days until the beans reached an average moisture content of 6%. The beans were then manually shelled to obtain the cotyledon ready for analysis. An AQUA BOY KPM (Intriago et al. 2019), was used to determine moisture content.

2.8. Physicochemical Tests

pH Determination

The hydrogen potential of the cotyledon from each treatment and repetition was determined by following these steps: taking 10g of cotyledon sample, crushing it with a mortar, adding 100 ml of distilled water, placing it in a flask with the crushed sample, mixing, then placing the pH meter in the mixture and recording the result (Vera et al. 2023).

Temperature Determination

Temperature was measured with a HANNA digital thermometer from the United State, directly placed in each sample and treatment.

Brix Degrees Determination

A part of the bean was placed in an OPTI digital refractometer, and the measurement was taken directly for all treatments and repetitions.

Ash Analysis

All materials used were thoroughly sterilized and cleaned before use. Following the experimental design, all treatments and repetitions were implemented: the porcelain crucible was carefully washed and dried with the prepared sample. A second wash was conducted, followed by drying the crucible in an oven set at 100°C for 30 minutes. It was then cooled in a desiccator and weighed with a precision of approximately 0.1 mg. About 2g of the sample was weighed with the same precision of 0.1 mg. The crucible with its content was placed near the open muffle door for a few minutes to avoid material loss due to projection if the crucible was introduced directly into the muffle. The crucible was then introduced into the muffle at 600°C until ash free of carbon particles was obtained, which took approximately 3 hours. Finally, the crucible with ashes was cooled in the desiccator and weighed again with a precision of 0.1 mg.

Formulation for Ash Determination

$$C = \frac{W2 - W1}{w0} x\ 100$$

Where:

W_0= Weight of the sample (g)

W_1= Weight of the empty crucible

W_2= Weight of the crucible plus the ashed sample (Alvarado et al., 2023).

Humidity Analysis

All materials used were thoroughly sterilized and meticulously cleaned. All necessary treatments and repetitions were performed. The procedure was as follows: a porcelain crucible was heated in an oven for 30 minutes, then the sample was placed in it. The crucible was allowed to cool to room temperature before weighing. The sample was homogenized, and 2 grams were taken with a precision of 0.1 mg. These

2 grams were placed in an oven at 130°C for two hours. After this period, the sample was removed and allowed to cool in a desiccator for half an hour before being weighed with precision.

Formula for humidity determination

$$\% \text{ H} = \frac{W2 - W1}{w0} x\ 100$$

Where:

W_0= Weight of the sample (g)

W_1= Weight of the crucible plus the sample after drying

W_2= Weight of the crucible plus the sample before dying

%MS= 100 – HT

2.9. Microbiological Tests

Sample preparation was carried out by maceration with a mortar, in order to carry out the physical-chemical and microbiological tests. To assess the microbiological quality of the cotyledon, two specific microbiological tests were carried out for each treatment and repetition: moulds/yeasts and Escherichia coli, tests that are essential due to the sun-drying process, in order to establish the microbiological quality of the cocoa.

During the fermentation of cocoa beans, there is the presence of various microorganisms, such as yeasts, lactic bacteria, acetic bacteria and filamentous fungi, which are essential for the fermentation process of the bean. However, during drying, their concentration decreases. The analyses carried out after shelling are crucial for quality control, since at that stage it is essential to ensure proper handling of the food (López, 2019).

Escherichia coli Microbiological Test

To prepare the culture medium, 25 g of MACCONKEY AGAR TM MEDIA were mixed in 500 ml of distilled water and the mixture was homogenized for 5 minutes. Petri dishes, the culture medium, yellow and blue pipette tips, and 2 ml microcentrifuge tubes were sterilized in an autoclave. All materials were then exposed to UV light for 15 minutes.

Aseptically, 0.5 g of each cotyledon sample were weighed, ground, and placed in a 2 ml dilution tube. The samples were then plated onto the Petri dishes, which were incubated in an oven at 25°C for 48 hours. Finally, Colony Forming Units (CFUs) were counted using a colony counter.

