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ORIGINAL

Modulation of the sensory and chemical profiles of cocoa liquor (Theobroma cacao L.) through efficient microorganisms and fruit extracts in fermented beans of the CCN-51 and Nacional varieties

Modulación de los perfiles sensorial y químico del licor de cacao (Theobroma cacao L.) mediante microorganismos eficientes y extractos frutales en almendras fermentadas de las variedades CCN-51 y Nacional

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ABSTRACT

Introduction: cocoa fermentation is a key biotechnological process that defines the chemical and sensory quality of the liquor. The incorporation of efficient microorganisms (EM) and fruit extracts has been proposed as a strategy to modulate fermentative metabolism and enhance the attributes of the final product.
Method: two cocoa varieties (CCN-51 and Nacional) were evaluated under a three-factor design with different EM concentrations (0, 40, and 80 %) and diluted extracts of banana, passion fruit, and jackfruit. Physicochemical variables (pH, titratable acidity, moisture, ash, protein, and energy) and sensory attributes (color, appearance, aroma, flavor, and aftertaste) were analyzed using ANOVA and interaction tests.
Results: pH and acidity showed highly significant differences for EM% and its interaction with the extracts, indicating greater lactic-acid and acetic-oxidative activity. Moisture was modulated by the combined action of EM and fruit matrices, particularly in treatments with passion fruit and banana. Ash content showed effects only in interactions, while protein and energy depended mainly on the genotype. Sensory analysis revealed that CCN-51 developed darker tones, satin surfaces, and fruity-cocoa profiles, whereas Nacional exhibited toasted, bitter notes and more persistent aftertastes.
Conclusions: the co-application of EM and fruit extracts significantly modulated the biochemical profile and sensory expression of the cocoa liquor, with contrasting responses between varieties. These findings support the use of combined bioprocesses to optimize functional and organoleptic attributes for specialty cocoa markets.

Keywords: Controlled Fermentation; Microbial Inoculants; Fruit-Based Matrices; Sensory Properties; Postharvest Quality; Cocoa Genotypes.

RESUMEN

Introducción: la fermentación del cacao es un proceso biotecnológico clave que define la calidad sensorial y química del licor. La incorporación de microorganismos eficientes (EM) y extractos frutales se ha propuesto como una estrategia para modular el metabolismo fermentativo y mejorar los atributos del producto final.

Método: se evaluaron dos variedades de cacao (CCN-51 y Nacional) bajo un diseño trifactorial con diferentes concentraciones de EM (0, 40 y 80 %) y extractos diluidos de banano, maracuyá y jackfruit. Se analizaron variables fisicoquímicas (pH, acidez titulable, humedad, ceniza, proteína y energía) y sensoriales (color, aspecto, aroma, sabor y regusto), mediante ANOVA y pruebas de interacción.
Resultados: el pH y la acidez mostraron diferencias altamente significativas para EM% y su interacción con los extractos, evidenciando una mayor actividad ácido-láctica y acético-oxidativa. La humedad fue modulada por la acción combinada de los EM y las matrices frutales, especialmente en tratamientos con maracuyá y banano. La ceniza presentó efectos solo en interacciones, mientras que proteína y energía dependieron principalmente del genotipo. Sensorialmente, CCN-51 mostró tonalidades más oscuras, superficies satinadas y perfiles frutales-cacao, mientras Nacional presentó notas tostadas, amargas y regustos más persistentes.
Conclusiones: la coaplicación de EM y extractos frutales moduló de forma significativa la bioquímica y el perfil sensorial del licor, con respuestas contrastantes según la variedad. Estos resultados respaldan el uso de bioprocesos combinados para optimizar atributos funcionales y organolépticos en mercados de cacao de especialidad.

Palabras clave: Fermentación Controlada; Inóculos Microbianos; Matrices Frutales; Propiedades Sensoriales; Calidad Poscosecha; Genotipos de Cacao.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a tree species grown mainly in tropical regions, and its plantations have a significant economic, social, and sensory impact. Cocoa beans or almonds, obtained from the tree's fruit, are the essential raw material for the production of liquor and chocolate, products whose value in international markets depends directly on the quality achieved during the fermentation and post-harvest process. This crop also serves as a fundamental pillar for the sustainability of rural economies and the conservation of tropical biodiversity. Fermentation is considered the most decisive phase in the development of the organoleptic profile of cacao, as it generates biochemical precursors that give rise to distinctive aromatic and taste notes during roasting and refining.[1] In this context, Ecuador is positioned as one of the primary references in the production of delicate and aromatic cocoa[2], with the Nacional and CCN-51 varieties standing out in particular, which present significant contrasts in chemical composition, fermentation response, and sensory attributes.[3]

Traditional fermentation relies on spontaneous microbiota and variable environmental conditions, leading to inconsistent expression of volatile compounds, organic acids, phenols, alkaloids, and peptides that contribute to the aroma and flavor of cocoa liquor.[4] Therefore, there has been growing interest in the application of biotechnological approaches that allow the metabolic pathways involved in the formation of desirable chemical and sensory profiles to be directed and enhanced.[5] Among these strategies, the use of efficient microorganism (EM) consortia has become a promising alternative, given their potential to optimize mucilage degradation, increase the synthesis of alcohols, aldehydes, and esters, and promote essential enzymatic processes during controlled fermentation.[6]

At the same time, incorporating fruit extracts as fermentation adjuvants offers additional advantages, as they provide fermentable carbohydrates, phenolic compounds, minerals, and organic acids that can stimulate microbial activity and positively influence volatile compound production.[7] This combined approach has shown potential to influence both sensory attributes, such as flavor, acidity, aromatic persistence, floral or fruity notes, and the chemical composition of the liquor, which directly impacts consumer perception and the commercial value of cocoa-derived products.[8]

Cocoa liquor is a complex matrix in which components such as theobromine, catechins, lipids, proteins, sugars, and aromatic precursors interact to produce specific sensory profiles. Several recent studies have shown that modifications induced during fermentation can generate significant differences between varieties and treatments, especially when selected microorganisms or complementary substrates are used.[9] However, most research has focused on traditional processes or a single type of intervention, leaving a gap in our knowledge of the combined effects of microbial consortia and fruit extracts on contrasting varieties such as CCN-51 and Nacional.

In this context, it is pertinent to analyze how the combined application of efficient microorganisms and fruit extracts affects the sensory and chemical profiles of cocoa liquor produced from fermented almonds.[10] Understanding these interactions allows not only to optimize organoleptic quality, but also to strengthen competitiveness in markets that demand differentiated, consistent products with a clear identity of origin. In addition, this type of research contributes to the design of sustainable fermentation models transferable to small- and medium-scale operations, with a focus on the high-value chocolate industry.

Consequently, the present study aims to evaluate the sensory profile of cocoa liquor made from fermented

CNN-51 and Nacional cocoa beans, treated with a consortium of efficient microorganisms (EM) and fruit extracts, to determine the influence of these treatments on the final product's organoleptic characteristics. This approach allows for the generation of scientific evidence applicable to innovative agro-industrial systems aimed at comprehensive product improvement, in line with current trends in functional food research, fermentative biotechnology, and sensory quality.

METHOD

Location and environmental conditions

This study was conducted at the facilities of the National Institute of Agricultural Research (INIAP) at the Pichilingue Tropical Experimental Station, located in the canton of Mocache, whose geographical coordinates are: Lat: 1° 4´ 26.00" and Long: 79° 29´ 20.47", at an altitude of 73 meters above sea level, where the fermentation of cocoa obtained in Santa Rosa, Province of El Oro, was carried out. Its geographical coordinates are: Lat: 3°23' 41.9" and Long: 79°50´ 23.6", and in the Province of Manabí, La Pipona Parish, Rocafuerte Canton, with coordinates: Lat: 0° 52´ 06.0" S" and Long: 80° 27' 41.2" W.

The variables for the physicochemical analyses were collected at the Bromatology Laboratory of the "La María" Campus, located on the grounds of the Technical State University of Quevedo. This laboratory is located at Kilometer 7 on the Quevedo-El Empalme road, San Felipe Campus, Mocache canton, with geographical coordinates of 01°06´00" S W, an altitude of 73 meters above sea level, and an average temperature of 25.8 °C.

The fruit used to make the diluted extracts was harvested on request at Recinto el Naranjo, in the canton of Mocache in the province of Los Ríos; its geographical coordinates are: Lat: 1°11´30.7" and Long: 79°35' 43.6".

The cocoa paste for the final product was produced in the Agri-Food Workshop, Confectionery and Chocolate section, located on the university's "La María" Campus.

The chemical analyses for evaluating the cocoa paste were conducted at the Industrial and Food Biotechnology Laboratory of the University of the Armed Forces of Ecuador (ESPE). – Santo Domingo de los Tsáchilas Campus, whose geographical coordinates are 0°24´47" south latitude and 79°18´34" west longitude at 625 meters above sea level, with a temperature of around 22-23 °C, determining the variables of pH, percentage of acidity, moisture, and ash.

Research Design

In this research study, a completely randomized two-factor design (CRD) was used, with 24 treatments and three replicates, yielding a total of 72 study objects. The first factor was the types of cocoa (*Theobroma cacao* L.), represented by two genetic materials widely cultivated in Ecuador: CCN-51 and Nacional. The second factor was the application of efficient microorganisms (0 % (0 mL; control), 40 % (400 mL), or 80 % (800 mL) per kilogram of fermentative liquid product). And finally, the third factor evaluated three types of fruit extract, adding a control without a diluted extract. Banana, jackfruit, and passion fruit extracts were used; each was prepared at a concentration of 3 % (30 mL) for the study.

Statistical Design

Infostat software was used for the analysis. This ensures robust, scientifically sound statistical estimates, and RStudio software was used to prepare the sensory analysis figures. This study used the experimental method to contrast the experimental factors, employing an experimental design and an ANDEVA scheme. Statistical analysis of the data was performed using ANOVA, and differences between means were determined using Tukey's multiple-range test at a significance level of $p \leq 0,05$.

Type of experimental design

The following mathematical model, known as a completely randomized three-factor design, was used for the statistical analysis, as shown in equation 1.

Equation 1. Mathematical model

$$Yijk = \mu_{+} \alpha_i + \beta_j + (\alpha.\beta)_{ij} + E_{ijk}$$

μ= The effect of the mean.
α_i= This is an effect of the "i-th" level of factor A.
β_j=This is an effect of the "jth" level of factor B.
$(\alpha.\beta)_{ij}$=It is an effect due to the interaction of the "i-th" level of factor A with the "j-th" level of factor B.
E_{ijk}= It is a random effect.[11]

ANDEVA diagram

Table 1 presents the analysis of variance (ANOVA), which includes the treatments, the factors evaluated,

and the interactions between them, providing a statistical basis for interpreting the main and combined effects of the experimental model.

