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APLICACIÓN DE *Saccharomyces cerevisiae* Y EXTRACTOS FRUTALES EN LA POSCOSECHA DEL CACAO (*Theobroma Cacao* L.) COMO ESTRATEGIA PARA MEJORAR LA CALIDAD DEL GRANO Y EL PERFIL SENSORIAL DE LA PASTA DE CACAO

APPLICATION OF *Saccharomyces cerevisiae* AND FRUIT EXTRACTS IN THE POST-HARVEST OF COCOA (*Theobroma Cacao* L.) AS A STRATEGY TO IMPROVE BEAN QUALITY AND THE SENSORY PROFILE OF COCOA PASTE

Luis Humberto Vásquez Cortez[1], Sanyi Lorena Rodríguez Cevallos[2], José Francisco Falconi Novillo[3]

{lvasquezc8@unemi.edu.ec[1], srodriguezc17@unemi.edu.ec[2], jfalconin@unemi.edu.ec[3]}

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RESUMEN: La fermentación constituye una etapa determinante para el desarrollo de la calidad del cacao, por lo que el empleo de cultivos iniciadores y matrices frutales representa una alternativa para mejorar el control del proceso postcosecha. El objetivo de esta investigación fue evaluar el efecto de *Saccharomyces cerevisiae* y extractos frutales durante la fermentación del cacao (*Theobroma cacao* L.) sobre la calidad fisicoquímica, fermentativa y sensorial del grano y de la pasta obtenida. Se evaluaron nueve tratamientos resultantes de la combinación de tres niveles de *S.c erevisiea* (0, 0,05 y 1 %) y tres conficiones de extracto (sin extracto, papaya y guanabana). La fermentación se desarrolló durante cinco días en cajas de microfermentación tipo Rohan, con seguimiento de temperatura, pH y sólidos solubles. Posteriormente, se determinaron las características físicas y la calidad fermentativo y de calidad. La combinación de una cantidad del 1 % de *S. cerevisiae* combinado con el extracto de papaya alcanzó un valor de 92 % de granos fermentados; 7 % de granos violetas y 6,13 % de humedad, lo cual favoreció el comportamiento tecnológico más correcto entre los tratamientos propuestos. Asimismo, el tipo de extracto ejerció un efecto significativo sobre el pH, acidez, humedad y cenizas de la pasta, y la modalidad de los tratamientos generó diferentes perfiles sensoriales como resultado de los mismos. Por tanto, se llega a la conclusión de que *S. cerevisiae* y los extractos de fruta pueden ser usados para modular la fermentación y las características de calidad del cacao, destacando el extracto de papaya como el más

[1]Universidad Estatal de Milagro, Facultad de Posgrado, Escuela de Ingeniería, Maestría en Ciencias y Tecnología de los alimentos, https://orcid.org/0000-0003-1850-0217

[2]Universidad Estatal de Milagro, Facultad de Posgrado, Escuela de Ingeniería, Maestría en Ciencias y Tecnología de los alimentos, https://orcid.org/0009-0003-4684-9587

[3]Universidad Estatal de Milagro, Facultad de Posgrado, Escuela de Ingeniería, Maestría en Ciencias y Tecnología de los alimentos, https://orcid.org/0000-0003-2623-115X

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interesante debido a su capacidad para regular las fermentaciones y las condiciones postcosecha.

Palabras clave: Saccharomyces cerevisiae; Theobroma cacao; fermentación; extractos frutales; calidad postcosecha; análisis sensorial.

ABSTRACT: Fermentation is a critical stage in the development of cocoa quality; therefore, the use of starter cultures and fruit-based matrices represents an alternative approach to improve the control of postharvest processing. This study aimed to evaluate the effect of *Saccharomyces cerevisiae* and fruit extracts during cocoa (*Theobroma cacao* L.) fermentation on the physicochemical, fermentative, and sensory quality of the beans and the resulting cocoa paste. Nine treatments were evaluated, resulting from the combination of three levels of *S. cerevisiae* (0, 0.5, and 1%) and three extract conditions (no extract, papaya, and soursop). Fermentation was conducted for five days in Rohan-type microfermentation boxes, with temperature, pH, and total soluble solids monitored throughout the process. Subsequently, the physical characteristics and fermentative quality of the beans, the physicochemical properties of the cocoa paste, and its sensory profile were evaluated. The combination of 1% S. cerevisiae mixed with papaya extract resulted in 92% fermented grains, 7% violet grains, and 6.13% moisture content, which yielded the best technological performance among the proposed treatments. Likewise, the type of extract had a significant effect on the paste's pH, acidity, moisture content, and ash content, and the treatment conditions resulted in different sensory profiles. Therefore, it is concluded that S. cerevisiae and fruit extracts can be used to modulate fermentation and the quality characteristics of cocoa, with papaya extract standing out as the most promising due to its ability to regulate fermentation and post-harvest conditions.

Keywords: Saccharomyces cerevisiae; Theobroma cacao; fermentation; fruit extracts; postharvest quality; sensory analysis

INTRODUCTION

Cocoa (Theobroma cacao L.) constitutes one of the tropical crops of greatest economic, social and environmental importance worldwide, because it represents the livelihood of millions of small producers and supplies a chocolate industry whose demand continues to increase, especially in the segment of fine and original chocolates. In this context, the quality of cocoa has acquired strategic relevance, since it determines the competitiveness of the product in specialized markets and the added value of the agroindustrial chain. Various studies agree that the final quality of cocoa does not depend only on the genetic material or cultivation conditions, but also on the proper management of post-harvest operations, with fermentation being the process with the greatest influence on the development of the physicochemical, biochemical and sensory characteristics of the grain. During this stage, the main precursors of the aroma and flavor are generated, which are subsequently developed during roasting through Maillard reactions, determining attributes such as the aroma, flavor, color and quality of the chocolate.(1)(2)(3)(4)

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Cocoa fermentation corresponds to a highly dynamic microbiological ecosystem in which yeasts, lactic acid bacteria and acetic acid bacteria participate sequentially. During the first 24 to 48 hours, yeasts predominate due to the high availability of sugars present in the mucilage and the initial conditions of low oxygen availability. Subsequently, the production of ethanol and the increase in aeration favor the establishment of acetic acid bacteria, responsible for the oxidation of ethanol to acetic acid and the increase in the temperature of the fermentative mass to values close to 45–50 °C. These changes lead to the death of the embryo and provoke a series of enzymatic reactions that favor the hydrolysis of proteins, carbohydrates and polyphenols into free amino acids, peptides and reducing sugars that are the main precursors of the aromatic profile of chocolate.(5)(6)(7)

However, in most producing countries, fermentation continues to be carried out using traditional and spontaneous methods, the efficiency of which depends on the native microbiota, environmental conditions and the management applied by producers. This high variability generates significant differences in the fermentation dynamics and in the physicochemical and sensory quality of the almonds, limiting the standardization of the process and the obtaining of grains with homogeneous characteristics. In response to this problem, in recent years directed fermentation using starter cultures has acquired growing interest as a biotechnological strategy to control microbial succession, optimize the formation of aromatic compounds and improve the quality of fermented cocoa.(8)(9)(10)(11)

In response to the need to increase the reproducibility of fermentation and improve the quality of cocoa, the use of starter cultures has acquired a relevant role within the biotechnological strategies applied to postharvest. Among the microorganisms evaluated, Saccharomyces cerevisiae has demonstrated high potential due to its rapid adaptation to mucilage conditions, its efficient sugar metabolism and its ability to produce ethanol, higher alcohols, esters and other volatile compounds that directly participate in the formation of the aromatic profile of cocoa. In addition to promoting alcoholic fermentation, this yeast presents pectinolytic activity that contributes to the degradation of mucilage, facilitating the aeration of the fermentative mass and promoting the establishment of acetic acid bacteria, responsible for the production of acetic acid and the increase in the temperature necessary to induce the internal biochemical transformations of the almond.(12)(13)(14)

Simultaneously, the search for sustainable alternatives to improve fermentation has led to the study of plant matrices that have a high content of nutrients with properties that are capable of stimulating microbial metabolism. In this context, tropical fruit extracts represent a promising alternative due to their content of fermentable sugars, vitamins, minerals, organic acids and bioactive compounds, which could favor the growth of beneficial microorganisms and modify the physicochemical conditions of the fermentation process. Among these fruits, papaya (Carica papaya L.) stands out for its high glucose, fructose and sucrose content, in addition to the presence of papain, a proteolytic enzyme widely recognized for its ability to hydrolyze proteins and generate peptides and amino acids that can potentially increase the availability of aromatic precursors during cocoa processing. On the other hand, soursop (Annona muricata L.)