2.10. Cadmium Analysis

Cadmium analysis in cocoa tissue was performed using graphite furnace atomic absorption spectrometry (GF-AAS). Cocoa samples were collected, air-dried, and ground into a fine powder. A 0.5 g portion of each sample was digested with 5 ml of nitric acid (HNO_3) and 2 ml of perchloric acid ($HClO_4$) in a beaker, gradually heated to 150-180°C until a clear solution was obtained. After cooling, the digested solution was filtered into a 50 ml volumetric flask and diluted to volume with distilled water.

Standard cadmium solutions (0, 1, 2, 5, 10, 20 ppb) were prepared and analyzed using GF-AAS to obtain absorbances and construct a calibration curve. The digested samples were analyzed in GF-AAS, and cadmium concentrations were determined using the calibration curve equation. Results were expressed in mg/kg of dry sample, ensuring precision and accuracy in cadmium determination in cocoa (Intriago et al., 2019).

3. RESULTS

3.1. Physiscal Characteristics of the Fruit

As shown in Table 2, the morphological variables of Macambo cocoa pods indicate a total pod weight ranging from a lower limit of 714 grams to an upper limit of 1443 grams. The length of the pod varies from 21.00 to 25.30 cm. The pod with the greatest length of 25.3 cm also had the highest weight, demonstrating a correlation between pod length and weight. There were no numerical differences in the number of ridges. In context, the circumference of the pod is greater than its weight, and its width is relatively similar, with values comparable to those found by (Gálvez et al. 2018).

Table 2.

Morphologycal variables of Macambo Cocoa Pod (Theobroma bicolor)

	Total pod weight	Pod lenght	Total pod diameter	Number of ridges	Diámeter
	714	21.0	42.5	10	10.8
	915	21.2	43.8	10	10.7
	999	22.3	46.1	10	11.1
	1060	23.0	46.5	11	11.0
	1109	23.7	48.2	11	10.8
	1148	24.7	48.5	11	10.8
	1153	24.7	48.5	10	10.8
	1240	24.1	49.6	10	11.5
	1443	25.3	52.6	10	11.5
Averages	**1087**	**23**	**47**	**10**	**11**

According Intriago et al. (2023), their research presents the characteristics of *Theobroma* cacao L.; when comparing these characteristics with those of common cocoa, notable differences are observed. The pods of *Theobroma cacao* L. generally weigh between 400 and 800 grams, although they can reach up to 1 kg in some varieties, indicating that Macambo is considerably heavier. In terms of length, common cocoa pods typically measure between 15 and 30 cm, so the length of Macambo pods falls within this range, although the longer ones tend to be heavier than those of common cocoa.

Morphology of Macambo

Table 3 shows the characteristics of Macambo cocoa beans (*Theobroma bicolor* Humb & Bonpl.). Regarding width, no statistically significant differences were observed ($p \leq 0.05$) according to Tukey's test; however, the upper part of the pod exhibited a larger size of 11.66 cm, compared to the lower part, which measured 9.64 cm.

Table 3.

Morphological Variables of Macambo Cocoa Pod and Fresh Cocoa Beans (Theobroma bicolor Humb & Bonpl.)

Factor		Variable				
Pod Division		**Width (Cm)**	**Thickness (Cm)**	**N° of almonds**	**Weight of fresh almonds (g)**	**Cobs without almonds (weight)**
High		11.16	1.01	9.93	89.07	212.36
Medium		10.14	1.05	17	176.10	220.64
Low		9.64	1	10.13	96.98	187.91
Probability	EEMM ±	1.05	1.24	0.4	4.32	7.33
	Morphological classification	0.3655	0.3748	<0.0001**	<0.0001 **	0.0056*

Nota: **= Highly significant ($p < 0.01$); *= Significant ($p < 0.05$).

For the other variable Thickness there was no significant statistical behavior in terms of Tukey's multiple range test p≤0.05 being the data similar in terms of ear division.

Regarding the variable number of beans, a highly significant statistical difference was found in the multiple range test of Tukey with a probability of p≤0.05, where the highest number of beans was observed in the middle part of the pod, averaging 17 Macambo beans, while the upper part had a lower average of 9.93 beans.