Table 1. ANDEVA scheme

Sources of variation (FV)		Degrees of freedom (DF)
Treatments	axb-1	71
Repetitions	r-1	2
Cocoa factor	(TP-1)	1
ME factor	(ME-1)	2
Fruit Extract Factor	(Ef-1)	3
Cocoa * ME factor	(Tp-1)(ME-1)	2
Cocoa*Fruit extracts	(TP-1)(Ef-1)	3
EM*Fruit extract		6
Cocoa*EM Factor*Fruit Extracts	(TP-1)(ME-1)(Ef-1)	6
Experimental error	(Tp*ME*Ef) (r-1)	47
Total	Me*EM*Ef r-1	71

Study factors

Table 2 details the structure of the experimental design, which consists of three main factors. Factor A corresponds to the types of cocoa and is represented by the CCN-51 and Nacional varieties. Factor B considers the application levels of efficient microorganisms (EM), distributed in three concentrations: 0 %, 40 %, and 80 %. Finally, Factor C includes the different fruit extracts used as fermentation adjuvants, which cover four conditions: no extract, banana, jackfruit, and passion fruit.

This factorial combination allows for the evaluation of the individual effects and possible interactions between cocoa varieties, levels of efficient microorganisms, and diluted fruit extracts on the response variables considered in the research.

Table 2. Factors and levels of study

FACTOR A Types of cocoa	FACTOR B Percentage of efficient microorganisms		FACTOR C Diluted fruit extract of fruit	
		0 %		Without fruit extract
CCN-51	Levels	40 %	Levels	Banana Jackfruit
National		80 %		Passion fruit

Combination of treatments

Table 3 organizes the 24 treatments evaluated using a coding system that allows each experimental combination to be accurately identified. The structure facilitates the ordering of the study units and their assignment during the fermentation process. This coded distribution optimizes the comparative analysis of the treatments and ensures the traceability of the results obtained.

Table 3. Study treatment arrangement

No.	Code	TYPES OF COCOA	Percentage of effective microorganisms	Diluted fruit extract
1	ccEM0Ef0	CCN-51	0	No diluted fruit extract
2	ccEM0Ef1	CCN-51	0	Banana
3	ccEM0Ef2	CCN-51	0	Jackfruit
4	ccEM0Ef3	CCN-51	0	Passion fruit
5	ccEM1Ef0	CCN-51	40	No diluted fruit extract
6	ccEM1Ef1	CCN-51	40	Banana
7	ccEM1Ef2	CCN-51	40	Jackfruit

8	ccEM1Ef3	CCN-51	40	Passion fruit
9	ccEM2Ef0	CCN-51	80	No diluted fruit extract
10	ccEM2Ef1	CCN-51	80	Banana
11	ccEM2Ef2	CCN-51	80	Jackfruit
12	ccEM2Ef3	CCN-51	80	Passion fruit
13	naEM0Ef0	NATIONAL	0	No diluted fruit extract
14	naEM0Ef1	NATIONAL	0	Banana
15	naEM0Ef2	NATIONAL	0	Jackfruit
16	naEM0Ef3	NATIONAL	0	Passion fruit
17	naEM1Ef0	NATIONAL	40	No diluted fruit extract
18	naEM1Ef1	NATIONAL	40	Banana
19	naEM1Ef2	NATIONAL	40	Jackfruit
20	naEM1Ef3	NATIONAL	40	Passion fruit
21	naEM2Ef0	NATIONAL	80	No diluted fruit extract
22	naEM2Ef1	NATIONAL	80	Banana
23	naEM2Ef2	NATIONAL	80	Jackfruit
24	naEM2Ef3	NATIONAL	80	Passion fruit

Experimental procedure

Post-harvest execution

Obtaining the cacao pod (raw material)

During the harvesting of cocoa pods, it is essential to ensure that the selected fruits are in optimal condition, strictly avoiding the harvest of those that show signs of contamination by Moniliasis (*Moniliophthora roreri*):

To guarantee 72 experimental units of 1 kg of fresh mass each, 36 kg were assigned to the CCN-51 variety and 36 kg to the Nacional array. Based on average yields reported for fresh almonds (0,12 kilograms per pod in CCN-51 and 0,10 kg in Nacional), approximately 300 and 360 pods were estimated, respectively. To compensate for physiological variability and ensure the availability of biological material, an additional 10 % margin was applied, resulting in 330 ears of CCN-51 and 400 ears of Nacional, for a total of 730 fruits.

Pulping

Once the required pods were obtained, the pulping process was carried out, which consists of separating the cocoa beans from the fruit placenta.

A longitudinal or transverse cut was also made in the pods to facilitate the extraction of the cocoa beans.

Once pulped, the cacao beans were separated and stored in clean, properly conditioned containers.

The beans were placed in the cells of the micro-fermentation boxes.

Experiment Management

Fermentation Process

Subsequently, the cocoa beans were placed in micro-fermenter boxes, each with a capacity of 1 kg of fresh cocoa mass,[12] made of white guayacán wood, with a total capacity of 36 boxes and dimensions of 125x175x10 centimeters. Two boxes were used in their entirety (72 cells), and 1 kg of almonds was used in each box, with the control treatments separated. The process involved a total of 72 kg of fresh cocoa-almond mass.[13] The fermentation stages lasted 5 days.[14]

Application of efficient microorganisms

Efficient microorganisms (Albiobacth) were induced in fresh cocoa almonds according to the specifications of an experimental sketch. This design includes the precise doses for each treatment and the corresponding repetitions, ensuring uniformity and methodological rigor throughout the experimental process.[5]

Albiobacth

Albiobacth microorganisms (trade name) were used, containing bacteria: $1,3x10^7$ Colony Forming Units/mL, while yeasts reached $4.0x10^6$ CFU/mL. The organisms identified include *Lactobacillus acidophilus, Saccharomyces cerevisiae, Rhizobium japonicum, and Azotobacter Chroococcum*.[5]

Percentage of application of efficient microorganisms

Following the methodology described by Vásquez[1], the amount of Albiobacth microorganisms to be applied was calculated using efficient organisms at the following proportions: 0 % (control), 40 % (400 mL), and 80 % (800 mL). These doses were applied per kilogram of fermentative liquid product mass, ensuring adequate distribution according to the established treatments.[15]

Preparation of diluted banana fruit extract

The banana extract (*Musa* spp.) was prepared as shown in figure 1 of the diagram, using four fruits in an advanced state of ripeness, with an average weight of 150 g each. From this quantity, 150 g of pulp was obtained, which was blended with 200 mL of distilled water at a controlled temperature below 40°C +/- 2°C.[16]

The final proportion corresponded to 42,85 % fruit in the mixture, obtained by applying the formula and following the methodology described by Viera.[17] This same equation 2 for the percentage of fruit was used in a similar way to calculate the proportion in the extracts made with the other fruits evaluated (banana, jackfruit, and passion fruit).

Equation 2. Fruit percentage

$$\text{ruta } \% = \frac{\text{g de pulpa}}{\text{g de pulpa} + \text{mL de agua}} \text{ x } 100$$

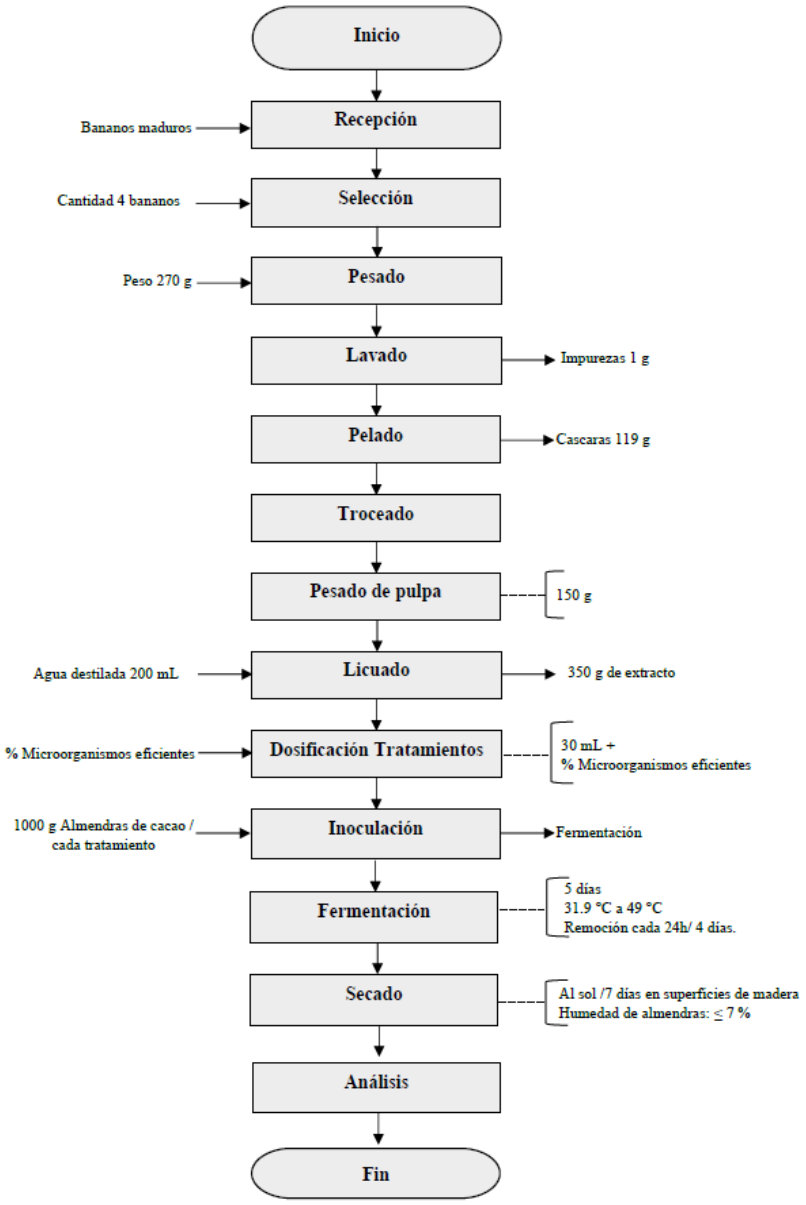

Figure 1. Diagram of the process of obtaining and applying diluted banana extract in the fermentation and drying of cocoa

To obtain the diluted banana extract, fruit in an advanced state of ripeness ("ripe") was used, characterized by a yellow color.[18]

Preparation of jackfruit extract

The preparation of diluted jackfruit extract (*Artocarpus heterophyllus*) is shown in figure 2. It was obtained from a natural extract at a concentration of 3 % (30 mL) in order to evaluate its effect on improving the quality of cocoa beans during the fermentation process. At the same time, a smoothie was prepared from jackfruit pulp, using 2700 g of fresh fruit, of which 150 g of pulp was processed and mixed with 200 mL of distilled water, following the methodologies proposed by Vásquez[19].