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contains carbohydrates, vitamin C, organic acids and phenolic compounds with antioxidant activity, characteristics that could contribute to maintaining a favorable environment for the development of the fermentative microbiota and favor the stability of the process.(4)(12)(15)(16)

Despite the growing interest in the directed fermentation of cocoa, most of the research has focused on the selection and evaluation of starter cultures, particularly yeasts and lactic acid or acetic acid bacteria, while the incorporation of fruit extracts as natural sources of nutrients during fermentation has been barely studied. Since the review of the recent scientific literature, no work has been carried out that has evaluated the combined effect of Saccharomyces cerevisiae with papaya and soursop extracts in the post-harvest fermentation of cocoa, which reveals a gap in knowledge and the development of innovative biotechnologies that seek to improve the physicochemical quality and sensory profile of cocoa and its derivatives.(5)(12)

The development of biotechnological strategies aimed at optimizing cocoa fermentation represents an opportunity to increase the quality of the bean and strengthen the competitiveness of the agro-industrial chain, especially in producing countries where traditional post-harvest systems predominate. The application of starter cultures in combination with natural sources of nutrients constitutes an innovative approach that could favor the microbiological stability of the process, promote a more uniform fermentation and improve the formation of aroma precursor compounds. Likewise, the use of tropical fruits such as papaya and soursop is framed within the principles of the bioeconomy and the circular economy, by encouraging the sustainable use of locally available plant resources with the potential to generate added value in cocoa production.(17)(18)(19)

From a scientific point of view, the combination of Saccharomyces cerevisiae with extracts of papaya (Carica papaya L.) and soursop (Annona muricata L.) poses a novel strategy based on the possible synergistic interaction between a highly efficient starter culture and natural matrices rich in fermentable sugars, vitamins, minerals and bioactive compounds. It is proposed that these extracts could favor the establishment and metabolic activity of yeast during the initial stages of fermentation, promoting greater ethanol production, facilitating the subsequent activity of acetic acid bacteria and favoring the biochemical transformations responsible for the formation of amino acids, peptides and reducing sugars, considered the main precursors of the aroma and flavor of chocolate. However, the existing scientific evidence on this combination remains scarce, so it makes sense to generate experimental information that allows establishing its effect on the physicochemical, fermentative and sensory quality of cocoa.(20)

The research presented below was carried out in order to offer scientific evidence on a new biotechnological strategy for improving cocoa postharvest, which has contributed, in this way, to advances in the improvement of fermentation processes that are more efficient, sustainable and reproducible. The results obtained may serve as a basis for future research aimed at optimizing fermentation through the use of starter cultures and natural resources, as well as for the design of technologies applicable to the cocoa industry and producers seeking to increase the quality and added value of their products. Consequently, the present study aimed to evaluate the effect of the

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application of Saccharomyces cerevisiae and fruit extracts during the postharvest fermentation of cocoa (Theobroma cacao L.) on the physicochemical, fermentative and sensory quality of the bean, as well as on the sensory profile of the cocoa paste obtained.

MATERIALS AND METHODS

Location

The cocoa pods (Theobroma cacao L.) used in the present investigation, as well as the fruits intended for the preparation of the extracts, corresponding to papaya (Carica papaya L.) and soursop (Annona muricata L.), were collected in the El Naranjo facility, Bajo Perdido sector, Mocache canton, province from Los Ríos, Ecuador (1°11'30.7" S; 79°35'43.6" W). Both the ears and the fruits were selected in an optimal state of physiological maturity, free of mechanical damage, diseases, insect attacks and other alterations that could affect the quality of the raw material or the development of the experimental process.

The post-harvest fermentation of the cocoa kernels was carried out according to the established experimental design, while the production of the cocoa paste was carried out at the Agroindustry Bromatology Plant of the Technical University of Babahoyo (UTB). Subsequently, the physicochemical determinations of the fermented almonds and cocoa paste, including pH, titratable acidity (%), humidity (%) and ash (%), were carried out in the Agroindustry Laboratory of the Technical University of Babahoyo, using standardized analytical methodologies for the analysis of products derived from cocoa.

Experimental design

The research was carried out using an experimental approach aimed at determining the effect of the incorporation of Saccharomyces cerevisiae and fruit extracts on the postharvest fermentation of cocoa (Theobroma cacao L.). The study contemplated the controlled manipulation of the independent variables with the purpose of establishing their influence on the fermentative dynamics, the physicochemical and fermentative properties of the almonds, and the quality of the cocoa paste obtained at the end of the process of the cocoa paste obtained.

The experimental trials were carried out under controlled conditions at the Agroindustry Bromatology Plant, where the working conditions were standardized to ensure the uniformity of the experimental process, favor the reproducibility of the results and minimize the influence of external factors. As raw material, fresh cocoa kernels were used, obtained from pods harvested in an optimal state of physiological maturity, free of mechanical damage, diseases, insect attacks and other defects that could compromise the normal development of fermentation.

The experiment was established using a completely randomized design (DCA) with a 3 × 3 factorial arrangement. The first factor corresponded to the concentration of Saccharomyces cerevisiae applied during fermentation (0, 0.5% "5 g" and 1.0% "10 g"),

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while the second factor was represented by the type of fruit extract (without extract, papaya extract (Carica papaya L.) and soursop extract (Annona muricata L.). The combination of both factors gave rise to nine experimental treatments, each with three repetitions, making up a total of 27 experimental units. The assignment of the treatments was carried out completely randomly, as seen in Table 1.

During the fermentation, the main variables of the process were continuously monitored, including the internal temperature of the fermentative mass, the pH and the total soluble solids. (°Brix), using previously calibrated equipment. Fermentation took place under the environmental conditions of the study area, with temperatures between 25 and 30 °C and relative humidity characteristic of the humid tropical climate.

Data collection was carried out longitudinally throughout the fermentation process, through periodic monitoring of the physicochemical and fermentative variables. Once the fermentation was completed and the product was prepared, a cross-sectional evaluation was carried out to determine the degree of fermentation of the almonds and the sensory characteristics of the cocoa paste, allowing the response of the different experimental treatments to be compared.

Table 1. Scheme of Study Factors.

Factor A *Saccharomyces cerevisiae (gr)*	**Factor B (Fruit extract)**
0%	Without fruit extract
0.5%	Papaya (Carica papaya L.)
1%	Soursop (Annona muricata L.).

ANOVA

In table number 2 you can find the ANOVA, where the treatments, the measured factors and the interactions are found, which offers a statistical basis to interpret the main effects and the combined effects of the experimental model.

Table 2. Analysis of variance (ANOVA) diagram.

Source variation (VS)			**Degrees of freedom (DOF)**
Treatment		axb-1	8[#]
Repetitions		r-1	2
Factor	Saccharomyces cerevisiae	(LE-1)	2

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Factor Extract	(Ex-1)	2
Yeast x Extract Factor	(LE-1)(EX-1)	4
Experimental Error	(LE x EX) (r-1)	18
Total	LE x EX r-1	27

Treatment arrangement

Table 3 presents the organization of the 9 treatments established in the experimental design, each one identified by a specified code according to the factors considered in the study. This nomenclature allowed us to clearly differentiate the combinations evaluated, maintain adequate control of the samples during fermentation and simplify the recording of information. Likewise, it contributed to reliably relating each experimental unit with the data generated, favoring the subsequent interpretation and comparison of the results.

Table 3. Treatment codes and study variables.

No.	Code	*Saccharomyces cerevisiae*	Fruit Extract
1	Sc0Ex0	0	Without fruit extract
2	Sc0Ex1	0	Papaya
3	Sc0Ex2	0	Soursop
4	Sc1Ex0	0.5	Without fruit extract
5	Sc1Ex1	0.5	Papaya
6	Sc1Ex2	0.5	Soursop
7	Sc2Ex0	1	Without fruit extract
8	Sc2Ex1	1	Papaya
9	Sc2Ex2	1	Soursop

Fermentation process

Fermentation was carried out in Rohan-type wooden microfermentation boxes (100 x 100 x 15 cm) that were assigned to carry out the experimental study factors; Each box had 27 compartments (1 kg per unit), which were used as experimental units. Cocoa beans, before fermentation, were inoculated with Saccharomyces cerevisiae and then fruit extracts were added inside low-density polypropylene covers for 24 hours. The casings were then removed and fermentation was carried out under aerobic conditions for five days. Manual turning was carried out daily to ensure homogenization of the dough and its oxygenation. The control physicochemical parameters (temperature, pH and soluble solids) were monitored daily. In total, 27 kg of fresh cocoa beans were processed. The amount of perishable grains was similar for both varieties.(21)(22)

Inocula (Saccharomyces cerevisiae)

Cocoa fermentation was carried out by applying active dry yeast of Saccharomyces cerevisiae as a starter culture. Three application levels were evaluated: (Control treatment) 0%, 0.5% (5 g) and 1% (10 g) per experimental unit, corresponding to 1000 g of fresh cocoa beans, in accordance with the established experimental design.(12)

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Prior to inoculation, the active dry yeast was rehydrated in 50 mL of sterile distilled water, previously conditioned at a temperature of 35 – 38 °C, for 10 minutes. Subsequently, the suspension obtained was incorporated uniformly into the fresh cocoa mass and mixed manually until a homogeneous distribution of the inoculum was achieved throughout the fermentation mass.(17)

Preparation of diluted fruit extracts

The diluted extracts of papaya (Carica papaya L.) and soursop (Annona muricata L.) were prepared following the experimental procedure described in the corresponding flow chart (Figure 1), with modifications adapted to the characteristics of each fruit. To guarantee the uniformity of the treatments, fruits were used in an optimal state of physiological maturity, free of mechanical damage, rot, microbiological contamination and visible alterations that could affect the composition of the extract or the development of fermentation.

