# Structural and color changes in the Criollo Cocoa beans during assisted fermentation

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## ABSTRACT

One of the key determinants of the sensory quality of cocoa by-products is the inclusion of fermentation as a critical unit operation in the production process. During fermentation, significant physical, chemical, biochemical, and structural changes occur, all of which are essential for the development of sensory precursors in cocoa and its derivatives. While the chemical and biochemical changes during fermentation are well documented in the literature, relatively little attention has been given to the structural changes occurring within the beans throughout this process. The aim of this study was to produce a photographic record of the structural changes in Criollo cocoa beans over seven days of fermentation, assisted by the addition of Acetic Acid Bacteria (AAB). In addition to bean swelling, darkening of the external surface through oxidation, and loss of the mucilaginous layer, Criollo cacao exhibits an ivory internal coloration throughout fermentation due to the absence or low concentration of anthocyanins. This characteristic coloration results in a light hue upon sun-drying ("light break"), as observed in the cutting test, giving Criollo cacao a unique identity that distinguishes it from other types such as Hybridized and Forastero, which display a deep brown color after sun-drying. The findings of this study provide a foundation for future research on aroma formation in cocoa during fermentation.

***Keywords:*** *Assisted fermentation, Cocoa bean structure, Criollo cocoa, Inoculated fermentation, Light break color, Starter culture, Theobroma cacao L.*

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## Highlights of this paper

- Assisted fermentation produces, cellular breakdown of Criollo cocoa beans, bean swelling, darkening of the external surface through oxidation, and loss of the mucilaginous layer without changing its ivory cotyledon color.
- The fermentation process drives pigment oxidation, turning the Criollo ivory cocoa bean a desirable light brown, or "light break," during sun-drying.
- The resulting modifications directly impact the development of cocoa's characteristic flavor and texture.

## 1. INTRODUCTION

Criollo cacao is a type of fine or super fine-aroma cacao originating in Venezuela [1] highly prized internationally for its flavor and aroma characteristics. As a region of origin [2, 3]. Venezuela boasts a wide variety of this cacao in terms of sensory n otes [4].

As reported by Jiménez, et al. [5] the cotyledons color of some Venezuelan cacaos is white or ivory, with some beans displaying a slight light-purple tint. This coloration is caused by the absence or very low presence of anthocyanins, and the beans are typically elliptical or oblong in shape. These are considered Criollo cocoa and after Despite its genetic origins, one of the greatest quality guarantees for obtaining chocolates with superior sensorial attributes is the application of the fermentation unit operation during the post-harvest process.

Cocoa fermentation is a metabolic process in which microbial populations, exhibiting successional behavior—such as yeasts and bacteria (mainly lactic acid bacteria [LAB] and acetic acid bacteria [AAB])—break down organic molecules (carbohydrates and sugars) into simpler compounds (alcohols, acids, and gases). This process can occur in the absence of air (anaerobically or facultatively) or in the presence of air, resulting in the production of ethyl alcohol, carbon dioxide, and various volatile and non-volatile compounds[6-13] .

Besides the production of the complex flavor precursors, which determine the sensorial quality of all of the cocoa by-products, due to the microbiological activities and cocoa beans' physiological conditions (oxidation and hydrolysis), cocoa beans must be fermented to eliminate the seed coat and kill the bean's germ [14].

In addition to producing complex flavor precursors that determine the sensory quality of cocoa by-products, the microbiological activities and physiological conditions of the cocoa beans (oxidation and hydrolysis) necessitate fermentation to eliminate the seed coat and kill the bean`s germ [15, 16].

Despite the consensus that raw, non-fermented cocoa beans are pale, astringent, and virtuallyTunjung Sari, et al. [17] and Fang, et al. [18] flavorless, and despite scientific research documenting the development of flavor precursors during fermentation, cocoa fermentation practices have not yet reached technological maturity. It has been demonstrated that, through several stages, the fermentation process transforms compounds in the seed into flavor precursors, thereby unlocking the rich, nuanced flavors of chocolate.

Fermentation in cacao involves three main types of transformations: chemical, biochemical, and physical.