For the variable weight of the beans, highly significant differences were found according to the Tukey test (p≤0.05), with the middle part of the pod showing the highest bean weight at 176.10 grams, while the upper part had the lowest weight at 89.07 grams.

In the case of the variable weight of the empty pod, a statistically significant behavior was also observed in the Tukey test (p≤0.05), with the middle morphological part having the highest weight without beans at 220.64 grams, and the lowest weight being in the lower part at 187.31 grams.

Parada et al. (2019), obtained very different data regarding the weight of the empty pod, which was 637.63 grams, falling within the weight range of the shell, with a value of 620.91 grams in their study. It can be mentioned that *Theobroma bicolor* (Patashte) from Honduras shows a thicker shell.

Temperature Variable

During the fermentation process, no statistically significant differences were detected in the temperature variable (p<0.05 according to Tukey's test). Table 4 presents the average temperatures recorded in Macambo cocoa beans (*Theobroma bicolor* Humb & Bonpl.) during the three consecutive days of fermentation. The initial temperature was highest in the lower part of the pod, registering 29.73°C, followed by the middle part with 29.70°C and the upper part with 29.50°C; the beans were taken from the lower, upper, and middle parts of the pod.

Table 4.

Temperature During the Fermentation Stage of Macambo Cocoa (Theobroma bicolor Humb & Bonpl.)

Factor		Variable		
Pod Division	**Fermentation Method**	**Temp1**	**Temp2**	**Temp3**
High	Rohan	29.50	39.30	45.30
	Polyethylene Bags	29.63	38.37	45.37
	Jute Bags	29.63	35.27	39.57
Medium	Rohan	29.57	39.40	45.57
	Polyethylene Bags	29.57	39.70	45.30
	Jute Bags	29.70	35.77	38.87
Low	Rohan	29.57	39.27	45.87
	Polyethylene Bags	29.73	39.17	45.73
	Jute Bags	29.60	36.17	39.77
Probability	EEMM±	0.05	0.18	0.07
	Morphological division Cob	0.8282	0.0491*	<0.0001**
	Fermentation Method	0.3007	<0.0001**	<0.0001**
	Cob division*Fermentation methods	0.6547	0.2642	0.0085*

Note: **= Highly significant (p < 0.01); *= Significant (p < 0.05).

On the last day of fermentation, temperature showed highly significant differences according to the morphological division of the pod. The lower part registered the highest temperature (45.87°C), followed by the middle part (45.57°C) and the upper part (45.37°C). Regarding the fermentation method, the Rohan boxes reached the highest final temperature (45.58°C), followed by polyethylene bags (45.46°C).

The jute bags had the lowest final temperature (39.40°C), showing highly significant differences. In the interaction factor between pod division*fermentation methods, no statistically significant difference was evidenced at probability.

According to Vásquez et al. (2022), in the fermentation of cocoa (*Theobroma cacao* L.) using the Rohan box method for the Nacional variety, a temperature of 41.16°C was recorded on the fourth (final) day of fermentation. In the case of Trinitario cocoa, the final value was 41.33°C, which is consistent with the findings of the present study. According to Alvarado et al. (2022) in the fermentation of cocoa using micro fermenter boxes, the value was 41.48°C, which is higher than the data found here, Cedeño et al. (2023) conducted a fermentation of cocoa (*Theobroma cacao* L.) in micro fermenter boxes and jute bags, measuring fermentation for 5 days, with values of 38.67°C and 37.67°C, respectively. Different values were presented by (Vera et al. 2022) in the fermentation of (*Theobroma cacao* L.) experimental hybrids, with a lower percentage regarding temperature.

pH variable

During the 3-day fermentation period of Macambo cocoa beans, no statistically significant differences were observed in the initial pH according to the morphological division of the pod, as shown in Table 5. However, among the different fermentation methods, it was found that the jute bags showed the highest pH (6.39), while the Rohan boxes recorded the lowest pH (6.18).

Table 5.