Prior to obtaining the extracts, the fruits were washed with drinking water to eliminate surface impurities and subsequently disinfected by immersion in a sodium hypochlorite solution at 100 mg L^{-1} for 2 min. They were then rinsed with sterile distilled water and allowed to drain at room temperature.

In the case of papaya (Carica papaya L.), the peel and seeds were manually removed, obtaining only the edible pulp. Subsequently, 150 g of fresh pulp was weighed and mixed with 200 mL of sterile distilled water. The mixture was homogenized for 2 min using a laboratory blender until obtaining a uniform extract.(11)

For soursop (Annona muricata L.), the peel and seeds were removed, keeping only the pulp. Similarly, 150 g of fresh pulp were weighed, which were mixed with 200 mL of sterile distilled water and homogenized for 2 min using a laboratory blender, obtaining a homogeneous extract.

The extracts of both fruits had an approximate final mass of 350 g, corresponding to a fruit concentration of 42.86% (w/w), calculated using the following equation:

$$\% \text{ de fruta} = \frac{\text{Pulpa (g)}}{\text{Pulpa (g)} + \text{Agua (mL)}} \text{x}100$$

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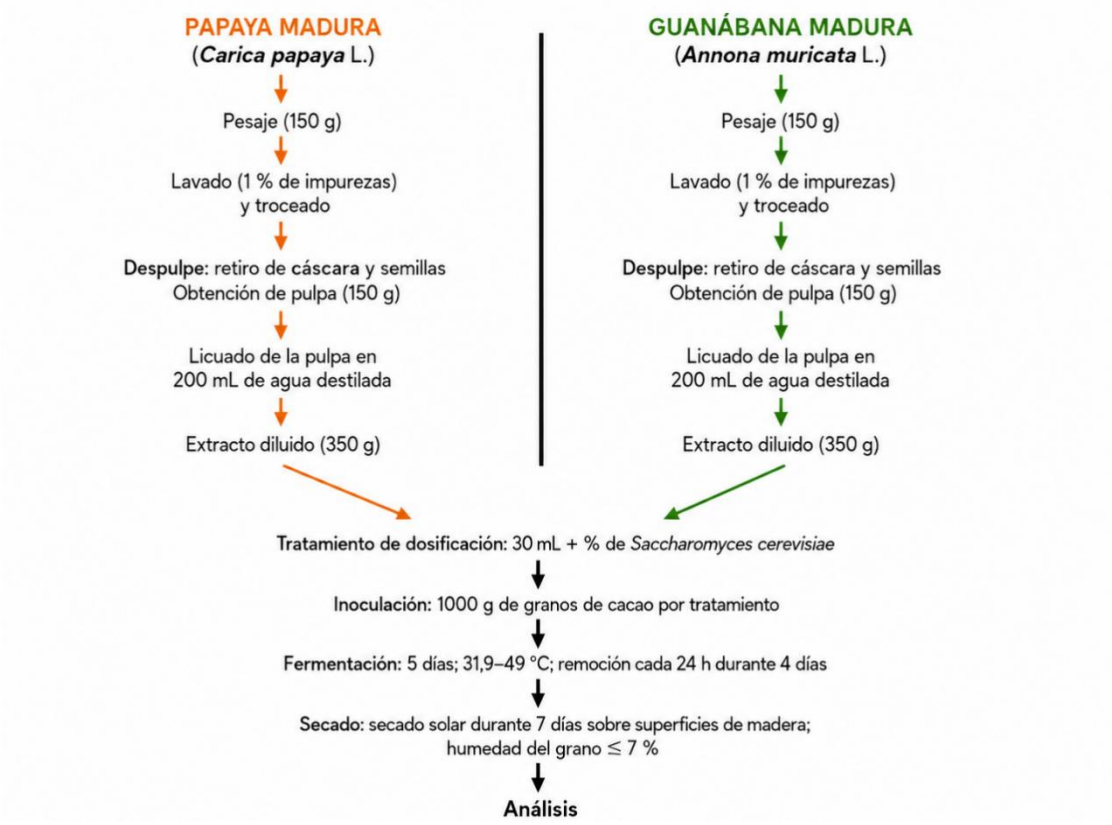


Figure 1. Diagram of the process for obtaining diluted fruit extracts.

Physicochemical variables of fermented and dried cocoa beans

pH analysis

The determination of pH was carried out using a standardized protocol. 10 g of finally ground cocoa beans were weighed, which were homogenized with 10 mL of distilled water previously heated to 40 °C. The mixture was prepared in glass containers and allowed to stabilize before measurement. The pH was determined with a digital meter (Thermo Orion Waterproof, USA), with buffer solutions of pH 4.0 and 7.0, according to the manufacturer's instructions. The determination was carried out in duplicate for each treatment.(11)

Determination of total soluble solids (°Brix)

The content of total soluble solids (°Brix) was determined by digital reflectometry. To do this, 10 g of cocoa beans were randomly selected, which were finally ground and homogenized with 10 mL of distilled water previously heated to warm temperature. Subsequently, the suspension was filtered to obtain a particle-free extract, which was used for analysis. Determinations were carried out using a digital refractometer (OPTI + 38-A2, Brix 0-95; Bellingham and Stanley, United Kingdom). It was previously calibrated for distilled water (0.00 °Brix) following the manufacturer's guidelines before each measurement. 1-3 drops of extract were placed on the surface of the prism for each sample, and readings were performed in triplicate.(23)

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Temperature determination

The temperature of the fermentative mass was recorded throughout the process using a previously calibrated digital thermometer. The continuous monitoring of this variable made it possible to evaluate the evolution of fermentation and verify that the values achieved were within the range of 45-50 °C, considered appropriate to favor the activity of fermentative microorganisms. Temperatures above this range can accelerate the process and affect the quality of the almonds, while lower values can decrease microbial activity and delay the development of fermentation.(4)

Drying of cocoa beans

At the end of the fermentation process, the cocoa beans were dried by direct exposure to solar radiation on wooden platforms, avoiding contact with surfaces that could generate contamination. Drying constitutes a critical stage of the postharvest benefit, since it allows humidity to be reduced, residual microbial activity to be stopped and the biochemical transformations associated with the development of the sensory profile of cocoa to be consolidated. The process was carried out during the approximate period of 7 to 8 days, until reaching a moisture content between 6 and 7%, a recommended value to guarantee the conservation and technological quality of the product.(24)

Storage of cocoa beans

After finishing drying, the cocoa beans were stored in paper bags, a material that facilitates air exchange and reduces the risk of moisture condensation during the storage period. These conditions allowed preserving the quality of the samples and maintaining the physicochemical and sensory characteristics acquired during the fermentation and drying process until the execution of the experimental analyses.(25)

Physicochemical variables of cocoa beans

Pod index

This variable corresponds to the number of fruits required to obtain 1 kg of dry cocoa (20 pods). The calculation was obtained from the equation that relates the weight of the pods to the dry cocoa yield with which we can estimate the effectiveness of the production process and the quality of the cocoa from the treatments applied. This parameter is key to determining the productivity of the farms and the sustainability of the crop.(12)

Equation 1. Pod index

$$IM = \frac{Numero\ de\ mazorcas}{peso\ en\ gramos\ de\ las\ almendras\ secas} * 1000$$

Seed indices

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Before the cutting test, the seed index (SI) was determined by weighing 100 previously fermented and dried cocoa beans using a high precision analytical balance (Mx Analytical Balance, Mettler Toledo, USA) calculating the average weight per grain using the formula .(12)

Equation 2. Seed index

$$IS = \frac{Peso\ en\ gramos\ de\ 100\ almendras\ de\ cacao\ fermentadas\ y\ secas}{100}$$

Testa and cotyledon test

The proportions of shell and cotyledon were determined using a 30 g sample of fermented and dried cocoa beans, weighed on a precision analytical balance and calculated according to the formula.(12)

Equation 3. % of testa and cotyledon $\%\ de\ testa = \frac{(Peso\ de\ la\ testa)}{Peso\ de\ 30\ gramos\ de\ cacao} * 100$

The cutting test was carried out on 100 randomly selected cocoa beans to evaluate the quality of fermentation. Beans were sorted longitudinally using a stylus and classified as well-fermented, moderately fermented, purple, slate, or moldy, following standard guidelines.