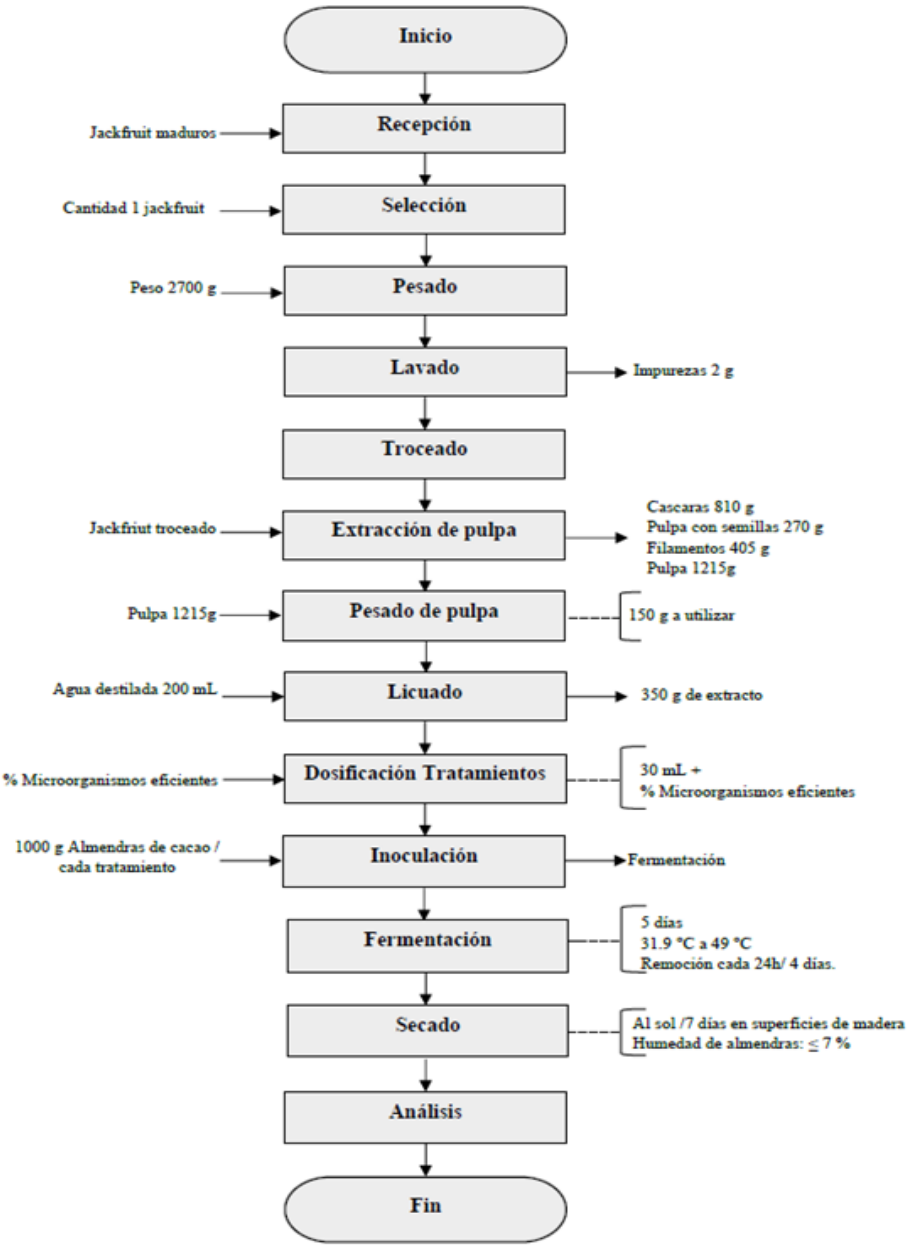

Figure 2. Diagram of the process of obtaining and applying the diluted jackfruit extract in the fermentation and drying of cocoa

Preparation of diluted passion fruit extract

At the same time, a smoothie was prepared using passion fruit pulp, as shown in figure 3, for which 400 g of fresh fruit was used (equivalent to approximately four passion fruits, considering an average weight of 100 g per fruit), of which 150 g of pulp was processed. The pulp was mixed with 200 mL of distilled water, ensuring that the process was carried out under controlled conditions to preserve enzymatic activity and to comply with the parameters established by the methodology proposed by Peña[10].

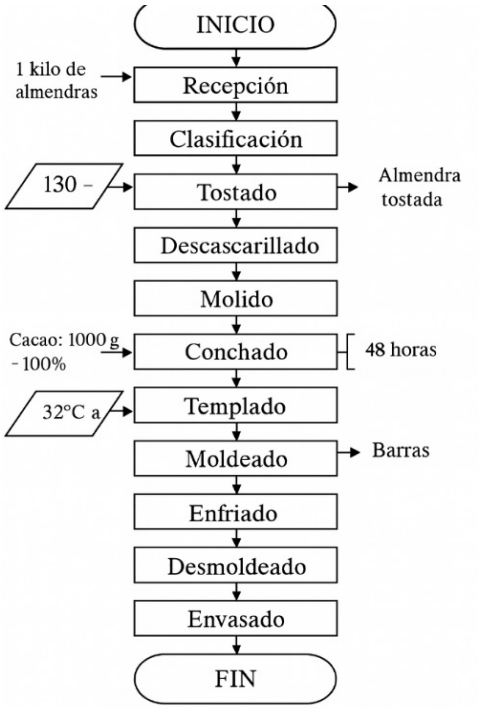

Figure 3. Diagram of the cocoa paste production process

Drying of the cocoa beans

The cocoa beans were then dried. This was done in direct sunlight, ensuring careful handling to avoid mixing beans from different treatments and farms. The cocoa drying process was carried out on wooden surfaces specially conditioned for this purpose, preventing contact with unsuitable surfaces that could lead to contamination by external agents.

During drying, the cocoa beans were stirred "Remociones" constantly and adequately to ensure uniform dehydration. This procedure was carried out for approximately 7 to 8 days, with the aim of achieving an optimal moisture content of 6 % to 7 %, thereby ensuring the final quality of the product.[1]

Storage of cocoa beans

Once the drying stage was complete, the cocoa beans were carefully stored, segregated by treatment and repetition. For preservation, they were stored in paper bags, which promote ventilation and prevent moisture accumulation, thereby maintaining and improving the quality of the cocoa beans. This method ensures that the characteristics acquired during the fermentation and drying process are optimally preserved until analysis or later.[15]

Process for obtaining 100 % cocoa paste

Selection and classification: the raw cocoa material was classified through a thorough visual and manual inspection, with contaminants and foreign bodies removed. This process guarantees the purity of the almonds used.

Roasting: the beans were roasted in a clay pot under controlled conditions at an average temperature of 120 °C. This process lasted 18 to 25 minutes, ensuring even heat distribution to prevent burning and facilitate the removal of residual moisture.

Shelling: the almonds were manually shelled, separating the hulls from the cotyledons. The shelled almonds were stored in properly labeled paper bags to prevent contamination and preserve their integrity.

Grinding: the cotyledons were ground using a traditional manual mill. This process reduced the particle size, facilitating subsequent refining and ensuring a uniform texture in the cocoa paste.

Refining: refining was carried out to achieve a particle size below 40 microns, ensuring a homogeneous texture and avoiding detectable granules. This step is critical to optimizing the organoleptic quality of the final product.

Conching: the refined cocoa mass was processed in a 5 kg capacity conching machine. The sample was introduced gradually to allow the equipment to capture all the raw material adhering to its walls, achieving uniform distribution and efficient emulsification. This process was maintained for 48 hours per treatment, ensuring the development of a superior sensory profile.

Tempering: once the refining process was complete, the cocoa paste underwent tempering, reducing its

temperature in a controlled manner. It was then poured into previously sanitized molds, where it was left to rest until it acquired the desired consistency.

Packaging and storage: finally, the cocoa paste was wrapped in aluminum foil for storage. Each unit was stored in bags identified with unique codes for traceability and stored under refrigerated conditions at 4 °C, minimizing the risk of cross-contamination and preserving its physicochemical and organoleptic properties,[15] as shown in figure 4.

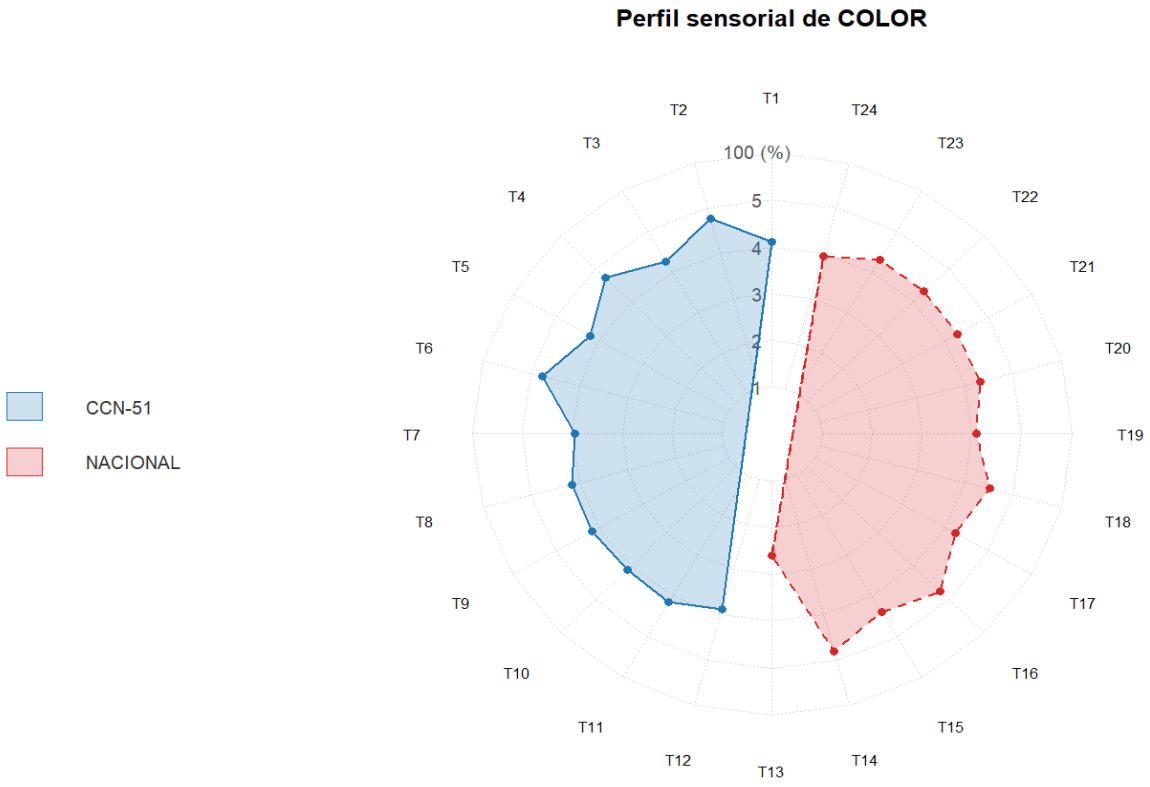

Figure 4. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the sensory color profile of CCN-51 and Nacional cocoa paste

Sensory analysis

For the sensory analysis, a semi-trained tasting panel of 50 tasters was used, who evaluated flavor, aroma, texture, bitterness, floral notes, and almond.[20]

The sensory analysis was performed using a structured descriptive evaluation of the cocoa liquor obtained from the experimental treatments. For this purpose, a sensory sheet with an ordinal hedonic scale from 1 to 5 was used, in which each value corresponded to a previously defined category for each attribute. Five main parameters were evaluated: color, appearance, aroma, flavor, and aftertaste, each with specific descriptors.