From a chemical and biochemical perspective, the first stage of the process is characterized by exothermic alcoholic fermentation carried out by yeasts, which are predominant due to the high sugar concentration (10–15%), the anaerobic environment of the cocoa mass, pH (4.5–4.8), and temperature conditions[7]. During this stage, yeast invertase enzymes hydrolyze sucrose in the pulp into fermentable monosaccharides, initiating alcoholic fermentation in which glucose is converted into ethanol and carbon dioxide. Furthermore, yeasts produce various aromatic precursor compounds, such as alcohols and esters, which positively contribute to the aromatic profile of chocolate [19, 20] .

Simultaneously with the action of yeasts, lactic acid bacteria (LAB) consume sugars, generating lactic acid and other metabolites (such as acetic acid and ethanol) [21]. LAB also consume citric acid, an organic acid present in the

cocoa pulp and responsible for its low pH, which causes the pH of the fermenting mass to increase slightly [22]. LAB are facultative microorganisms; although they tolerate the presence of oxygen, to carry out fermentation and efficiently produce lactic acid, they require air-free conditions. LAB are also known to affect the sensory properties and composition of chocolate [23].

The consumption of pulp by yeasts creates a more aerobic environment, which, in combination with increased pH and temperature, favors the proliferation of acetic acid bacteria (AAB). AAB promote the exothermic oxidation of ethanol produced by yeasts into acetic acid, resulting in a further rise in cocoa mass temperature and a decrease in pH [24]. Under these conditions, acetic acid penetrates the cotyledon, inducing embryo death and triggering a series of enzymatic and biochemical reactions that lead to the development of flavor and color. Through these reactions, AAB cause structural changes and promote bean swelling.

From a physical perspective, several structural changes occur in the beans during fermentation that are critical to the development of desirable qualities. One such change is the degradation of pectin in the pulp by yeast pectinase enzymes, along with endogenous pectinase. As the pulp heats up and begins to liquefy, it eventually drains away, allowing air to enter the fermenting mass. External microbial processes (mainly by AAB) further heat the mass and produce acetic acid, which penetrates the bean`s Testa (hull), alters the internal pH, and causes embryo death. Moreover, the cocoa bean becomes fleshy and swells during fermentation due to embryo death, the absorption of external acidic liquids, and the rupture of internal cells. This halts germination and disrupts cell walls, allowing the bean`s enzymes to interact freely with other substances.

Furthermore, the initial white, beige, or deep purple color of the cotyledons in non-fermented cocoa is determined by the absence or presence of anthocyanins, respectively, and reflects the genetic origin of the cocoa. As fermentation acidifies the bean and oxidizes these compounds, the color shifts to the characteristic rich brown seen in high-quality Forastero and hybridized cocoas.

However, Criollo cocoa develops a light brown color.

Additionally, the breakdown of cell walls creates internal pockets and fissures in the cotyledon, resulting in a denser and more rubbery texture.

Structural changes in cocoa beans during fermentation are crucial because they eliminate astringency and bitterness, and create the chemical precursors responsible for chocolate`s flavor and aroma. Without this physical and biochemical transformation, the raw bean would retain a tasteless structure and remain looser and more porous.

Therefore, the aim of this research is to monitor the physical changes in Criollo cocoa beans over seven days of fermentation in an assisted process, with acetic acid bacteria added on the third day.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

Raw material:

- A micro-batch of 13 kg of wet, mucilage-covered Criollo cocoa (*Theobroma cacao* L.) beans from a commercial farm, Hacienda *La Porcelana*, located in Caño Amarillo, Táchira state, at the south-central end of Lake Maracaibo, Venezuela.
- 13 g of Bayanus WB06 yeast, a species with high alcohol tolerance.
- 90ml of acetic acid bacteria (AAB) developed in an artesian reactor using old wine biomass

Design: Experimental and descriptive.

Approach: Monitoring and control of biophysical variables.

The study was conducted at the VSL (Varietales Sur del Lago) Clonal Garden using vegetative propagation of Criollo cacao from the region.

The Criollo cacao pods were harvested at the optimal stage of physiological maturity, and all beans (with pulp) were obtained from the shelled pods. A selection process was performed on the pulped beans mass, to manually discard overripe, diseased, or mechanically damaged pods. The selected biological material was then extracted and immediately placed into the fermentation system.

### *2.2. Procedure*

Day 0: System Conditioning and Loading.