Determination of the number of cocoa beans in 100 g

The number of poop beans per 100 g was determined by randomly selecting a representative sample and counting the number of beans needed to complete 100 g.(12)

Humidity

The humidity of the cocoa beans was determined after fermentation and drying. This variable is decisive for storage and commercial quality, being within the optimal range of 6 to 7%. No lower percentage of this parameter, which is considered optimal, is economical for the cocoa industry, as it influences the price and processing properties. The measurement was carried out with a moisture meter (AQUA BOY III, United Kingdom), specific for grains, placing the sample on top of the sensor of the device and taking the reading.(26)

Processing to obtain 100% cocoa paste

Figure 2 is a flow diagram that represents the procedure for obtaining 100% cocoa paste. The particularities of each of the phases are described below.

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Figure 2. Diagram of the process for obtaining 100% cocoa paste.

Selection and classification: The cocoa raw material was classified through an exhaustive manual inspection, eliminating any contaminants or foreign bodies attached to the beans. This process guarantees the purity of the grains used.

Roasting: The beans underwent a roasting process with the help of a clay pot under controlled conditions at an average temperature of 120°C for 18 to 25 minutes, ensuring uniform heat distribution to prevent burns and facilitate the removal of residual moisture.

Hulling: A manual process of hulling the beans was carried out, which made it possible to separate the hulls from the cotyledons. The hulled beans were preserved in paper to avoid contamination and alteration.

Grinding: The grinding of the cotyledons was carried out with a traditional manual mill. Through this phase, the intended particle size was reached to facilitate subsequent refining and ensure a homogeneous texture of the cocoa paste.

Refining: The refining process was carried out using a melanger type cocoa refiner (Spectra 11 Stone Melanger, Spectra, Coimbatore, India) for a particle size less than 40 µm, thus guaranteeing a homogeneous texture without the existence of granules that could extend on the surfaces of the palate. This phase is key to optimize the organoleptic characteristics of the product to be obtained.

Conching of the cocoa mass: The refined cocoa mass was introduced into a 5 kg conching machine, during which the sample was slowly introduced into the machine in such a way

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that the machine collected all the raw material adhered to its walls, thus achieving a uniform emulsion of the cocoa mass. The temperature control and stirring notably strengthened the emulsion and its homogeneity of the cocoa mass. This process was extended for 48 h for each of the treatments and the cocoa mass produced had a development that marked an outstanding sensory profile.

Tempering: Once the refining process was finished, the cocoa paste was subjected to the tempering process where, after heating it, its temperature must be lowered little by little. Subsequently, the cocoa paste was extracted from the previously sterilized molds and allowed to rest until it acquired the desired consistency.

Packaging and storage: Subsequently, the cocoa paste was packaged in aluminum-type paper with unique codes for traceability, in addition to being stored at 4 °C, which reduces the risk of cross contamination and also maintains its physicochemical or organoleptic properties.

Physicochemical analysis of cocoa paste

Determination of the pH of cocoa paste

The pH of cocoa paste was determined through standardized processing. A previously homogenized cocoa sample (approximately 10 g) was dispersed in 10 mL of analytical grade distilled water to obtain a uniform suspension suitable for semisolid matrices. The mixture was allowed to equilibrate before measurement. The pH was measured in duplicate using a digital potentiometer (pH meter) equipped with a glass electrode (Thermo Orion, Waterproof, Beverly, USA), previously calibrated with standard buffer solutions (pH 4.0 and 7.0; Merck, Darmstadt, Germany) according to the manufacturer's instructions.(12)

Determination of titratable acidity and total acidity in cocoa paste.

Titratable acidity was determined by acid-base titration using a previously homogenized cocoa paste sample. Approximately 10 g of sample was weighed and transferred to a 250 mL Erlenmeyer flask, adding 250 mL of distilled water (analytical grade, Merck, Darmstadt, Germany). The mixture was subjected to constant stirring using a magnetic stirrer to ensure complete dispersion. Subsequently, it was titrated with a 0.1 N sodium hydroxide (NaOH) solution, added gradually until reaching a final pH of 8.3, considered the titration end point for this type of matrix. The volume of NaOH consumed was recorded and the titratable acidity was calculated using formula (4), expressing the results as percentages of acidity.(22)

Equation 4. Titratable acidity and total acidity. $\% \, Acidez = \frac{V1 \, x \, N1 \, x \, M \, x \, 100}{V2}$

Where:
V1= Volume of NaOH used in the titration (mL);
N1= Normality of the NaOH solution;

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M= Molecular weight of the acid considered as reference (g mol-1);
V2= Volume of the sample taken for analysis (mL).

Determination of moisture in cocoa paste

The samples were presented in porcelain crucibles in an oven for about 30 min and then cooled to room temperature, and their weight was recorded with a precision analytical balance. Next, 2 g of previously homogenized cocoa paste were taken from the crucibles and dried in an oven at 130 °C for 2 hours. After two hours, the crucibles were placed in a desiccator and allowed to cool for about 30 min, and their weight was determined. Finally, the humidity of the cocoa paste was determined using formula 5.(22)

$$\% \, Humedad = \frac{M2 - M3}{M2 - M1} \, X \, 100$$

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

Determination of ashes in cocoa paste

Approximately 2 g of homogeneous cocoa paste were taken from pretreated porcelain crucibles (as described in the section “Determination of moisture in cocoa paste” and heated in a muffle oven at 600 ± 20 °C for 3 hours. The weight of the crucible was determined before and after heating, as described in the previous section. From the weights obtained, the ash content was determined by applying the formula.(22)

$$\% \, Cenizas = \frac{M3 - M1}{M2 - M1} \, X \, 100$$

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

Sensory analysis

In the sensory analysis we carried out a semi-trained tasting panel which was made up of 30 tasters, who determined: Flavor, Smell, Texture, Bitterness, Floral, Almond. The sensory analysis was carried out through a structured descriptive evaluation of the cocoa liquor obtained from the experimental treatments. To this end, a sensory sheet with an ordinal hedonic scale from 1 to 5 was used, where each of the values referred to some predefined category for each of the attributes. Five parameters of main interest were taken into consideration: Color, Appearance, Aroma, Flavor and Aftertaste, each subject to a specific descriptor.(22)

The scale used for each of the parameters was the following:

Color: Caramel coffee, 2= Brown, 3= Walnut, 4: Carob, 5= Black coffee.

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Appearance: 1= Very shiny, 2= shiny, 3= satin, 4= opaque, 5= very opaque.

Aroma: 1= Tropical fruits, 2= cocoa, 3= citrus fruit, 4= toasted, 5= nutty.

Flavor: 1= fruity, 2= roasted cocoa, 3= dry fruity, 4= bitter, 5= very bitter.

Aftertaste: 1= Smooth persistence, 2= bitter, 3= citrus not prolonged, 4= astringent, 5= spotty sensation.

The samples were subjected to sampling, identifying them with codes (T1-T9) and in this way maintaining a blind tasting to avoid perceptual biases. The tasting session was carried out under neutral lighting conditions, in a temperature-controlled room of 20 ± 2 °C and without visual or olfactory interference. Each sample was served in coded containers and was evaluated individually and independently.

The data obtained were organized for subsequent statistical analysis according to the experimental design.

RESULTS

Temperature dynamics during cocoa fermentation

The temperature of the cocoa mass was progressively increasing during the fermentation time as can be seen in Table 4. On day 0 of temperature measurement, the values were homogeneous between the treatments and ranged between 27.45 and 27.56 °C. At this stage, no significant effects of Saccharomyces Cerevisiae ($p = 0.8530$), the type of fruit extract ($p = 0.4623$) or the interaction between both factors ($p = 0.9990$) were observed.

From day 1, a differentiation in the thermal response was evident. Both S. cerevisiae and the type of fruit extract exerted significant effects on temperature ($p < 0.0001$), a behavior that was maintained until day 4. During this period, temperature increased from an interval of 31.48-34.07 °C on day 1 to 43.20-47.01 °C at the end of the evaluation. Among the experimental combinations, the treatment with 1% S. cerevisiae and papaya extract consistently recorded the numerically highest values from day 1, with temperatures of 34.07; 40.64; 44.92 and 47.01 °C on days 1, 2, 3 and 4, respectively. However, the treatment without S. cerevisiae and without extract presented the lowest records during the same period, with 31.48; 36.70; 40.77 and 43.20 °C.