The scale used for each parameter was as follows:

- Color: 1 = caramel brown, 2 = brown, 3 = walnut, 4 = carob, 5 = black coffee.
- Appearance: 1 = very shiny, 2 = shiny, 3 = satiny, 4 = opaque, 5 = very opaque.
- Aroma: 1 = tropical fruit, 2 = cocoa, 3 = citrus fruit, 4 = roasted, 5 = nutty.
- Flavor: 1 = fruity, 2 = roasted cocoa, 3 = dry fruity, 4 = bitter, 5 = very bitter.
- Aftertaste: 1 = mild persistence, 2 = bitter, 3 = short-lived citrus, 4 = astringent, 5 = staining sensation.

The samples were identified by codes (T1–T24) to ensure a blind evaluation and avoid perceptual biases. The session took place in an environment with neutral lighting, controlled temperature (20 ± 2 °C), and no visual or olfactory interference. Each sample was served in coded containers and evaluated individually and independently.

The data obtained were organized for subsequent statistical analysis in accordance with the established experimental design.

Physical and chemical tests on cocoa paste

Determination of pH and acidity in cocoa paste

The pH analysis was performed in duplicate using the same previously homogenized sample. Before determining this variable, the glass electrode was rinsed several times with distilled water until the reading stabilized at pH 6. A representative portion of the sample was then placed in a volumetric flask with a capacity of 25-100 cm^3, in which the electrode was immersed. First, 10-25 cm^3 of 0,1 N sodium hydroxide (NaOH) solution was added, stirring constantly until the pH reached 6. Neutralization was continued with slower additions of the same solution until a pH of 7 was reached. Then, 0,1 N NaOH was added dropwise at about four drops per addition, sequentially reporting both the total volume added and the resulting pH, until a pH of approximately 8,3 was reached.

The volume of 0,1 N NaOH was determined precisely by interpolation, allowing the pH point at 8,1 to be identified. From this value, the percentage of acidity was calculated using the equation designated as the following formula:[21]

Equation 3. Determination of pH and acidity in cocoa paste

% Acidity = (V1N1M) * 10V2

Where:
V1= cm3 of NaOH used for titration of the sample
N1= Normality of the NaOH solution
M= Molecular weight of the acid considered as a reference
V2= Volume of the sample taken for analysis.

Determination of moisture in cocoa paste

The moisture analysis was determined in duplicate. First, the porcelain crucibles intended to contain the samples were conditioned in the oven for about 30 minutes. Once removed, they were allowed to cool to room temperature, and their weight was recorded with analytical precision.

The previously homogenized sample was weighed to approximately 2 g, with an accuracy of 0,1 mg. Subsequently, the crucibles with the samples were dried in an oven at 130 °C for two hours, in accordance with the established procedure.

After drying, the crucibles were transferred to a desiccator and left to cool for around 30 minutes to prevent the absorption of ambient moisture. However, in the same context, the final weighing was carried out, and the moisture content of the sample was determined using equation.[21]

Equation 4. Determination of moisture in cocoa paste

$$\% \mathrm{H} = \frac{\mathrm{M2} - \mathrm{M3}}{\mathrm{M2} - \mathrm{M1}} \mathrm{x}\ 100$$

Where:
M1= Mass of the empty container (g)
M2 = Mass of the container plus the sample (g)
M3= Constant mass of the dry sample (g)

Determination of ash in cocoa paste

The ash analysis was carried out in duplicate; the crucibles were then cleaned and conditioned in an oven at 100 °C for 30 minutes. After drying, they were left to rest in a desiccator until they reached room temperature, and their weight was recorded to 0,1 mg.

Approximately 2 g of a homogeneous sample was added to each previously tared crucible, and the mass was verified with the same accuracy. The samples were then incinerated in a muffle furnace at 600 ± 20 °C, maintaining this temperature until uniform ashes free of carbonaceous residues were obtained after approximately 3 hours of combustion. Finally, the crucibles were removed, cooled again in a desiccator, and weighed with a sensitivity of 0,1 mg. Based on the measurements obtained, the ash content was determined by applying the established formula.[21]

Equation 5. Determination of ash

$$\% \text{ Ceniza} = \frac{\text{M3} - \text{M1}}{\text{M2} - \text{M1}} \text{ x } 100$$

Where:
M1: Mass of empty crucible (g)
M2: Mass of crucible plus sample (g)
M3: Mass of crucible with ashes (g)

Determination of protein in cocoa paste

The protein content was determined using the Kjeldahl method, a procedure that quantifies the total nitrogen present in the sample. The technique consists of digesting the sample with sulfuric acid in the presence of a catalyst, commonly mercury or selenium, following the basic methodology used in Vera's research.[22]

Sample preparation:

Grind approximately 100 g of sample in a micro mill fitted with a 1 mm sieve, ensuring that 95 % of the material passes through the mesh.

The previously ground and homogenized samples were immediately transferred to an airtight container and kept until analysis.

The sample was homogenized by repeatedly shaking the container to ensure uniform distribution.

Digestion procedure

Next, approximately 0,3 g of the previously conditioned sample was weighed onto nitrogen-free paper and placed into the micro-digestion tube.

A catalyst tablet was added to the micro-digestion tube along with 5 mL of concentrated sulfuric acid. The digestion tubes containing the respective samples were placed in the digestion block. The fume collector was checked to ensure it was functioning correctly for the equipment's proper operation.

The digester was turned on, and the corresponding caps were placed promptly to ensure the process developed correctly. The digester was turned on and calibrated to 350-400 °C, and the sample was kept in the equipment until clarification, as evidenced by a light green color. The sample was allowed to cool to room temperature. The risk of precipitation was minimized through occasional stirring.

Distiller

15 mL of distilled water was added to each microtube.

The microtube and receiving flask containing 50 mL of a 2 % boric acid solution were inserted into the Kjeltec distillation system.

The system was activated, and then 30 mL of 40 % sodium hydroxide was added, ensuring proper water circulation at all times, which is essential to guarantee the stability of the process and the reliability of the analytical results.

Approximately 200 mL of the distillate obtained was collected, and once the process was complete, the accessories were carefully removed from the system, and the equipment was turned off.

Titration

Three drops of the corresponding indicator were added to the previously collected distillate in the flask to continue the analytical procedure.

The sample was titrated with 0,1 N hydrochloric acid, with a mechanical stirrer to ensure a homogeneous mixture throughout the titration. The volume of acid consumed was recorded.

Calculations

Crude protein content in food was quantified using the formula below.

Equation 6. Determination of protein

$$\% \text{ PB} = \frac{(\text{VCHI} - \text{Vb}) * 1.401 * \text{NHCl} * \text{F}}{\text{g. muestra}}$$

Where:

1,401 = Atomic weight of nitrogen
NHCl = Normality of hydrochloric acid 0,1 N
F= Conversion factor (6,25)
VHCl = Volume of hydrochloric acid consumed in the titration
Vb = Volume of the blank (0,1)

Determination of energy in cocoa paste

The energy variable was determined using a calorimetric bomb, a technique that quantifies the heat of combustion of the samples. This method is based on measuring the temperature increase generated during the complete combustion of the sample pellet in an oxygen-rich environment.

Sample preparation

A compact sample pellet was prepared using a press, weighing 1,0-1,5 g, and then weighed on an analytical balance with a sensitivity of 0,1 mg.

Combustion procedure

The sample was placed in the ignition pump, the system was sealed, and it was pressurized with oxygen to 30 atmospheres. 2000 mL of distilled or demineralized water was placed in the calorimeter cuvette, ensuring that the initial temperature was kept below the ambient temperature where the research was being conducted (laboratory).

The ignition pump was installed in the cuvette, connecting the electrical current conduction electrodes. Next, the calorimeter lid was placed on, and the stirring arm was attached, which remained in operation for 3 min to stabilize the water temperature.

Temperature recording

The initial temperature was recorded, and the pump's electric ignition was activated, initiating the combustion of the sample. During the process, the temperature was monitored every minute until it reached a stabilization point.

Finally, the maximum temperature reached was recorded, the strap and cover of the calorimeter were removed, and the pump was placed on the standard support to cool down.

Post-combustion procedure

The upper valve of the bomb was slowly opened to release the residual gases. Once the internal pressure was eliminated, the cap was removed, and the inside of the bomb was examined, rinsing the residues with distilled water. This rinsing solution was transferred to an Erlenmeyer flask.

Then, 1 mL of 2 % phenolphthalein solution was added, and the solution was titrated with 0,1 N sodium carbonate to quantify the acids generated during ignition, mainly nitric and sulfuric acids.

Calculations

Equation 7. Energy *determination*

$$g = \frac{Tw - e1 - e2 - e3}{m}$$

Where:
Hg= represents the calorific value, expressed in calories per gram (Cal/g).
T= Final temperature – Initial temperature.
W= is the equivalent energy of the calorimeter, with a value of 2410,16.
e1= represents the milliliters consumed of the sodium carbonate solution.
e2= (13,7 X 1,02) weight of the sample.
e3= cm of wire x 2,3.
m= Weight of the sample.

RESULTS

Chemical variables of cocoa paste

The analysis of variance showed contrasting responses of the physicochemical variables to the factors evaluated (variety, concentration of efficient microorganisms, and type of fruit extract), as well as their interactions. For the pH variable, highly significant differences were observed for the EM% factor (p = 0,0004) and the EM%×Extracts% interaction (p = 0,0055), indicating that bean acidification depends on microbial load

and the fruit substrate used. The variety did not show any significant effects (p > 0,05), suggesting similar responses to the treatments between CCN-51 and Nacional. Titratable acidity was significant for EM% (p < 0,0001), Extracts% (p = 0,0078), and the interaction EM%×Extracts% (p = 0,0024).