The mass of wet cocoa beans was initially weighed to establish the baseline yield. The fresh, pulped, and selected beans were carefully transferred (to minimize initial air pockets) into a solid-wall plastic bins (FDA compliant for food use). At the point, 13g of yeast Bayanus WB06 was added under the Criollo cocoa mass and mixed; the temperature of the system was 35 °C, with 17 °Brix and a pH of 4.5. Once mixed the surface was completely covered with the plastic bins covert to optimize retention of metabolic heat.

Day 1: Anaerobic Stage (0 to 48 hours).

During the first 48 hours, the system was maintained under strict anaerobic conditions (absence of oxygen), with pH 4.5, 31 °C, and 17 °Brix. Activity from the inoculated yeasts was recorded during this period, as they metabolized the sugars in the mucilage, converting them into ethanol and carbon dioxide. Throughout this phase, the mass was not stirred or turned. However, the process was monitored to control the formation of excessive acids, since a total absence of oxygen could lead to excessive lactic and butyric acid production, thereby impairing the final flavor of the beans.

Days 2 to 6: Aerobic Stage (48 to 144 hours).

At this stage, a portion of the mucilage was removed from the Criollo cocoa mass. The °Brix had diminished to 6%, and the temperature had increased to 37 °C. Beginning at the 48-hour mark, aeration was induced by manually turning the cocoa mass and transferring it to another container; this procedure was repeated every 24 hours. On day 3, 90 mL of acetic acid bacteria was added to facilitate the oxidation of ethanol during this phase. As alcohol formed through sugar fermentation and the temperature and pH increased, acetic acid bacteria began to predominate. Furthermore, the introduction of oxygen stimulated the proliferation of acetic acid bacteria, initiating the oxidation of ethanol into acetic acid. On day 4, the °Brix remained at 6, and the temperature was maintained at 37 °C. On day 6, the temperature reached 38 °C, and by day 7, it increased from 38 to 42.2 °C.

As a result of this second fermentation, both temperature and the concentration of acetic acid increased, giving rise to the characteristic vinegar smell. The acetic acid penetrated the seed coat, reacting with internal compounds and triggering the series of reactions previously described.

Day 8: Drying Operation.

Once the fermentation cycle (144 hours) was complete, the mass temperature reached 40 °C and the solid concentration was 6 °Brix. The beans were then spread onto solar drying beds in thin layers approximately 5 cm thick. They were turned regularly throughout the day to ensure uniform dehydration, and the process was concluded once the beans reached a moisture content of approximately 7–7.5%.

## 3. RESULTS AND DISCUSSION

As shown in Table 1 and Figure 1A and B, the beans at days 0 and 1 exhibit external moisture due to the presence of mucilage, and have a wet, compact, and smooth appearance with a white outer color. The cotyledons are ivory-

colored with some light brown oxidized zones, as observed in Figure 1C. This coloration occurs because, upon immediate contact with oxygen from the air, the polyphenols within the cotyledons undergo oxidation catalyzed by polyphenol oxidase enzymes.

After 2 days of fermentation, the external appearance of the beans is similar to that of the first days. The differences observed in cotyledon color are due to the low concentration of anthocyanins Figure 2. Thus, the ivory-white color of pure Criollo cotyledons occurs because they completely lack, or have very low levels of, anthocyanins (specifically cyanidin-3-galactoside and cyanidin-3 -arabinoside), with a prevalence of (−)-epicatechin, which is white or pale yellow in color [5].

**Table 1.** Physical characteristics of the beans during the fermentation process.

| **Procedure of fermentation** | | **VSL Criollo Cocoa** | | |
|---|---|---|---|---|
| **Days** | **Characteristics** | **Beans appearance** | **External Color*** | **Internal Color** |
| 0 -1 | Swollen with abundant mucilage | Wet, compact and smooth | White | White* |
| 2 | Swollen with dark mucilage | Compact smooth | Ivory | Lightly violet |
| 3 | Swollen with amber-colored mucilage | Compact smooth | Ivory | Ivory |
| 4 | Swollen with little mucilage | Compact smooth | Ivory | white |
| 5 | Swollen with little mucilage | Compact smooth | Ivory | ivory |
| 6 | Swollen with little mucilage | Corrugated | Ivory | ivory |
| 7 | Swollen with little mucilage | Corrugated | Ivory | ivory |