Despite the differences observed for the main effects, the S. cerevisiae x extract interaction was not significant on any of the days evaluated ($p= 0.9990$; 0.6319; 0.1536 and 0.2853 for days 0, 1, 2, 3 and 4). This behavior indicates that, under the experimental conditions evaluated, no statistical evidence was detected that the effect of S.cerevisiae on temperature depended on the type of extract incorporated, or vice versa. Altogether, the results show an ascending thermal evolution of the cocoa mass during fermentation and evidence significant effects of the factors S. cerevisiae and fruit extract type from day 1, without evidence of significant interaction between both factors.

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Table 4. Effect of Saccharomyces cerevisiae and fruit extracts on temperature dynamics during cocoa fermentation.

Factor		Variable				
Saccharomyces Cerevisiae	**Fruit Extract**	**Temp-day 0**	**Temp-day 1**	**Temp-day 2**	**Temp-day 3**	**Temp-day 4**
0	**Without fruit extract**	27,47	31,48	36,70	40,77	43,20
0	**Papaya**	27,50	32,13	37,39	41,57	44,12
0	**Soursop**	27,45	31,94	37,17	41,27	43,87
0,5	**Without fruit extract**	27,50	32,77	38,58	42,97	45,18
0,5	**Papaya**	27,56	33,32	39,48	43,97	46,16
0,5	**Soursop**	27,47	33,17	39,24	43,72	45,92
1	**Without fruit extract**	27,47	33,65	39,92	44,32	46,35
1	**Papaya**	27,55	34,07	40,64	44,92	47,01
1	**Soursop**	27,45	33,98	40,51	44,82	46,89
	EEM ±	0,08	0,07	0,08	0,08	0,07
	CV	0,49	0,38	0,36	0,31	0,28
Probability	**Saccharomyces Cerevisiae**	0,8530	ns	<0,0001	***	<0,0001	***	<0,0001	***	<0,0001	***
	Extract	0,4623	ns	<0,0001	***	<0,0001	***	<0,0001	***	<0,0001	***
	S.Cerevisiae x Extract	0,9990	ns	0,6364	ns	0,6319	ns	0,1536	ns	0,2853	ns

Dynamics of total soluble solids during cocoa fermentation

Soluble solids were those with the greatest progressive decrease during cocoa fermentation, as seen in Table 5. On day 0, the values ranged between 20.56 and 20.66 °Brix, with no difference attributable to Saccharomyces cerevisiae ($p = 0.9664$), the type of fruit extract ($p = 0.8011$) or the interaction between both factors ($p = 0.8815$), which shows homogeneous initial conditions between the treatments.

From day 1 onwards, a general reduction in soluble solids was observed, accompanied by a significant effect of S. cerevisiea ($p < 0.0001$). At this time, the values were between 18.15 and 19.40 °Brix, while the effect of the fruit extract was not significant ($p = 0.5641$). On day 2, soluble solids decreased to a range of 14.39 to 16.81 °Brix, with significant effects of both S. cerevisiea ($p < 0.0001$) and the type of extract ($p = 0.0004$) being recorded. This behavior was maintained during days 3 and 4, in which both factors presented completely significant effects ($p < 0.0001$).

At the end of the evaluation, the values ranged between 7.93 and 11.78 °Brix. The treatment with 1% S. cerevisiae and papaya extract presented the lowest numerical

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value on day 4 (7.93 °Brix), followed by the treatments with 1% S. cerevisiae without extract (8.64 °Brix). However, the treatment without S. cerevisiae and without extract retained the highest content of soluble solids at the end of the process (11.78 °Brix).

The S. cerevisiae x extract interaction was not significant on any of the days evaluated (p = 0.8815; 0.0717; 0.1257; 0.3926 and 0.3668 for days 0, 2, 3 and 4). Therefore, the results show a sustained reduction in soluble solids during fermentation, with a significant effect of S. cerevosoae during day 1 and the type of fruit extract from day 2 onwards, with no evidence of significant interaction between both factors.

Table 5. Effect of Saccharomyces cerevisiae and fruit extracts on the dynamics of total soluble solids (°Brix) during cocoa fermentation.

Factor		Variable				
Saccharomyces Cerevisiae	**Fruit Extract**	**Brix-day 0**	**Brix-day 1**	**Brix-day 2**	**Brix-day 3**	**Brix-day 4**
0	**Without fruit extract**	20,60	19,24	16,81	13,92	11,78
0	**Papaya**	20,66	19,40	16,74	13,55	11,34
0	**Soursop**	20,56	19,22	16,79	13,84	11,53
0,5	**Without fruit extract**	20,63	18,76	15,69	12,39	9,84
0,5	**Papaya**	20,60	18,61	15,27	11,91	9,33
0,5	**Soursop**	20,63	18,75	15,49	12,16	9,54
1	**Without fruit extract**	20,57	18,34	14,84	11,33	8,64
1	**Papaya**	20,64	18,15	14,39	10,74	7,93
1	**Soursop**	20,61	18,29	14,56	10,95	8,15
	EEM ±	0,07	0,07	0,08	0,08	0,07
	CV	0,58	0,63	0,85	1,06	1,29
Probability	**Saccharomyces Cerevisiae**	0,9664	ns	<0,0001	**	<0,0001	**	<0,0001	**	<0,0001	**
	Extract	0,8011	ns	0,5641	ns	0,0004	**	<0,0001	**	<0,0001	**
	S.Cerevisiae x Extract	0,8815	ns	0,0717	ns	0,1257	ns	0,3926	ns	0,3668	ns

pH dynamics during cocoa fermentation

The pH of the cocoa mass showed a gradual increase during the fermentation period, shown in Table 6. On day 0, the values ranged between 3.64 and 3.73. At this time, Saccharomyces cerevisiae did not show a significant effect on pH (p = 0.7209), while the type of fruit extract presented a highly significant effect (p < 0.0001). The S. cerevisiae x extract interaction was also not significant (p = 0.9131).

From day 1 onwards, pH increased in all treatments and a significant effect of S.cerevisiea was observed (p = 0.0008), while the effect of fruit extract remained highly

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significant ($p < 0.0001$). The values recorded on this day were between 3.70 and 3.83. The upward trend continued during days 2 and 3, when the pH reached ranges of 3.82 – 4.02 and 3.97 – 4.26, respectively. At both times, both S. cerevisiae and the type of extract presented highly significant effects ($p < 0.0001$).

At the end of the evaluation, the pH reached values between 4.14 and 4.49. Among the experimental combinations, treatment with 1% S. cerevisiae and papaya extract recorded the highest numerical value (4.49), followed by 1% S. cerevisiae with soursop extract (4.42) and 0.5% S. cerevisiae with papaya extract (4.40). On the contrary, the treatments without S. cerevisiae and without extract, and without S cerevisiae with soursop extract, presented the lowest numerical values on day 4 (4.14 in both cases). At this last moment, both S. cerevisiae and the type of extract maintained highly significant effects on pH ($p < 0.0001$).

The S. cerevisiae x extract interaction was not significant on any of the days evaluated ($p = 0.9131$; 0.8731; 0.8829; 0.6970 and 0.4729 for days 0, 1, 2, 3 and 4). Therefore, under the experimental conditions evaluated, no statistical evidence of interaction between both factors on pH was found. Altogether, the results show an upward evolution of pH during fermentation, with a significant effect of the type of fruit extract from day 1 and of S. cerevisiea from day 1.

Table 6. Effect of Saccharomyces cerevisiae and fruit extracts on the pH dynamics during cocoa fermentation.

Factor		Variable				
Saccharomyces Cerevisiae	Fruit Extract	pH-day 0	pH-day 1	pH-day 2	pH-day 3	pH-day 4
0	Without fruit extract	3,70	3,76	3,87	4,00	4,14
0	Papaya	3,73	3,80	3,92	4,08	4,23
0	Soursop	3,64	3,70	3,82	3,97	4,14
0,5	Without fruit extract	3,70	3,78	3,92	4,11	4,29
0,5	Papaya	3,73	3,82	3,99	4,20	4,40
0,5	Soursop	3,65	3,73	3,89	4,09	4,31
1	Without fruit extract	3,69	3,79	3,96	4,17	4,38
1	Papaya	3,72	3,83	4,02	4,26	4,49
1	Soursop	3,64	3,74	3,93	4,17	4,42
	EEM ±	0,01	0,01	0,01	0,01	0,01
	CV	0,41	0,40	0,50	0,50	0,48
Probability	Saccharomyces Cerevisiae	0,7209	ns	0,0008	*	<0,0001	*	<0,0001	*	<0,0001	*
	Extract	<0,0001	*	<0,0001	*	<0,0001	*	<0,0001	*	<0,0001	*

S.Cerevisiae x Extract	0,9131	ns	0,8731	ns	0,8829	ns	0,6970	ns	0,4729	ns

Physical and fermentative quality of cocoa beans

The physical and fermentation quality of cocoa beans was mainly influenced by the individual effects of Saccharomyces cerevisiae and fruit extract (Table 7). The analysis of variance revealed a highly significant effect of S. cerevisiae on dry weight, percentage of cotyledons, seed index, fermented, violet and slate-colored grains, as well as on moisture content ($p < 0.0001$). The pod index was the only variable that did not show significant differences with respect to this factor ($p = 0.5264$).