In addition, the three-way interaction Variety×EM%×Extracts% was significant (p = 0,0276), suggesting that both genotype and the bioactive compounds present in the extracts and the inoculated microbiota modulate organic acid production. In moisture, significant effects were observed for EM% (p = 0,0001) and the EM%×Extracts% interaction (p = 0,0038), along with significance for Variety×Extracts% (p = 0,0214), indicating a combined influence on water retention and loss during fermentation.

No differences in ash content were detected among the main factors; however, the interaction EM%×Extracts% was significant (p < 0,05), indicating a slight alteration in mineral composition associated with mucilage degradation and microbial activity. For protein, the only significant effect was related to variety (p = 0,0001), with protein concentration being more dependent on genotype than on fermentation treatment. In energy content (kcal/g), variety was significant (p = 0,038), while ME% showed a trend (p = 0,1121), with no influence attributable to extracts or interactions.

In general terms, the variables pH, acidity, moisture, and ash were the most sensitive to the combined action of the fermentation factors, while the genetic basis of the material mainly determined protein and energy. The presence of second- and third-order interactions supports the multifactorial nature of the cocoa fermentation process. The coefficients of variation (4–25 %) and the mean standard errors confirmed the experimental precision and reliability of the data. As can be seen in table 4, the chemical variables of cocoa liquor.

Sensory Variables

Color sensory profile

Analysis of the sensory profile of the color parameter revealed marked differences between the treatments corresponding to the CCN-51 (T1–T12) and Nacional (T13–T24) varieties. The scale used (1 = caramel coffee, 2 = brown, 3 = walnut, 4 = carob, 5 = black coffee) allowed the chromatic intensity associated with each experimental unit to be identified.

In treatments T1 to T12 (CCN-51), values predominantly ranged from 3 to 5, indicating shades from walnut to black coffee, with slight variation between treatments with and without fruit extracts or efficient microorganisms. Treatments T3, T4, and T5 showed the highest scores within the variety, suggesting a darker coloration attributable to a more uniform fermentation process or positive interaction between the extracts and the applied microbiota. In contrast, treatments T7, T8, T9, and T10 had slightly lower values, indicating variations in pigmentation possibly associated with differences in polyphenol degradation or differentiated microbial activity.

In treatments T13 to T24 (Nacional variety), the values ranged mainly between 2 and 4, indicating lower color intensity than in CCN-51. Treatments T18, T19, T20, and T21 recorded medium-high levels (≈4), suggesting a greater expression of carob tones in the presence of fruit extracts such as passion fruit and jackfruit. However, treatments T14, T15, and T16 showed lower values in this variety, indicating shades closer to brown or walnut. This differential response may be influenced by the polyphenolic composition of the Nacional genotype, whose response to microbial consortia appears less intense than that of CCN-51.

Compared with CCN-51, CCN-51 showed greater homogeneity and higher color scale values, which can be attributed to greater oxidative activity of phenolic compounds and more consistent fermentation. In contrast, the Nacional variety showed greater dispersion between treatments, suggesting a more dependent interaction between the type of fruit extract and the concentration of efficient microorganisms.

Table 4. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the chemical variables of cocoa paste

Factors			Variable					
Variety	EM%	Extracts%	pH	Acidity	% Moisture	% Ash	% Protein	Energy kcal/g
CCN-51	0	Without extract	5,88	0,87	4,07	3,02	8,55	10,35
CCN-51	0	Banana	6,54	0,58	3,46	2,97	9,05	9,28
CCN-51	0	Jackfruit	6,46	0,60	3,47	3,05	9,71	9,05
CCN-51	0	Passion fruit	6,40	0,61	3,39	3,73	10,3	8,51
CCN-51	40	No extract	5,89	0,86	4,07	3,01	9,03	6,90
CCN-51	40	Banana	6,54	0,59	3,44	2,98	10,58	6,75
CCN-51	40	Jackfruit	6,48	0,62	3,44	3,04	10,5	5,90

CCN-51	40	Passion fruit	6,40		0,58		3,38		3,77		11,19		6,44	
CCN-51	80	No extract	5,89		0,87		4,07		3,01		11,47		6,82	
CCN-51	80	Banana	6,54		0,59		3,45		2,98		10,69		5,98	
CCN-51	80	Jackfruit	6,47		0,61		3,46		3,04		11,95		5,67	
CCN-51	80	Passion fruit	6,40		0,60		3,39		3,75		12,91		5,37	
National	0	No statement	6,16		0,75		3,75		3,03		8,37		7,08	
National	0	Banana	6,17		0,74		3,74		3,03		8,64		7,09	
National	0	Jackfruit	6,16		0,74		3,75		3,03		8,55		7,02	
National	0	Passion fruit	6,49		0,57		3,34		3,06		8,20		6,61	
National	40	No statement	6,49		0,58		3,35		3,05		9,40		6,14	
National	40	Banana	6,48		0,58		3,35		3,05		9,07		6,61	
National	40	Jackfruit	6,27		0,65		3,34		3,15		9,07		6,20	
National	40	Passion fruit	6,28		0,65		3,35		3,15		10,00		5,80	
National	80	No statement	6,30		0,64		3,34		3,15		9,23		6,14	
National	80	Banana	6,53		0,55		3,69		3,41		9,75		5,94	
National	80	Jackfruit	6,51		0,54		3,71		3,40		10,52		6,07	
National	80	Passion fruit	6,52		0,56		3,73		3,43		10,00		5,87	
		DMS	0,86091		0,38125		1,05061		0,93015		3,81649		5,44568	
		EEM ±	0,16		0,07		0,19		0,17		0,70		0,70	
		CV	4,31		18,67		9,36		9,30		12,29		25,39	
		Variety	0,5578	ns	0,2263	ns	0,5396	ns	0,6121	ns	0,0001	**	0,0380	*
		EM%	0,0004	*	<0,0001	**	0,0001	**	<0,0001	**	0,0003	*	0,1121	ns
		Extracts%	0,0388	*	0,0078	*	0,4033	ns	0,0564	ns	0,6772	ns	0,8482	ns
		Variety*EM%	0,1547	ns	0,2908	ns	0,0304	*	0,0827	ns	0,9612	ns	0,9908	ns
		Variety* Extracts%	0,4704	ns	0,2170	ns	0,0214	*	0,3320	ns	0,9999	ns	>0,9999	ns
		EM%* Extracts%	0,0055	*	0,0024	*	0,0038	*	0,1192	ns	0,4679	ns	0,9986	ns
		Variety*EM% *Extracts	0,0354	*	0,0276	*	0,1206	ns	0,3334	ns	>0,9999	ns	>0,9999	ns

These findings indicate that both the genetic material and the technological factors applied (EM% and extracts) directly influence the color expression of the cocoa liquor, with direct implications for the sensory acceptance of the final product and its potential for industrial standardization, as shown in figure 4 of the sensory color note.

Sensory profile appearance

Analysis of the appearance parameter revealed consistent differences between treatments T1–T12 (CCN-51 variety) and T13–T24 (Nacional variety), using the sensory scale:

1 = very shiny, 2 = shiny, 3 = satin, 4 = opaque, and 5 = very opaque. In the treatments corresponding to CCN-51 (T1–T12), scores predominantly ranged from 2 to 4, indicating surfaces with a shiny to satin finish and slight tendencies toward opacity in certain treatments. The lowest values (T8, T9, T10, and T11) indicate a cleaner, more uniform appearance, possibly associated with improved mucilage degradation and greater microbial activity during fermentation. On the other hand, treatments T1, T2, and T4 showed values close to 4, suggesting a slightly more opaque appearance, possibly influenced by the interaction between the fruit extracts and the applied microbial load.

In the Nacional variety (T13–T24), there was a tendency toward higher scores, ranging from 3 to 5, indicating predominantly satin to very opaque surfaces. Treatments T18, T19, T20, and T21 had the highest values, associated with a less shiny appearance, which may be due to lower efficiency in mucilage disintegration or varietal differences in the phenolic and lipid composition of the grain. In contrast, T13, T14, and T15 showed slightly lower values within the variety, revealing a more moderate response to fruit extracts.

A direct comparison between the two varieties shows that CCN-51 has a more uniform appearance and lower levels of opacity than Nacional, which may be related to more homogeneous fermentation and greater ease of mucilage breakdown. In contrast, the Nacional variety showed greater dispersion across treatments, suggesting that the liquor's visual appearance depends more on the type of extract and the level of efficient microorganisms.

Overall, the results indicate that cocoa liquor appearance responds to both genotype and fermentation management, being more favorable in CCN-51, where satiny and shiny surfaces predominate. These findings have direct implications for the visual perception of the final product and its acceptability for high-value-added industrial and sensory applications, as shown in figure 5 of the sensory note on appearance.

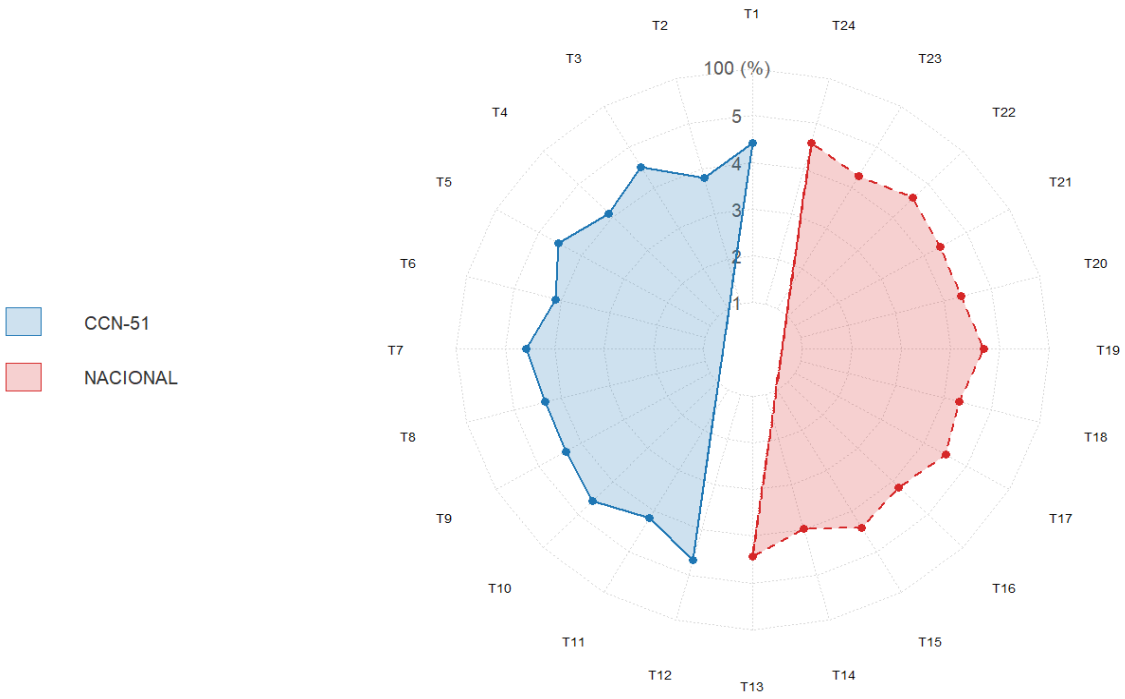

Figure 5. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the sensory profile of the appearance of CCN-51 and Nacional cocoa paste

Sensory profile aroma

Analysis of the aroma profile revealed clear differences between the treatments corresponding to the CCN-51 (T1–T12) and Nacional (T13–T24) varieties, using the sensory scale:

1 = tropical fruit, 2 = cocoa, 3 = citrus fruit, 4 = roasted, and 5 = nutty.