**Note:** *Light brown coloration is due to the oxidation for air exposure.

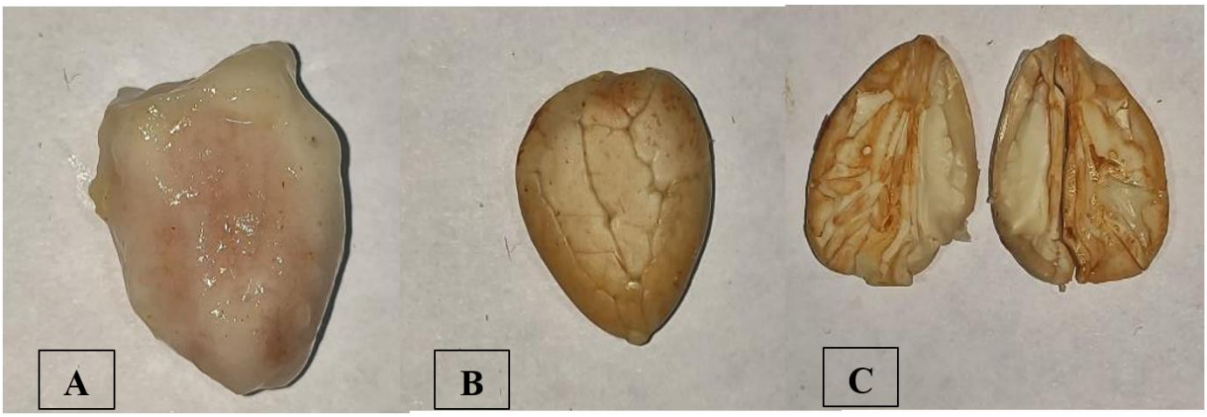


**Figure 1**. A Criollo cocoa bean; A. with mucilage, B. without mucilage, and C. transversely cut at the 0-1 day.

It is well established that the cotyledons of pure Criollo cacao contain negligible or extremely low levels of anthocyanins, which accounts for their characteristic white or ivory color [25, 26].

Unlike Forastero or Trinitario varieties, which accumulate high concentrations of this water- soluble pigment, resulting in dark purple cotyledons; Criollo cacao is notable for its low astringency and bitterness, precisely because of its lower content of pigmented phenolic compounds.

These findings are illustrated in Figures 3 and 4 where Criollo cocoa is classified and compared. This document includes a structured technical comparative matrix detailing the fermentation guidelines established by Ramos, et al. [26] (project with global commodity standards (ICCO, CFC) and specialized fine-flavor protocols outlined by INIA Venezuela).