The type of fruit extract also significantly influenced most of the variables. Differences were observed in dry weight ($p = 0.0012$) and highly significant effects on percentage of cotyledons, seed index, fermented grains, purple grains and moisture content ($p < 0.0001$). Similarly, the percentage of slate gray kernels showed significant differences ($p = 0.0004$), while the cob index was not affected by the extract ($p = 0.3540$).

Among the treatments evaluated, the incorporation of 1% of Saccharomyces cerevisiae resulted in the highest percentages of fermented grains, with values of 85.00%, 92.00% and 90.00% for the treatments without extract, with papaya and with soursop, respectively. This response was accompanied by a reduction in the percentage of purple beans, which decreased to 13.00%, 7.00% and 8.33%, respectively. A similar trend was observed for slate gray grains, which reached 2.00%, 1.00%, and 1.67% under the same conditions. In particular, treatment with 1% Saccharomyces cerevisiae gave the best results. The treatment that combined Saccharomyces cerevisiae and papaya extract presented the most favorable technological profile, with the highest percentages of fermented grains (92.00%), cotyledons (88.97%) and seed index (1.71), as well as the lowest percentages of purple (7.00%) and slate gray (1.00%) grains. Furthermore, this treatment had a water content of 6.13%, lower than the 6.90% recorded for the treatment without Saccharomyces cerevisiae or extract.

In contrast, the treatment without Saccharomyces cerevisiae or extract showed the lowest percentage of fermented grains (68.33%) and the highest proportions of purple (26.00%) and slate gray (5.67%) grains. In general, by increasing the concentration of Saccharomyces cerevisiae, better performance was observed. The presence of Saccharomyces cerevisiae was associated with a progressive increase in the percentage of fermented grains and a decrease in purple and slate gray grains.

Despite these differences between treatments, no significant interaction was observed between Saccharomyces cerevisiae and the fruit extract for any of the variables studied ($p > 0.05$). Interaction probability values were 0.9072 for dry weight, 0.4435 for cotyledons, 0.9674 for seed index, 0.3412 for fermented kernels, 0.1157 for purple kernels, 0.6674 for slate gray kernels, 0.7640 for ear index, and 0.3054 for moisture. For all these reasons, the observed difference was mainly attributed to the individual effects of the factors tested with the absence of statistical evidence of interactions between them. The variation coefficients showed a low behavior in the vast majority of the

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variables, obtaining 0.66% in the dry weight, 0.30% in the cotyledons, 0.92% in the seed index, 1.58% in the fermented grains, 0.69% in the spike index and 0.64% in humidity, the highest being those that belonged to the purple (6.07%) and slate gray grains. (14.34%).

Table 7. Effect of Saccaromyces cerevisiae and fruit extracts on the physical characteristics and fermentative quality of cocoa beans (Theobroma cacao L.)

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Factor		Variable							
Saccharomyces Cerevisiae	Fruit Extract	Dry Weight (g)	Cotyledon (%)	Seed index	Fermented (%)	Violets (%)	Slates (%)	Pod index	Moisture (%)
0	Without fruit extract	552,47	85,72	1,62	68,33	26,00	5,67	24,99	6,90
0	Papaya	546,53	86,45	1,66	72,33	23,00	4,67	24,99	6,72
0	Soursop	551,12	86,07	1,65	71,00	24,33	4,67	52,20	6,69
0,5	Without fruit extract	549,40	86,98	1,65	77,33	19,67	3,00	25,00	6,60
0,5	Papaya	539,95	88,19	1,69	82,33	15,67	2,00	24,91	6,40
0,5	Soursop	544,71	87,58	1,67	81,00	16,67	2,33	24,99	6,44
1	Without fruit extract	542,88	87,81	1,66	85,00	13,00	2,00	25,06	6,31
1	Papaya	535,67	88,97	1,71	92,00	7,00	1,00	24,94	6,13
1	Soursop	539,32	88,61	1,69	90,00	8,33	1,67	25,02	6,18
	EEM ±	2,08	0,15	0,01	0,73	0,60	0,25	0,10	0,02
	CV	0,66	0,30	0,92	1,58	6,07	14,34	0,69	0,64
Probability	Saccharomyces Cerevisiae	<0,0001	**	<0,0001	**	<0,0001	**	<0,0001	**	<0,0001	**	0,5264	ns	<0,0001	**
	Extract	0,0012	*	<0,0001	**	<0,0001	**	<0,0001	**	<0,0001	**	0,0004	*	0,3540	ns	<0,0001	**
	S.Cerevisiae x Extract	0,9072	ns	0,4435	ns	0,9674	ns	0,3412	ns	0,1157	ns	0,6674	ns	0,7640	ns	0,3054	ns

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Physicochemical characteristics of cocoa paste

Physicochemical characteristics of cocoa paste presented variations associated with the factors evaluated as It is seen in Table 8. The type of fruit extract had a highly significant effect on the pH, acidity, humidity and ash content ($p < 0.0001$). On the other hand, Saccharomyces cerevisiae did not show significant effects on pH ($p = 0.2657$), acidity ($p = 0.4258$) or ash ($p = 0.7568$), although it did present a significant effect on humidity ($p = 0.0353$).

The pH of the paste was between 5.87 and 6.47. The numerically highest values corresponded to the treatments with papaya extracts, with a record of 6.44; 6.46 and 6.47 for 0.5 or 1% S. cerevisiae, respectively. On the contrary, the pastas from the treatments without extract presented values between 5.87 and 5.88. The acidity ranged between 0.57 and 0.88%, with the lowest numerical value observed in the treatment with 1% S. cerevisiae and papaya extract (0.57%) while the treatments without extracts recorded the highest values, between 0.87 and 0.88%.

Humidity presented values between 3.36 and 4.02%. The lowest numerical record turned out to be 1% of S. The assembly of S. cerevisiae and papaya extract (3.36%), and the treatments of 0.5 and 0% of S. cerevisiae linked in isolation with papaya (3.38 and 3.42% respectively) presented a balanced development between these interactions. However, treatments without extract showed values between 3.99 and 4.02%. The statistical analysis confirmed significant effects of both S. cerevisiae ($p = 0.0353$) and the type of fruit extract ($p < 0.0001$) on this variable.

The ash content showed a narrower variation, with values between 2.99 and 3.14%. The highest numerical records were observed in the pastas from the treatments with soursop extract (3.12 - 3.14%), while the combinations with papaya extract presented the lowest values (2.99 - 3.01%). For this variable, the type of extract exerted a highly significant effect ($p < 0.0001$), while S. cerevisiae did not show a significant effect ($p = 0.7568$).

No significant interaction was detected between S. cerevisiae and the type of extract for any of the physicochemical properties analyzed: pH ($p = 0.8336$), acidity ($p = 0.7878$), humidity ($p = 0.9940$) and ash ($p = 0.9784$). Together, these results show that the type of fruit extract had a significant effect on the four physicochemical characteristics evaluated in the cocoa paste, while the effect of S cerevisiae was statistically limited to humidity. The absence of significant interaction indicates that, under the conditions of the study, no statistical dependence was detected between both factors for the variables analyzed.

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Table 8. Effect of Saccharomyces cerevisiae and fruit extracts on the physicochemical characteristics of cocoa paste.

Factor		Variable			
Saccharomyces Cerevisiae	Fruit Extract	pH	Acidity (%)	Moisture (%)	Ash (%)
0	Without fruit extract	5,87	0,88	4,02	3,02
0	Papaya	6,44	0,59	3,42	2,99
0	Soursop	6,30	0,64	3,47	3,12
0,5	Without fruit extract	5,88	0,87	4,00	3,01
0,5	Papaya	6,46	0,58	3,38	3,00
0,5	Soursop	6,32	0,63	3,43	3,13
1	Without fruit extract	5,87	0,88	3,99	3,02
1	Papaya	6,47	0,57	3,36	3,01
1	Soursop	6,33	0,62	3,41	3,14
	EEM ±	0,02	0,01	0,02	0,02
	CV	0,47	2,97	1,00	1,03
Probability	Saccharomyces Cerevisiae	0,2657 ns	0,4258 ns	0,0353 *	0,7568 ns
	Excerpt	<0,0001 *	<0,0001 *	<0,0001 *	<0,0001 *
	S.Cerevisiae x Extract	1 *	<0,0001 *	<0,0001 *	1 *
		0,8336 ns	0,7878 ns	0,9940 ns	0,9784 ns

Sensory profile of the cocoa paste

The sensory analysis allowed us to characterize the descriptive profile of the cocoa paste corresponding to the nine experimental treatments as evidenced in Figure 3. The joint representation of the attributes color, appearance, aroma, flavor and aftertaste showed variations in the configuration of the profiles between the different combinations of Saccharomyces cerevisiae and fruit extracts.