In the CCN-51 variety, treatments T1 to T12 presented values mostly between 2,5 and 4,0, with a tendency towards cocoa, citrus fruit, and slightly toasted notes, indicating a moderate and balanced aromatic expression. Treatments T3, T4, and T5 stood out for their higher values within the group, suggesting that the combination of efficient microorganisms and fruit extracts favored the development of volatile compounds associated with the intermediate phase of fermentation (aldehydes and alcohols that are precursors of toasted aromas). In contrast, treatments T8, T9, and T10 showed lower intensities, associated with less complex profiles or less active fermentation.

In the National variety (T13–T24), there was a greater tendency toward values between 3,5 and 4,5, indicating a predominance of toasted and nutty notes, typical of longer fermentations or greater transformation of aromatic precursors. Treatments T18, T19, T20, and T21 showed the highest scores within the group, reflecting greater aromatic complexity possibly associated with the interaction between residual mucilage, the lipid content characteristic of the genotype, and the fruit extracts applied. Treatments T14, T15, and T17 showed slightly lower values, reflecting intermediate profiles dominated by citrus and cocoa aromas.

A direct comparison between varieties indicates that Nacional developed more intense aromas closer to the "nut" category. At the same time, CCN-51 presented predominantly fruity and cocoa profiles, with less evolution towards deep toasted notes. This behavior suggests a differential response in aromatic potential depending on the bean's genetics and the combined effects of the extracts and efficient microorganisms. As indicated in figure 6 of the sensory aroma note. These results confirm that the interaction between plant

material, applied microbiota, and bioactive compounds in fruits modulates the aromatic expression of cocoa liquor, which is a key criterion for its sensory and industrial value.

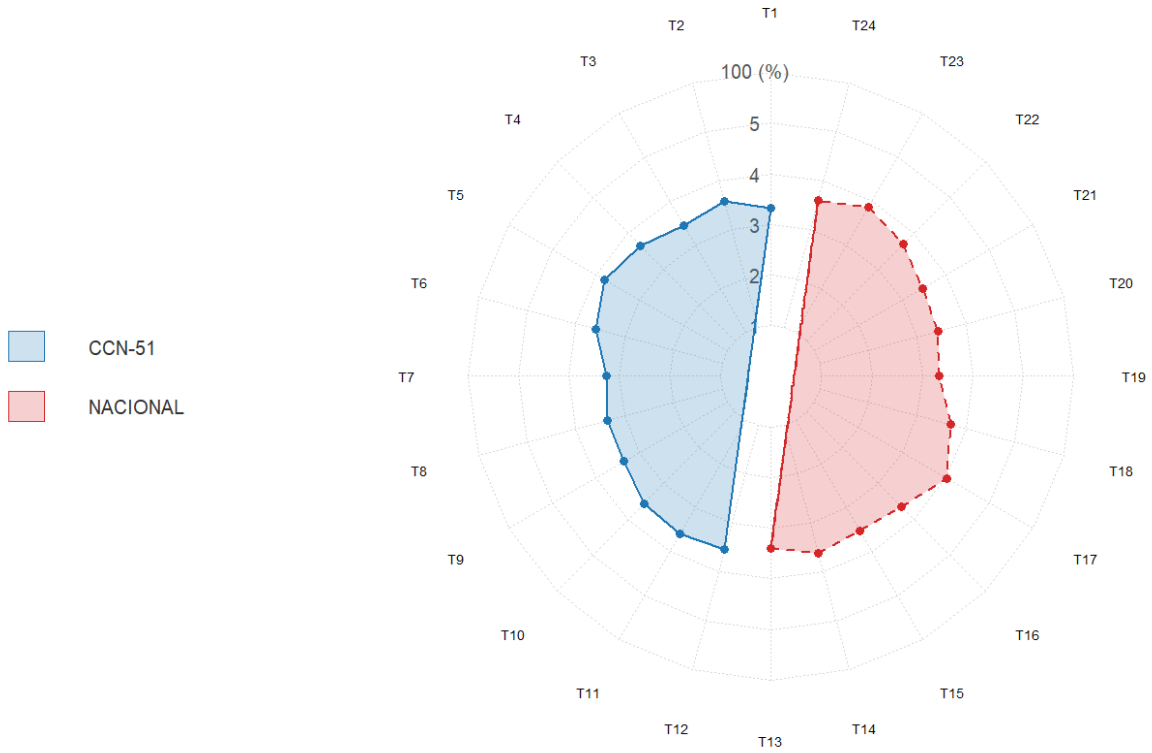

Figure 6. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the sensory aroma profile of CCN-51 and Nacional cocoa paste

Sensory flavor profile

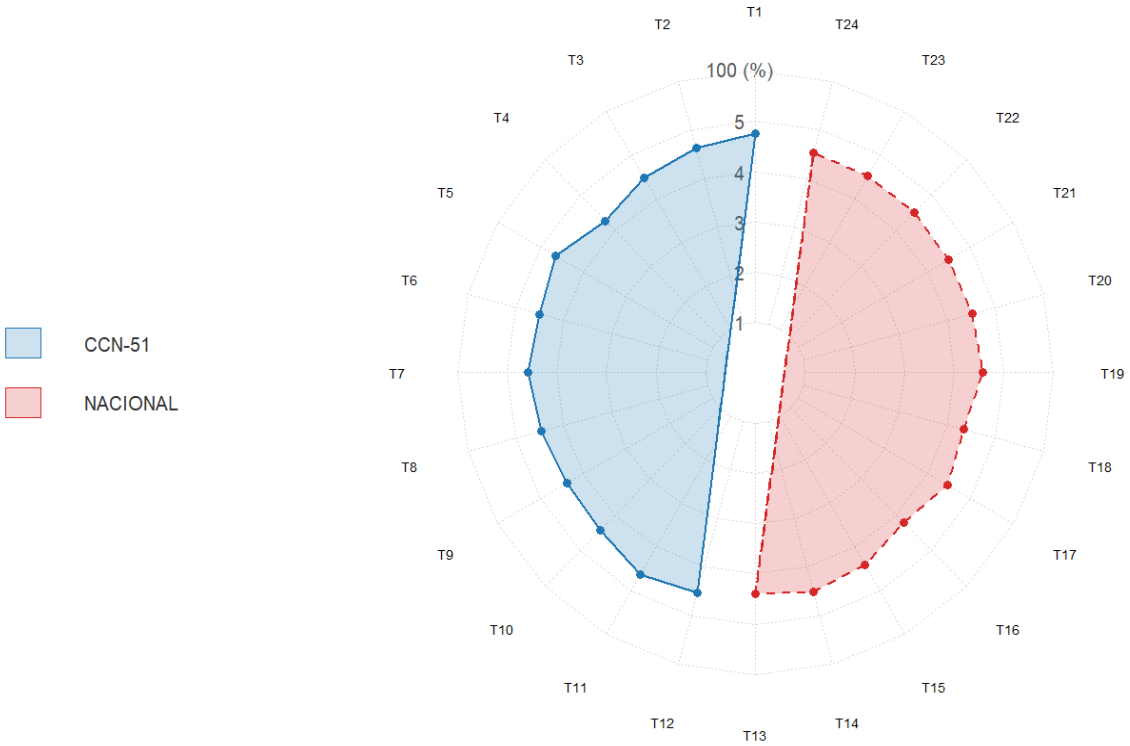

Figure 7. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the sensory flavor profile of CCN-51 and Nacional cocoa paste

The analysis of the flavor parameter showed consistent differences between the treatments evaluated for the CCN-51 (T1–T12) and Nacional (T13–T24) varieties, reflecting the combined effect of genetic material, microbial consortia, and fruit extracts.

In the CCN-51 variety, treatments T1 to T12 showed scores mainly between 2,5 and 4,0, indicating a sensory transition from notes of roasted cocoa and dry fruitiness to moderate bitter nuances. Treatments T3, T4, and T5 achieved the highest scores within the group (close to 4), suggesting an increase in bitter compounds linked to the degradation of alkaloids and phenolic precursors during fermentation. In contrast, treatments such as T8, T9, T10, and T11 showed values closer to 2–3, associated with milder profiles and the presence of residual fruity notes.

In the Nacional variety, treatments T13 to T24 showed a general trend towards higher values, between 3,5 and 4,5, with a predominance of dry, bitter, fruity profiles and even approaching “very bitter” in some cases (e.g., T18, T19, T20, and T21). This indicates a greater expression of phenolic compounds and alkaloids characteristic of this variety, reinforced by microbial activity during fermentation. Treatments T14, T15, and T16 recorded slightly lower values, remaining in a medium range of taste intensity.

The contrast between the two varieties shows that CCN-51 maintains a more balanced, less aggressive sensory profile. At the same time, Nacional tends to express more pronounced, intense flavors, with a shift towards the bitterness characteristic of fine cocoa. These results reflect differences in the chemical composition that precedes flavor, as well as in the fermentative response to efficient microorganisms and fruit extracts, as shown in figure 7 of the sensory flavor note.

Overall, the data confirm that the flavor of cocoa liquor is determined by the interaction between genotype and biofermentative conditions, with the Nacional variety developing more intense sensory attributes associated with cocoa beans with high phenolic concentration and specialty potential.