Determination of pH in Postharvest of Theobroma bicolor

Factor		Variable		
Pod Division	**Fermentation Method**	**pH1**	**pH2**	**pH3**
High	Rohan	6.23	5.88	5.59
	Polyethylene Bags	6.23	5.78	5.57
	Jute Bags	6.39	6.19	5.99
Medium	Rohan	6.28	5.79	5.53
	Polyethylene Bags	6.29	5.75	5.53
	Jute Bags	6.20	6.06	5.91
Low	Rohan	6.18	5.74	5.56
	Polyethylene Bags	6.24	5.72	5.52
	Jute Bags	6.30	6.09	5.91
Probability	EEMM±	0.02	0.01	0.01
	Morphological division Cob	0.2191	<0.0001**	0.0047*
	Fermentation Method	0.0370*	<0.0001**	<0.0001**
	Cob division*Fermentation methods	0.0035*	0.2246	0.7580

Note: **= Highly significant ($p < 0.01$); *= Significant ($p < 0.05$).

The interaction between the morphological part of the pod and the fermentation methods revealed significant differences, highlighting lower acidity (pH 6.23) in the upper part of the Macambo pod fermented in Rohan boxes. On the second day, there were highly significant differences in pH between the upper part (6.19) and the lower part (5.72) of the Macambo pod.

Vásquez et al. (2022), indicated that the initial value on the first day of fermentation for Nacional cocoa was 3.83, and for Trinitario cocoa it was 3.50, which is very different from those found in *Theobroma bicolor* fermented in Rohan boxes, where the final pH values are higher than *Theobroma cacao* L., reaching 5.10 for Nacional and 4.60 for Trinitario. These pH values do not correlate with the final pH of this

research. According to Alvarado et al. (2022); their values are close to the final pH during the fermentation stage, ranging from 5.44 to 5.52 for Macambo cocoa..

In the research conducted by Gálvez et al (2016), in Mexico on the chemical determination of *Theobroma bicolor*, they found a pH of 6.03, a value higher than that observed in the present study, which could be attributed to geographical factors and specific fermentation conditions. During cocoa fermentation, various microorganisms such as yeasts, lactic bacteria, and acetic bacteria metabolize sugars from the pulp, producing alcohols and organic acids that penetrate into the cocoa beans, causing significant biochemical changes.
Externally, fermentation breaks down the pulp and produces acetic acid, while internally these acids and the increase in temperature break down the cell walls of the cocoa beans, releasing enzymes and flavor precursors. These biochemical processes tend to lower the pH of the cocoa beans, although the magnitude of this change depends on the duration and control of fermentation, environmental conditions, and the initial composition of the pulp, which vary according to the region and cocoa variety (Vera et al., 2023).

Brix Degrees

En la tabla 6, during the fermentation of Macambo almonds, soluble solids were evaluated. In the morphological division of the pod, there were no significant differences, with the lower part showing the lowest value (13.70 °Brix) and the middle part showing the highest (14.63 °Brix). In the fermentation methods, no statistical differences were observed. The interaction between pod division and fermentation methods did not show significant statistical behavior according to the Tukey test.

Table 6.
Brix in the fermentation stage of Theobroma bicolor

Factor		Variable		
Pod Division	**Fermentation Method**	**Brix1**	**Brix2**	**Brix3**
High	Rohan	14.43	9.40	5.87
	Polyethylene Bags	14.50	9.36	6.16
	Jute Bags	13.93	10.86	7.22
Medium	Rohan	14.23	9.53	5.55
	Polyethylene Bags	14.23	9.70	5.13
	Jute Bags	14.63	11.03	7.04
Low	Rohan	13.70	9.73	5.34
	Polyethylene Bags	14.26	9.66	5.69
	Jute Bags	14.50	11.16	7.16
Probability	EEMM±	0.13	0.07	0.06
	Morphological division Cob	0.5221	0.0210*	<0.0001**
	Fermentation Method	0.3943	<0.0001**	0.0013*
	Cob division*Fermentation methods	0.0673	0.8874	0.0013*

Note: **= Highly significant ($p < 0.01$); *= Significant ($p < 0.05$).

Regarding the °Brix variable 2, it was observed that the upper part of the pod had the highest percentage of soluble solids, with 9.36 in the beans, while the lower part of the pod recorded 11.16 soluble solids. Concerning the fermentation methods factor, a highly significant effect was observed according to the Tukey test ($p \leq 0.05$). The fermentation method using jute bags showed the highest percentage of soluble solids, with 11.16 °Brix, while the lowest value was for beans fermented in Rohan boxes, with 9.40 °Brix.