In the representation obtained, a progressive differentiation was observed between the treatments, particularly in those corresponding to the highest level of application of S. cerevisiae. Treatments T8 (Sc2Ex1: 1% S. cerevisiae + papaya extract) and T9 (Sc2Ex2: 1% S. cerevisiae + soursop extract) presented more external profiles for the set of attributes represented, while T7 (Sc2Ex0: 1% S. cerevisiae without extract) showed an intermediate configuration. Similarly. Within the 0.5% yeast level, differences were observed between T4, T5 and T6, reflecting descriptive variations associated with the different experimental combinations.

On the other hand, T1 (Sc0Ex0: without S. cerevisiae and fruit extract) presented a comparatively more internal and homogeneous profile. However, the relative position of the treatments. The scores represent descriptive sensory categories and not

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increasing levels of acceptability; Therefore, a greater extension of the polygon does not necessarily imply better quality or sensory preferences. For example, increasing scores in flavor approach the descriptor “very bitter,” while in appearance they correspond progressively to greater opacity.

Altogether, the radar diagram allows us to visualize the differentiation of the sensory profile of the cocoa paste between the nine experimental combinations and facilitates the identification of simultaneous changes in the evaluated attributes.

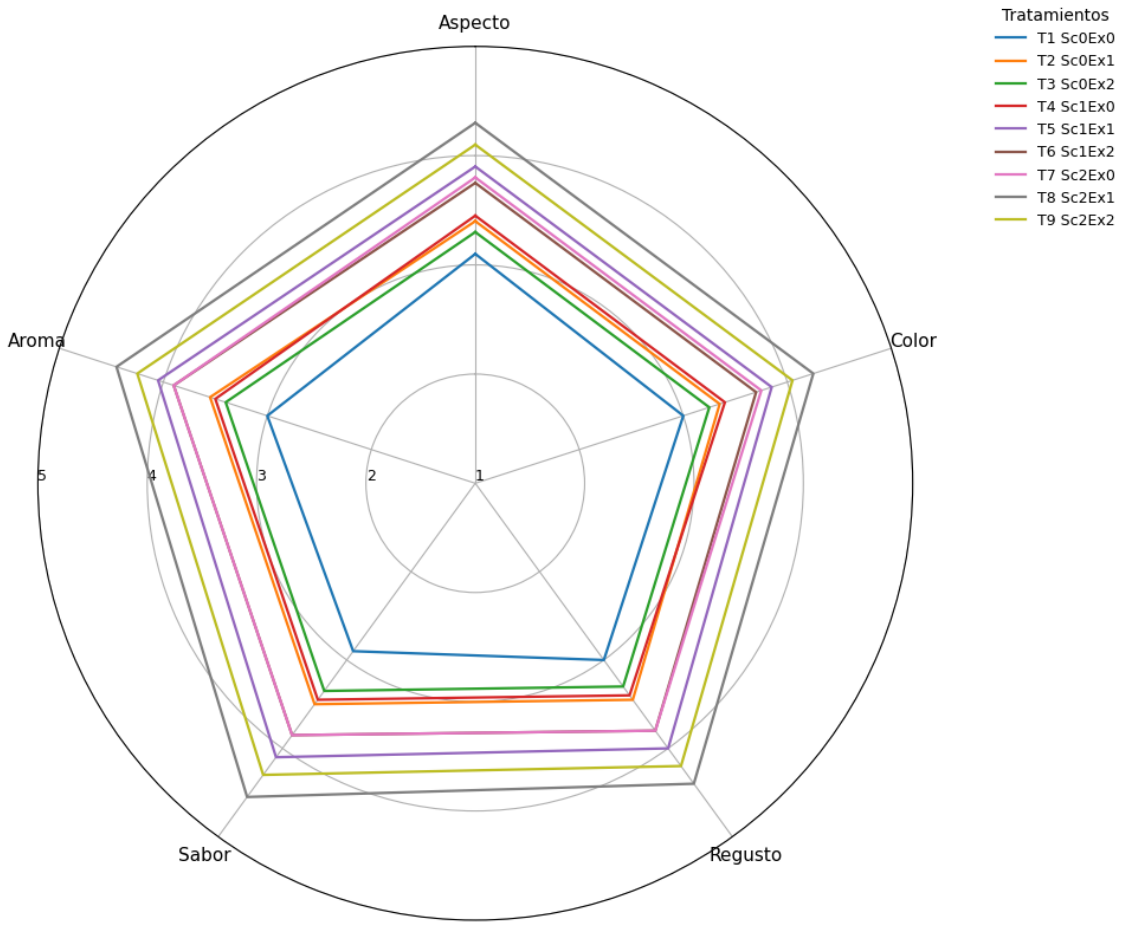


Figure 3. Sensory profile of cocoa paste obtained using different combinations of Saccharomyces cerevisiae and fruit extracts.

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DISCUSSION

The observed evolution is consistent with the microbial succession typical of cocoa fermentation. During the early stages, Saccharomyces cerevisiae actively participates in the consumption of sugars from mucilage and in the production of ethanol. Subsequently, greater aeration favors acetic acid bacteria, whose oxidations of ethanol generate heat and lead to an increase in the temperature of the dough. This behavior has been described by De Vuyst and Leroy et al. point out that this metabolic transition normally leads from initial conditions close to ambient temperature to values typical of an active fermentation, where the oxidation of ethanol and subsequent overoxidation of organic acid contribute decisively to the generation of heat.(13)

The observed influence of S. cerevisiae on the thermal behavior from the first active stages of fermentation finds support in studies that show that the temperature level is closely related to the performance of this yeast, the influence of this yeast observed from the first day also coincides with Kouamé et al. , using a predictive model developed specifically for the alcoholic fermentation of cocoa, precisely identified the initial concentration of the inoculum and the thermal evaluation as determining variables of the growth of S. cerevisiae. This relationship allows us to interpret that a greater initial presence of yeast can favor a faster colonization of the mucilage and a more efficient transformation of the available carbohydrates, generating favorable metabolic conditions for the continuity of microbial succession. However, one should avoid attributing the thermal increase exclusively to S. cerevisiae, because the more pronounced increase in temperature is subsequently associated with oxidative metabolism of acetic acid bacteria.(27)

Comparable results were reported by Streule et al. in Ecuadorian cocoa fermentations, where an adequate temperature progression was associated with more complete fermentation processes. Similarly, Gutiérrez et al. indicate that the aerobic stage can raise the temperature of the dough to approximately 40-52 °C, a range related to the activity of acetic acid bacteria and the internal biochemical transformations of the cotyledon.(28)(29)

The lack of significant interactions between S. cerevisiae and extracts suggests that S. cerevisiae and papaya acted independently on temperature, avoiding demonstrating a synergistic effect not confirmed statistically. Diaz et al. They also showed that the effect of a starter culture depends on its implantation capacity and the particular conditions of the process, so the fermentative response does not always result from additive interactions between the evaluated components.(30)

Overall, the thermal response obtained is compatible with active fermentation and functional microbial succession. The role of S. cerevisiae seems to be mainly related to the initial stages, while the subsequent increase in temperature is associated with the oxidative metabolism of acetic acid bacteria.

Concordantly, Plua et al. observed that the directed fermentation of Nacional and CCN-51 cocoa with Weisella confusing and Lactiplantibacillus plantarum was accompanied by

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a progressive increase in temperature, associated with the metabolic activity developed during the process. The authors highlight that thermal evolution constitutes a relevant indicator of fermentative progress, due to its relationship with the transformation of mucilage substrates and the succession of microbial groups. This evidence supports the importance of microbiological control of fermentation and suggests that the use of selected cultures can contribute to more uniform dynamics; However, the magnitude of the response also depends on the fermentation system and the conditions under which the process takes place.(20)

The progressive reduction of soluble solids is consistent with the use of mucilage carbohydrates as a substrate for microbial metabolism. Viesser et al. demonstrated that glucose and fructose constitute the main fermentable sugars of cocoa pulp, observing a preferential consumption of glucose during the first 48-72 hours. This behavior allows the loss of soluble solids to be related to the advancement of the fermentation phase and the transformation of available sugars.(31)

The participation of S. cerevisiae is particularly important in this dynamic. What is indicated by Muñoz and De Vuyst et al. It agrees with the functioning of yeasts that used as starter cultures affect the utilization of carbohydrates in the pulp, as well as the production of ethanol and compounds involved in aroma. Consequently, a more intense reduction of soluble solids in inoculated fermentations may reflect greater metabolic activity on the available substrates.(32)

This interpretation also coincides with recent research on controlled fermentation. A 2025 study with different strains of S. cerevisiae showed a progressive decrease in °Brix during cocoa fermentation and a higher residual concentration of soluble solids in natural fermentation compared to inoculated treatments. This supports the use of °Brix as a complementary indicator of the use of substrates during fermentation.