Sensory profile aftertaste

The evaluation of the aftertaste allowed us to identify clear contrasts between the treatments corresponding to the CCN-51 (T1–T12) and Nacional (T13–T24) varieties, applying the sensory scale:

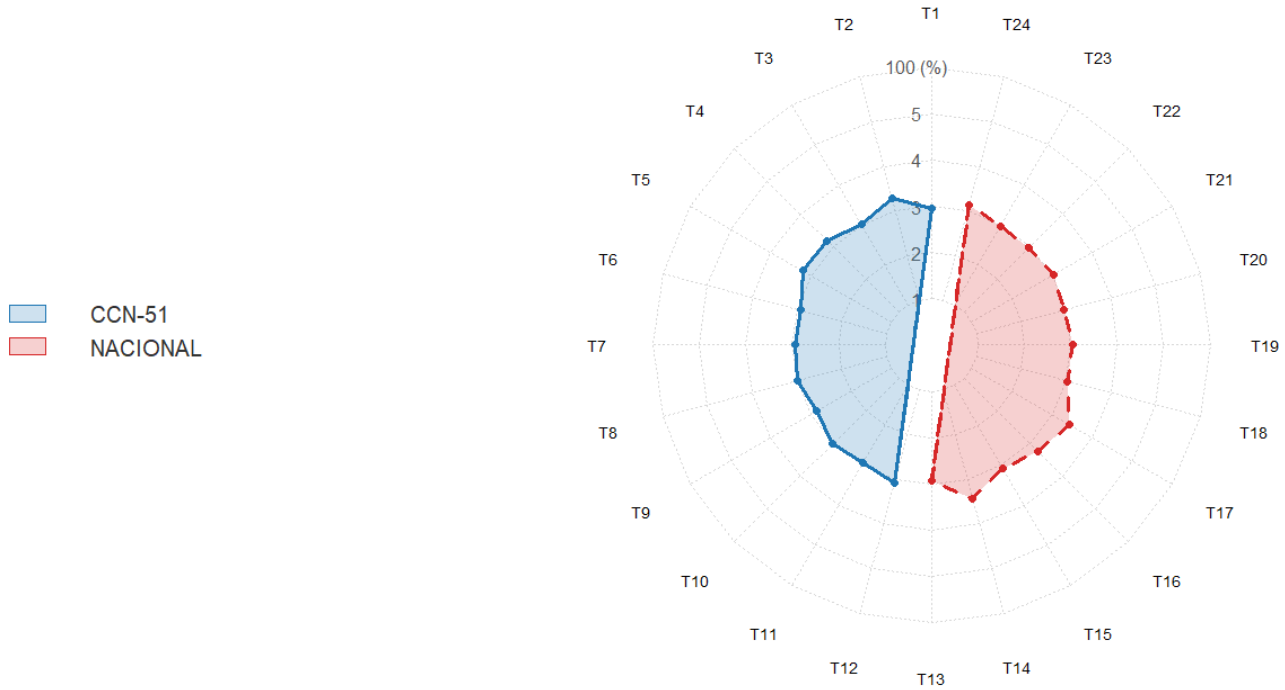

Figure 8. Effect of the interaction of efficient microorganisms through the application of diluted fruit extracts on the sensory profile of the aftertaste of CCN-51 and Nacional cocoa paste

1 = mild persistence, 2 = bitter, 3 = short-lived citrus, 4 = astringent, 5 = grainy sensation.

In the CCN-51 variety, treatments T1 to T12 recorded values mainly between 2,0 and 3,5, with a predominance of moderate bitter perceptions and non-prolonged citrus notes, indicating a short and controlled taste persistence. Treatments T1, T2, and T3 achieved the highest values within the group, close to 3,5, denoting a more pronounced aftertaste possibly associated with partially degraded phenolic compounds. In contrast, treatments T8, T9, T10, and T11 showed values below 2,5, suggesting a smoother, less persistent sensation, likely favored by microbial action in combination with certain fruit extracts.

In the Nacional variety, treatments T13 to T24 showed higher and more consistent values, ranging from 3,0 to 4,2, with a predominance of prolonged citrus-like sensations, moderate astringency, and a slight perception of bitterness in some cases. Treatments T18, T19, T20, and T21 were the most notable for their intensity, indicating a greater accumulation of metabolites derived from the oxidation of polyphenols and alkaloids during fermentation. Treatments T14–T16 showed slightly lower values, within the medium-high range, indicating that even under less intense conditions, the Nacional variety maintains a more persistent aftertaste than CCN-51.

The comparison between the two varieties indicates that CCN-51 has a shorter, less aggressive aftertaste, associated with the gradual reduction of astringent compounds. At the same time, Nacional develops a more intense, prolonged residual sensation, resulting from its chemical composition and its interactions with extracts and efficient microorganisms, as shown in figure 8.

These findings confirm that the aftertaste of cocoa liquor is determined by both genetic material and the applied biofermentative modulation, which is essential for predicting its sensory and industrial acceptance in specialty chocolates.

DISCUSSION

The physicochemical results obtained confirm that cocoa fermentation responds differently to the interactions among the inoculated microbiota, fruit extracts, and genetic material. The significant decrease in pH associated with the increase in EM% and its combination with the extracts shows an intensification of the lactic acid and acetic acid-oxidative pathways. This behavior coincides with that reported by Díaz[23], who demonstrated that selected starter cultures and microbial consortia can direct acid dynamics and the formation of precursor compounds during cocoa fermentation, thereby modifying its acidity and sensory profile. Complementarily, Peña[10] observed that the addition of fruit matrices rich in fermentable sugars alters the availability of energy substrates and regulates the synthesis of organic acids, supporting the modulating role of fruit extracts in microbial metabolic activity evidenced in this study.

Furthermore, Voorde et al.[24] demonstrated that the selection of native yeast strains used as starter cultures in controlled cocoa fermentation significantly influences sugar conversion and the formation of organic acids and volatile compounds responsible for aromatic development. The authors observed that strains of *Saccharomyces cerevisiae and Hanseniaspora opuntiae* promote more homogeneous fermentation and greater aromatic complexity, associated with increased levels of fruit esters and higher alcohols. These findings support the relevance of the microbial control applied in this work, confirming that managing fermentative communities allows metabolic dynamics to be directed and enhances the sensory quality of cocoa liquor.

Titratable acidity showed a clear response to the interaction between factors, reinforcing the hypothesis that extracts provide fermentable compounds that favor the action of yeasts and lactic acid bacteria. Field and laboratory studies have reported increases in acidity and changes in the organic acid profile when complementary substrates or targeted inoculations are used.[25] In addition, recent work emphasizes that the acidogenic response is modulated by the genotype×microbiota interaction, which explains the three-way significance Variety×EM%×Extracts found in our data.[26]

In the moisture variable, the influence of EM% and its interaction with extracts confirms that mucilage degradation depends on both the applied microbiota and the pectinolytic compounds present in fruits such as banana and passion fruit. Chagas[27]. described how fruit matrices rich in pectins and related enzymes facilitate the breakdown of the pectic matrix, accelerating the liquefaction of mucilage and water loss, a mechanism consistent with the treatments that showed the greatest decrease in moisture in this study.

In this context, Rahayu[28] demonstrated that the joint inoculation of yeasts and lactic acid bacteria (*Saccharomyces cerevisiae, Lactiplantibacillus platarum, and Acetobacter pasteurianus)* promotes faster hydrolysis of the mucilage and improves the release of secondary metabolites during cocoa fermentation. The authors observed that metabolic cooperation between yeasts and lactic acid bacteria accelerates pectin degradation and increases the availability of organic acids, alcohols, and reducing sugars, thereby promoting a more homogeneous and efficient fermentation process. This behavior coincides with the results of the present study, in which the combination of efficient microorganisms (EM%) and fruit extracts rich in pectinolytic compounds (banana and passion fruit) facilitated mucilage liquefaction and moisture loss. The synergistic action of these biotas accelerates the transfer of metabolites to the bean, thereby directly influencing the generation of aromatic precursors and the uniformity of the color and flavor of the cocoa liquor.

Complementarily, Morales[29] demonstrated that the incorporation of the enzyme *Pectin Trans-Eliminase* (PTE) during the fermentation of the Nacional and CCN-51 genotypes significantly reduced cadmium levels in the beans without compromising the sensory quality of the cocoa, maintaining an adequate balance between acidity and characteristic cocoa and nut notes. The authors attribute this effect to the modification of the pectin matrix through the breakdown of α-(1→4)-galacturonic bonds, which promotes greater permeability of the mucilage and the release of complexed metals, in addition to optimizing conditions for microbial metabolism. In the context of our study, these results support the hypothesis that biotechnological interventions targeting

the mucilage matrix, either through exogenous enzymes such as PTE or naturally pectin-rich fruit extracts, can influence fermentation dynamics and the generation of chemical aroma precursors. This parallelism suggests that the synergy among inoculated microbiota, fruit extracts, and pectic components not only creates a more efficient fermentation environment but also promotes the formation of volatile metabolites responsible for the fruity, toasty, and nutty notes observed in higher-quality cocoa liquor.

The protein and energy variables responded mainly to the genetic component, consistent with recent chemical characterizations showing intrinsic differences in lipid and protein fractions between genotypes such as CCN-51 and other fine materials. Vera[30] documented variations in fatty acid and phenolic compound profiles among cultivars, reinforcing the interpretation that protein and energy content depend mainly on genetics and indeed also indicates that with respect to ash, the lack of main effects and the exclusive significance in the EM%×Extracts interaction reflects indirect mineral modifications associated with mucilage leaching processes.

From a sensory perspective, the results show that the organoleptic profile can be modulated by integrating biotechnological and genetic factors. Vásquez[31] reported changes in the volatile and sensory profiles of Ecuadorian cocoa beans depending on fermentation practice and genotype, which support our observations: CCN-51 showed a more homogeneous coloration and chromatic intensity, linked to greater polyphenol oxidative activity. In contrast, the Nacional variety showed greater variability across extracts.

Finally, the development of differentiated aromas, flavors, and aftertastes between treatments confirms that fruit extracts and efficient microorganisms act as sensory enhancers. Vásquez[32] has documented that controlled inoculation and supplementation with sugar-rich substrates favor the formation of volatile precursors (aldehydes, alcohols, esters, and pyrazines), which, after roasting, contribute to the fruity, toasted, and nutty notes observed by Vásquez[7]. In similar studies, Korcari[8] showed that inoculation with selected lactic acid bacteria enhances the expression of fruity notes and reduces bitterness, confirming the potential of fruit additives as sensory modulators. Taken together, the data indicate that integrating efficient microorganisms and fruit extracts enables the chemical and sensory development of cocoa liquor to be directed, enhancing attributes that differ by genotype and supporting the application of combined bioprocesses for the production of cocoa with high sensory and technological value.[33]

CONCLUSIONS

In summary, controlled cocoa fermentation via co-application of efficient microorganisms (EM) and natural fruit extracts significantly modulated the fermentative biochemistry and sensory attributes of cocoa liquor, demonstrating that the interaction among microbiota, fruit substrates, and genotype is decisive in generating differentiated attributes. Treatments with 80 % EM combined with passion fruit and banana extracts promoted greater acidification, moisture reduction, and color homogeneity, supported by the intensification of lactic acid and acetic-oxidative pathways. The genetic component mainly influenced protein and energy composition, defining the capacity to accumulate structural metabolites and aromatic precursors, while technological factors determined fermentative transformation and final sensory expression.

From a sensory point of view, CCN-51 showed balanced fruit-cocoa profiles and uniform chromaticity. At the same time, Nacional expressed greater complexity, with toasted notes and persistent aftertastes associated with its phenolic composition and microbial behavior. Finally, the integration of biotechnological strategies, such as targeted inoculation and the use of fruit extracts, constitutes a sustainable way to optimize the chemical and sensory profiles of fermented cocoa, strengthening the production of cocoa with high sensory and technological value aimed at differentiation of origin and specialized markets.