The ANDEVA analysis with the Tukey test revealed highly significant differences ($p \leq 0.05$) in the °Brix of Macambo cacao beans. The upper part of the pod showed the highest value (7.22), while the middle part recorded the lowest (5.13). The fermentation method also influenced the results, with jute bags achieving

the highest value (7.22) and Rohan boxes the lowest (5.34). The interaction showed that beans from the upper part fermented in jute bags had the highest value, while those from the lower part fermented in Rohan boxes had the lowest value.

In line with the above, according to the study by Vásquez et al. (2022), the initial °Brix in the fermentation of Nacional cacao over 4 days of study ranged from 13.12 °Brix to 10.03 °Brix, and for Trinitario it was from 12.78 to 8.19 °Brix. This contrasts with *Theobroma bicolor*, which on the third day of study showed initial values of 14.43 °Brix and final values of 5.87 °Brix. Similarly, Alvarado et al. (2022) mention that soluble solids are consumed by microorganisms during the post-harvest process, with values of 13.47 °Brix on day 1 and 6.18 °Brix on day 3, while Cedeño et al. (2023) indicate that soluble solids increase as fermentation progresses.

Physical Variables

According to Table 7, the physical variable data for *Theobroma bicolor* is presented, specifically regarding the ANOVA results for the moisture content after drying the cacao beans. Based on the morphological division factor of the pod, no statistically significant differences were recorded according to the Tukey test (p≤0.05). The same behavior was observed for the fermentation method factor. Additionally, the interaction between the pod and the fermentation method did not show any statistical significance.

Table 7.
Physical Variables of Theobroma bicolor

Factor		Variable		
Pod Division	**Fermentation Method**	**Humidity**	**Ash**	**pH of Dried Almond**
High	Rohan	7.49	5.00	5.82
	Polyethylene Bags	7.48	4.94	5.87
	Jute Bags	7.41	4.82	6.12
Medium	Rohan	7.62	4.91	5.77
	Polyethylene Bags	7.71	5.08	5.81
	Jute Bags	7.50	5.14	6.06
Low	Rohan	7.55	5.07	5.63
	Polyethylene Bags	7.52	4.89	5.84
	Jute Bags	7.53	5.22	6.10
Probability	EEMM±	0.09	0.12	0.02
	Morphological division Cob	0.5334	0.6459	0.0449
	Fermentation Method	0.7675	0.864	<0.0001**
	Cob division*Fermentation methods	0.9707	0.7007	0.0971

Note: **= Highly significant (p < 0.01); *= Significant (p < 0.05).

No significant statistical differences were found in the ash content variable related to the morphological division of the pod or among the different fermentation methods. The interaction between the pod division and fermentation methods also showed no significant differences, although a numerical increase was observed. The highest ash content was recorded in the fermentation with jute bags from the lower part of the pod, while the lowest value was observed in the fermentation with jute bags from the upper part of Macambo.

The pH variable of dried Macambo cotyledons, considering the morphological division of the pod, showed significant differences (p≤0.05), being higher in the upper part (5.87) and lower in the middle part (5.06). Regarding the fermentation method, the pH was significantly higher in jute bags (6.09) and lower in Rohan boxes (5.63). The interaction between pod division and fermentation method did not show significant statistical differences.

In comparison to the findings of Paradas et al. (2019) their humidity value was 4.33%, which is similar to the values found by Gálvez (Galvez & Diaz, 2016) with a humidity of 3.55% in non-fermented samples. In the current research, humidity ranged from 7.41% to 7.62%. According to the cocoa standard INEN 176 (INEN, 2018) this value should neither exceed nor be below 7%. A higher value is not recommended as it can promote the proliferation of microorganisms that affect chocolate quality. Conversely, if the value is below 7%, the yield decreases because aroma precursors may be impacted, making it unacceptable in international markets. Additionally,(Tinajero et al. 2021) found humidity in Pataxte beans ranging from 3.1% to 20.5%, indicating that Macambo has a high mucilage content Galvez & Diaz, (2016) found that the ash content was lower than 3.52%. However, this value is similar to that found by Tinajero et al. (2021) 2.23 a 2.35 which ranged from 2.23 to 2.35 in the evaluation of ash content in unfermented *Theobroma bicolor* beans. In contrast, the ash content data from (Parada et al., 2019) in Honduras analyzed the ash content in Macambo beans, which was lower than the values found in the present study at 4.33%.