On the other hand, the incorporation of fruit matrices can modify the composition of the fermentation medium. Vizcaino et al. By replacing cocoa mucilage with fruit pulps in unconventional fermentations, they verified important transformations in sugars and organic acids as the process progressed. This suggests that fruit extracts can alter the availability of substrates for the microbiota; However, its specific contribution must be confirmed by the individual quantification of glucose, fructose, sucrose, ethanol, organic acid, given that °Brix represents the set of soluble solids or exclusively sugars.(33)

The ascending evaluation of pH can be related to the transformations of the organic acids present in the mucilage and to the microbial succession during fermentation. Diaz et al. point out that the consumption of citrate, mainly associated with the activity of lactic acid bacteria, progressively reduces the initial acidity of the pulp and modifies the conditions for the establishment of the microorganisms that participate in later stages.(18)

This trend has also been observed experimentally. In controlled cocoa fermentations, initial values close to pH 3.5 and final values around 4.0 – 4.3 have been reported, attributed to the microbial transformation of citrate and carbohydrates present in the

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pulp. Likewise, recent research with S. cerevisiae as a starter culture shows that the pH can fluctuate during the process and subsequently increase, confirming its behavior depends on the metabolic activity and the fermentation stage.(34)(35)

The effect of fruit extracts may be associated with differences in their composition of sugars and organic acids. Vizcaino et al. , by replacing cocoa mucilage with fruit pulps, verified changes in pH, titratable acidity and concentrations of citric, lactic and acetic acid during fermentation. These antecedents support that the incorporation of fruit matrices can modify the chemical environment of the fermentative mass; However, the specific mechanism will depend on the composition of each extract.(36)

The physical quality of the grain is closely related to the efficiency of fermentation, particularly with internal transformations of the cotyledon and the reduction of defects associated with incomplete fermentation. Ecuadorian National cocoa, Falconí et al. demonstrated that the use of a native microbial cocktail as a starter culture favored the development of the fermentative process compared to conventional fermentation. This background supports the use of selected microorganisms as a strategy to improve the uniformity and quality of cocoa processing.(10)

The participation of Saccharomyces cerevisiae is particularly relevant due to its ability to establish during the first stages of fermentation and transform the available substrates into mucilage. Diaz et al. verified that fermentations inoculated with S. cerevisiae led to a more advanced process than those inoculated with Hanseniaspora opuntiae, in addition to generating richer and more reproducible aromatic profiles. This supports the potential of S. cerevisiae as a starter culture to promote a more consistent transformation of the grain.(30)

The decrease in violet grains takes on special importance, because their presence is associated with an incomplete transformation of the cotyledon. Vizcaino et al. , in unconventional fermentations of CCN-51 cocoa with replacement of mucilage by fruit pulps, obtained more than 81% of fermented beans and less than 18.4% of violet beans after five to six days. The methodological proximity of this study is particularly relevant, since it demonstrates that the modification of the fermentative matrix using fruit pulps can be accompanied by favorable changes in the quality determined by cutting test.(37)

The absence of interaction between S. cerevisiae and fruit extracts indicates that there is no statistical evidence to suggest a synergistic effect between both factors. Their contributions on physical characteristics and fermentative quality must be interpreted independently. In applied terms, the available evidence supports the use of starter cultures to promote a more uniform fermentation, while the use of fruit matrices constitutes a promising alternative to modulate the process and deserves a more in-depth microbiological and metabolic characterization.

The physicochemical variations of cocoa paste reflect that the changes induced during fermentation and drying are subsequently maintained in the processed product. In this sense, pH and acidity are particularly sensitive to the accumulation and diffusion of organic acids during fermentation. Various studies have shown that the acidification of

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the cotyledon depends mainly on the penetration of acetic and lactic acid, which subsequently affects the cocoa liquor or paste and can modify its sensory quality.(38)

The influence of the type of extract on pH and acidity can be related to the composition of each plant matrix. Fruit extracts add sugars and organic acids which would modify the conditions of the mucilage and therefore the metabolic trajectories during fermentation, while this adjusts to what was found in previous works where the addition or substitution of matrices rich in carbohydrates modified the metabolism of sugars and organic acids in the fermentation of cocoa, later finding differences in the chemical nature of the product. However, establishing which compounds were responsible for these variations would require individually quantifying citric, lactic and acetic acid.

Humidity constitutes another parameter of technological interest, since it is related to the stability of the raw material and subsequent transformation operations. In studies carried out with fermented cocoa, the moisture content showed variations attributable to both the fermentation process and drying, while reduced contents favored greater stability during storage. Similarly, recent research with induced fermentations has highlighted that post-harvest handling determines water retention and the final composition of processed cocoa.(39)

For its part, the differences in ash can be linked mainly to the mineral composition of the raw material and the added matrices. Unlike pH or acidity, this variable does not directly depend on the fermentative activity, because it represents the residual inorganic fraction of the product. Studies on fermentation dynamics have shown that ash content can vary between processing conditions and cocoa materials, although these changes are usually less pronounced than those observed in humidity or acidity.(11)

The absence of interaction between Saccharomyces cerevisiae and the fruit extracts indicates that there is no evidence of a synergistic effect on the physicochemical properties of the paste. Therefore, the influence of both factors must be interpreted independently. Overall, the literature supports that the physicochemical composition of cocoa paste constitutes the cumulative result of fermentation, drying and the nature of the raw material, with the balance between pH and acidity being particularly important due to its subsequent relationship with the technological and sensory quality of cocoa.

The differences observed between the sensory profiles confirm that fermentation conditions can modify the perceptible characteristics of the cocoa paste. The activity of yeast plays an important role in this process, due to its participation in the formation of alcohols, esters and other metabolites that subsequently contribute to aroma and flavor. In this sense, Díaz et al. demonstrated that the use of Saccharomyces cerevisiae as a starter culture produced richer and more reproducible aromatic profiles of cocoa and chocolate liquor than other fermentative conditions, associated with differences in the composition of the volatile organic compound.(30)

The influence of yeasts on the sensory profile also depends on their metabolic capacity. Chagas et al. , when evaluating indigenous yeasts from cocoa fermentations, identified

sensory differences linked to the production of compounds such as ethyl acetate, isoamyl acetate and 2-phenylethyl acetate, related to fruity and floral notes. This shows that the selection of the starter microorganism can qualitatively modify the aromatic profile developed during fermentation.(40)

In Ecuadorian National cocoa Streule et al. They also verified the fermentation and drying conditions have a direct impact on the sensory profile of the liquor. Insufficient fermentations were associated with bitter, astringent, woody and earthy attributes, confirming that postharvest handling conditions the sensory expression of the final product.(28)

CONCLUSIONS

The application of Saccharomyces cerevisiae and extract of papaya (Carica papaya L.) and soursop (Annona muricata L.) allowed modifying the conditions of the postharvest fermentation of cocoa, showing variations in temperature, pH and soluble solids. The results obtained presented a greater guarantee of fermentative development given the more transformative character that the almonds showed and demonstrated the potential of directed fermentation as an alternative to have a more controlled and uniform postharvest process.

The treatments influenced the fermentative quality of the grain and the physicochemical characteristics of the cocoa paste. Among the combinations evaluated, the treatment consisting of 1% of S. cerevisiae and papaya extract presented the most favorable technological behavior, with 92% of fermented grains, 7% of violet grains and 1% of slate grains, in addition to a paste characterized by lower acidity and humidity. These results allow us to consider this combination as the one with the greatest potential to optimize postharvest quality within the experimental conditions studied.

The different fermentation conditions generated variations in the sensory attributes of the cocoa paste, confirming that fermentative management has an impact on the perceptible characteristics of the final product. However, the absence of a statistically significant interaction between S cerevisiae and fruit extracts indicates that a synergistic effect between both factors was not proven, so their effects must be interpreted mainly independently.

The results support the potential of S. cerevisiae and, particularly, papaya extract as a tool to guide biotechnological strategies aimed at improving the fermentation and quality of cocoa.

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DECLARATION OF INTEREST (OPTIONAL)

The authors declare that they have no personal or conflicts of interest that could have influenced the development, analysis, interpretation or publication of the results of this research.

AUTHOR CONTRIBUTIONS (OPTIONAL)

Luis Humberto Vásquez Cortez and Sanyi Lorena Rodríguez Cevallos: conceptualization, research design, methodology, experimental development, conceptualization, research design, methodology, experimental development of treatments, data collection and processing, statistical analysis, interpretation of results, completed research, generation of figures, tables, writing of the original draft, review and editing of the manuscript. José Francisco Falconi Novillo: methodology, supervision, critical review and approval of the final version of the manuscript. All authors read and approved the final version of the article.

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