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BIBLIOGRAPHIC REFERENCES

1. Vásquez L, Alvarado K, Intriago F, Raju N, Prasad R. Banana and apple extracts with efficient microorganisms and their effect on cadmium reduction in cocoa beans (Theobroma cacao L.). Discover Food. 2024;4(163):1–13. https://doi.org/10.1007/s44187-024-00205-5

2. Intriago F, Alvarado K, Vera J, Vásquez L, Tigselema S, Verduga C. Post-harvest quality of cacao (Theobroma cacao L.) for the chocolate industry. In: García L, Naga M, Zambrano F, Aguilar D, editors. Sustainable Cacao Cultivation in Latin America. 1st ed. London: Routledge; 2024. p. 270–91. https://www.taylorfrancis.com/chapters/edit/10.4324/9781003381761-17/post-harvest-quality-cacao-theobroma-cacao-chocolate-industry-frank-intriago-flor-kerly-alvarado-vásquez-jaime-vera-chang-luis-vásquez-cortez-solanyi-tigselema-zambrano-cristhian-verduga

3. Vera J, Vásquez L, Alvarado K, Intriago F, Raju M, Radice M. Physical and Organoleptic Evaluation of 12 Cocoa Clones (Theobroma cacao L.) of National Type, in Cocoa Liquor—a Study from Ecuador. Syst Smart Technol Innov Soc. 2024;870:199–211. 10.1007/978-3-031-51982-6_18

4. Campos S, Martínez W, Reis G, Ocán D, Santos G, Rosas F, et al. The role of microbial dynamics, sensorial compounds, and producing regions in cocoa fermentation. Microbiol Res (Pavia). 2025;16(4):1–34. https://www.mdpi.com/2036-7481/16/4/75?utm_source=chatgpt.com

5. Vera J, Benavides J, Vásquez L, Alvarado K, Reyes J, Intriago F, et al. Effects of two fermentative methods on cacao (Theobroma cacao L.) Trinitario, induced with Rhizobium japonicum to reduce cadmium. Rev Colomb Investig Agroindustriales. 2023;10(1):95–106. https://doi.org/10.23850/24220582.5460

6. Vásquez L, Vera J, Erazo C, Intriago F. Induction of Rhizobium japonicum in the fermentative mass of two varieties of cacao (Theobroma Cacao L.) as a strategy for the decrease of cadmium. Int J Health Sci (Qassim). 2022;3(April):11354–71. https://sciencescholar.us/journal/index.php/ijhs/article/view/8672/5762

7. Vásquez L, Montoya E, Cadenas L, Plua J, Camacho C, Cobos F, et al. Evaluación físisco química y sensorial del cacao (Theobroma cacao L.) CCN51 fermentado con Saccharomyces cerevisiae y extracto de fruta (Mango manila) en diferentes grados de madurez. Nutr Clin y Diet Hosp. 2025;45(3):117–28.

8. Korcari D, Fanton A, Ricci G, Rabitti N, Laureati M, Hogenboom J, et al. Fine cocoa fermentation with selected lactic acid bacteria : composition and sensory properties. Foods. 2023;12(1):1–15. https://www.mdpi.com/2304-8158/12/2/340

9. Cedeño J, Parraga C, Intriago F. Induction of effective microorganisms (EM) in the fermenting mass of cacao (Theobroma cacao L.) and their impact on physicochemical and antioxidant characteristics. Biotecnia. 2023;26(e2422):1–8. https://doi.org/10.18633/biotecnia.v26.2422

10. Peña M, Ortiz J, Santander F, Lazo M, Caroca R. Physicochemical changes during controlled laboratory fermentation of cocoa (CCN-51) with the inclusion of fruits and on-farm inoculation. Brazilian J Food Technol. 2023;26:1–9. https://www.scielo.br/j/bjft/a/yc7QZvLwpxx8JZS639ycszH/?lang=en

11. Vera F, Vera J. Resumen de principios de diseños experimentales. 1st ed. Compás G, editor. Guayaquil Ecuador; 2018. 102 p. https://repositorio.uteq.edu.ec/handle/43000/3764

12. Vásquez L, Erazo C, Vera J, Intriago F. Induction of Rhizobium japonicum in the fermentative mass of two varieties of cacao (Theobroma cacao L .) as a strategy for the decrease of cadmium. Int J od Heal Sci. 2022;6(3):11354–71. 10.53730/ijhs.v6nS3.8672

13. Torres A, Vera J, Vásquez L, Alvarado K, Intriago F. Extraction of Cocoa Powder for the Preparation of a Drink by Adding Mucilage and Guava. Sarhad J Agric. 2023;39(2):10–8. https://dx.doi.org/10.17582/journal.sja/2023/39/s2.10.18

14. Vera J, Vásquez L, Alvarado K, Intriago F, Raju M, Radice M. Physical and organoleptic evaluation of 12 cocoa clones (Theobroma cacao L .) of National type , in Cocoa liquor — a study from Ecuador. Syst Smart Technol Innov Soc. 2024;870:8–15.

15. Intriago F, Cedeño J, Parraga C, Alvarado K, Vásquez L, Revilla K, et al. Induction of effective microorganisms (EM) in the fermenting mass of cacao (Theobroma cacao L.) and their impact on physicochemical and antioxidant characteristics. Biotecnia. 2024;26(2422):1–8. https://doi.org/10.18633/biotecnia.v26.2422

16. Morales W, Morante J, Herrera R, Ayuso M, Bernalte M. Efecto de la adición de levaduras y enzimas durante la fermentación sobre la calidad fisicoquímica de los granos de cacao fino de aroma. J Agric Food Res. 2024;16(101126):1–43.

17. Viera W, Shinohara T, Samaniego I, Terada N, Sanada A, Ron L, et al. Pulp Mineral Content of Passion Fruit Germplasm Grown in Ecuador and Its Relationship with Fruit Quality Traits. Plants. 2022;11(5):1–19. https://www.mdpi.com/2223-7747/11/5/697#B93-plants-11-00697

18. Kinetics E, Polyphenol OF, From O, Michel G, Different T, Stages M. Cinética enzimática de la polifenol oxidasa del banano gros michel en diferentes estados de maduración. Rev La Fac Química Farmecéutica. 2006;13(2):13–9. https://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0121-40042006000200002

19. Vásquez L, Durazno L, Rivadeneira C, Vera J, Arboleda L, Intriago F. Utilización De extracto de Jackfruit (Artocarpus heterophyllus) como estategia para mejorar la calidad del grano de cacao. Rev Científica Multidiscip InvestiGo. 2023;4(8):95–117. https://www.revistainvestigo.com/EditorInvestigo/index.php/hm/article/view/67

20. Vera J, Vasquez L, Zapata K, Cevallos R. Caracterización morfológica , fisicoquímica y microbiológica del cacao Macambo (Theobroma bicolor Humb & Bonpl .) en Ecuador. Rev Agrotecnología Amaz. 2024;4(2):1–17. https://doi.org/10.51252/raa.v4i2.657

21. Thairy L. Estudio de las características organolépticas del chocolate influenciado por el efecto de diferentes agentes fermentadores (Microorganismos). Universidad de las Fuerzas Armadas; 2024. https://repositorio.espe.edu.ec/items/9120e2fa-d653-4ba8-8c4f-e77e358f3b85

22. Vera J, Radice M, Vásquez L, Intriago F. Perfil químico de 12 clones tipo nacioal de pasta de cacao (Theobroma cacao L.). Univ y Soc. 2024;16(1):126–36. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2218-36202024000100126

23. Díaz C, Luc V. Functional yeast starter cultures for cocoa fermentation. J Appl Microbiol. 2022;133(1):39–66. https://academic.oup.com/jambio/article/133/1/39/6989019

24. Voorde D, Díaz C, Hernandez C, Weckx S, Vuyst L. Yeast strains do have an impact on the production of cured cocoa beans, as assessed with Costa Rican Trinitario cocoa fermentation processes and chocolates thereof. Front Microbiol. 2023;14:1–27.

25. Herrera F, Cala M, Aguirre J, Rodríguez C, Chica M, Olarte H, et al. Dissecting fine-flavor cocoa bean fermentation through metabolomics analysis to break down the current metabolic paradigm. Sci Rep. 2021;11(1):1–18. https://www.nature.com/articles/s41598-021-01427-8

26. Vásquez L, Cevallos C, Uvidia M, Segobia S, Plua J. Evaluación de métodos fermentativos de cacao Nacional y CCN-51, induciendo microorganismos en yute y cajas Rohan para mejora organoleptico. Rev Luna Azul. 2025;(60):168–81.

27. Chagas R, Andrade S, Denadai M, Nunes M, Narain N. Evaluation of bioactive compounds potential and antioxidant activity in some Brazilian exotic fruit residues. Food Res Int. 2017;102:84–92.

28. Rahayu E, Triyadi R, Khusna R, Djaafar T, Utami T, Marwati T, et al. Indigenous yeast, lactic acid bacteria, and acetic acid bacteria from cocoa bean fermentation in indonesia can inhibit fungal-growth-producing mycotoxins. Fermentation. 2021;7(192):1–11.

29. Morales W, Morante J, Carranza M, Ormaza D, Concepción M, Bernalte M. Fermentation with pectin trans-eliminase to reduce cadmium levels in nacional and CCN-51 cocoa bean genotypes. Plants. 2025;14(16):1–23. https://www.mdpi.com/2223-7747/14/16/2553

30. Vera J, Radice M, Vásquez L, Intriago F. Peril químico de 12 clones tipo Nacional de pasta de cacao (Theobroma cacao L.). Univeridad Y Soc. 2024;16(1):126–36.

31. Vásquez L, Rodríguez S, Cadena L, PLua J, Camacho C, Cobos F, et al. Aroma, Ciencia y Tradición: Nuevas fronteras en la fermentación del cacao. 1st ed. Rodríguez S, Rojas J, editors. Ediciones GESICAP.; 2025. 1–126 p. https://edicionesgesicap.com/index.php/gesicap/catalog/book/47

32. Vasquez L, Alvarado K, Intriago F, Vera J, Raju N, Prasad R. Banana and apple extracts with efficient

microorganisms and their effect on cadmium reduction in cocoa beans (Theobroma cacao L.). Discov Food. 2024;4(1):1–13. https://link.springer.com/article/10.1007/s44187-024-00205-5

33. Intriago F, Vera J, Vásquez L, Alvarado K. Inducción anaérobica de Bradyrhizobium japonicum en la postcosecha de híbridos experimentales de cacao y su mejoramiento en la calidad fermentativa. J Sci Res UTB. 2022;7(2):19–23.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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