According toAmores et al. (2009), the pH in dry beans of Nacional cacao is 5.20, indicating that the pH behavior of Macambo is lower than that of conventional cacao..

According to Vera et al. (2024), in a study by Quinteros in Peru, the moisture content was found to be 7.09%, which is consistent with the findings of the present investigation. The same author indicates that for high-quality cacao, the safe moisture values are in the range of 7 to 8%, which supports the fermentative activity and helps reduce the water content in the Macambo beans.

3.2. Microbiological Analysis

Mold and Yeast Analysis in the Cotyledon of Cacao Beans

According to the results found in Table 8, the mold and yeast analysis determined that there was the growth of one CFU in four treatments. This means that the levels are within the permissible ranges according to INEN 176 standards for human consumption or for further processing of the beans, such as chocolate production (INEN, 2018).

Table 8.
Results of Mold and Yeast Analysis in Fermented and Dried Macambo Cacao Beans (Theobroma bicolor Humb & Bonpl.)

TRAT	MEDIAS
T8	9.67e+00 a
T5	9.67e+00 a
T2	6.33e+00 ab
T9	5.00e+00 b
T3	3.33e+00 bc
T4	3.30e-01 c
T6	3.30e-01 c
T1	0.00e+00 c
T7	0.00e+00 c

Note: Means with a common letter are not significantly different (p≤0.05).

Escherichia coli Analysis in Cacao Beans

It was observed that there was a total absence of Colony Forming Units in any of the treatments and repetitions of the fermented cacao beans. This indicates that there was no contamination and no development of microorganisms during the fermentation and drying processes of the cacao beans.

Cadmium Analysis in Cacao Bean Cotyledon

The findings of this study, as shown in Table 9, reveal that the cadmium content is below the permissible limits of 0.80 mg/kg of cadmium.

Table 9.
Analysis of cadmium in almonds

Laboratory Number	Sample Identification	Cd/mg kg
1189	High-Medium-Low	0.19

According to the European Union (EU) Commission Regulation 2021/1323, the maximum allowable cadmium content is 0.80 mg/kg(Comisión Europea, 2021).

The compliance of cadmium levels in Macambo cacao (*Theobroma bicolor*) underscores its potential as an alternative to common cacao (*Theobroma cacao*), especially in Latin American markets where cadmium presents a significant challenge. This finding highlights Macambo's ability to meet international regulatory standards, ensuring the economic viability and sustainability of the crop in Ecuador and other producing countries.

CONCLUSIONS

The research on Macambo cacao pods (*Theobroma bicolor*) revealed that the fermentation method in jute sacks resulted in almonds with the lowest moisture content (7.41%) and the highest pH (6.12). Throughout the study, the degree of fermentation showed a linear decrease in Brix degrees, with T4 (7.22) being the highest and T5 (5.13) the lowest. Although no significant statistical changes in pH were observed over time, the temperature increased as the study progressed.

Microbiological analyses did not detect Escherichia coli, but mold and yeast growth was observed in treatments without air during fermentation. Measuring physical and morphological parameters is crucial for standardizing and ensuring fruit quality, allowing the identification of consistent characteristics that ensure product uniformity and optimize yield. There is a significant correlation between physical parameters and physicochemical results; for instance, lower moisture content improves product preservation and pH influences organoleptic properties.

To enhance this crop, it is necessary to optimize fermentation methods, implement sustainable agricultural practices, and control post-harvest parameters. Additionally, it is important to investigate the impact of different fermentation and drying methods, the genetic variability of Macambo, the sustainability of agricultural practices, and the nutritional and functional potential of the cacao, which could open new markets and applications for the product.

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CONFLICTO OF INTEREST

There is no conflict of interest related to the subject matter of the work.

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