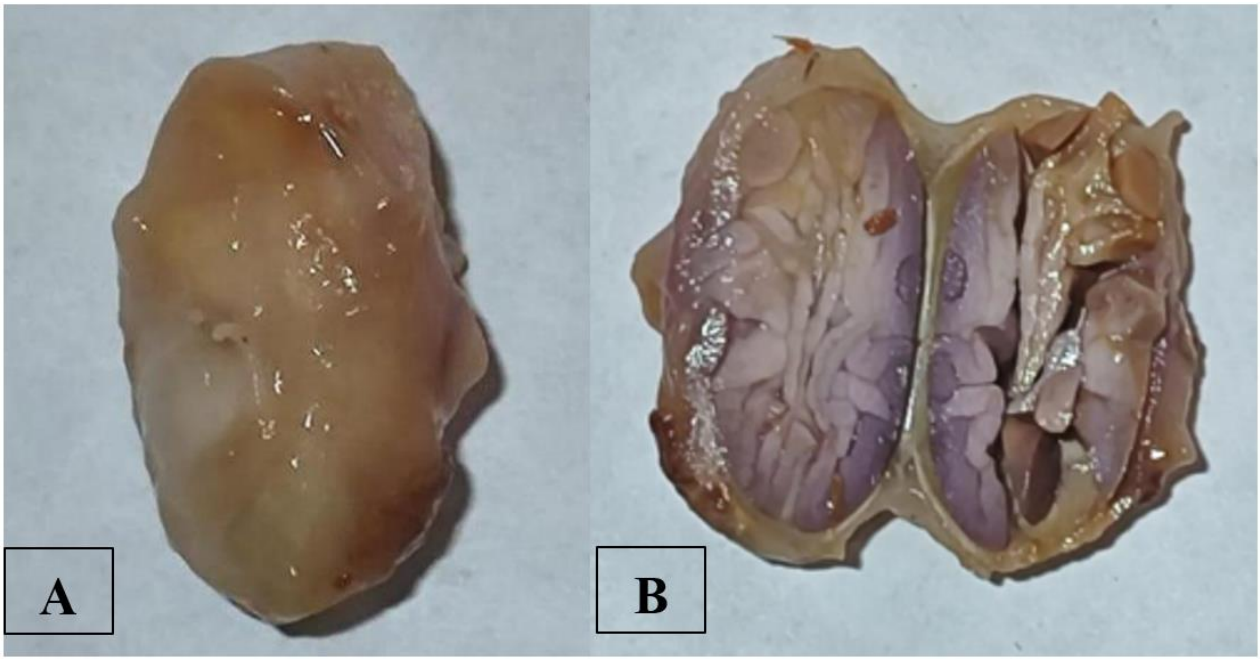


**Figure 2.** A Criollo cocoa bean; A. with mucilage, B. transversely cut at the 2 days.

Figure 5 shows the internal and external structural changes at 3 days of fermentation. Externally, the bean is swollen with amber-colored mucilage. When the mucilage is removed, the beans show a compact structure similar to that seen at day 1 and retain a smooth appearance. Internally, the cotyledons begin to show changes, with the tissue becoming slightly more segmented and open, as seen in Figure 2 (day 2).

## Cocoa Fermentation Protocols

| Type | Fermentation days | Turning frequency |
|---|---|---|
|  Criollo | 3 to 4 days | 1st turning at 24 hours ..... (1st day)<br>2nd turning at 48 hours ..... (2nd day)<br>Start drying at 72 hours ..... (3rd day) |
|  Trinitario | 4 to 6 days | 1st turning at 24 hours ..... (1st day)<br>2nd turning at 72 hours ..... (3rd day)<br>3rd turning at 120 hours ..... (5th day)<br>Start drying at 144 hours ..... (6th day) |
|  Forastero | 7 days | 1st turning at 24 hours ..... (1st day)<br>2nd turning at 72 hours ..... (3rd day)<br>3rd turning at 120 hours ..... (5th day)<br>Start drying at 168 hours ..... (7th day) |
| | 8 days | 1st turning at 24 hours ..... (1st day)<br>2nd turning at 72 hours ..... (3rd day)<br>3rd turning at 120 hours ..... (5th day)<br>4th turning at 168 hours ..... (7th day)<br>Start drying at 192 hours ..... (8th day) |

### Drying Protocol for All Types of Cocoa

Spread the fermented beans in layers no thicker than 3 cm on a drying patio or platform, considering that one square meter of drying area can hold 20 kg of wet cocoa beans. Remove any foreign matter and broken beans. Stir the beans regularly to ensure uniform drying. The drying process should be gradual and begin in the early hours of the morning (6:30 a.m.). Its duration may vary depending on weather conditions in the area, and exposure to sunlight should be as follows:

Day 1 ..... 2 hours
Day 2 ..... 2 to 3 hours
Day 3 ..... 3 to 4 hours
Day 4 ..... 4 to 5 hours
Day 5 ..... All day

Protect the beans from humidity and rain.

This publication is the product of the different research studies developed under the Project to Establish Physical, Chemical, and Organoleptic Parameters for the Characterization of Cocoa (*Theobroma cacao* L.) and Its Optimization for International Markets (ICCO), funded by the Common Fund for Basic Commodities (CFC) and implemented by the National Institute for Agricultural Research (INIA-Mérida).



**Figure 3.** Fermentation protocols for cocoa developed through the International Cocoa Organization (ICCO), Cocoa Baba Flower, the Common Fund for Commodities) and National Institute of Agricultural Research (INIA), Venezuela.
**Source:** Ramos, et al. [26].

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**Figure 4.** Comparison of the beans separated from Criollo cocoa pods from Palmarito, (Sur del Lago de Maracaibo, Venezuela; Seboruco genetic) with those identified in the fermentation protocols for cocoa according to the project by the International Cocoa Organization (ICCO), Cocoa Baba Flower, Common Fund for Commodities) and National Institute of Agricultural Research (INIA), Venezuela.
**Source:** Ramos, et al. [26].

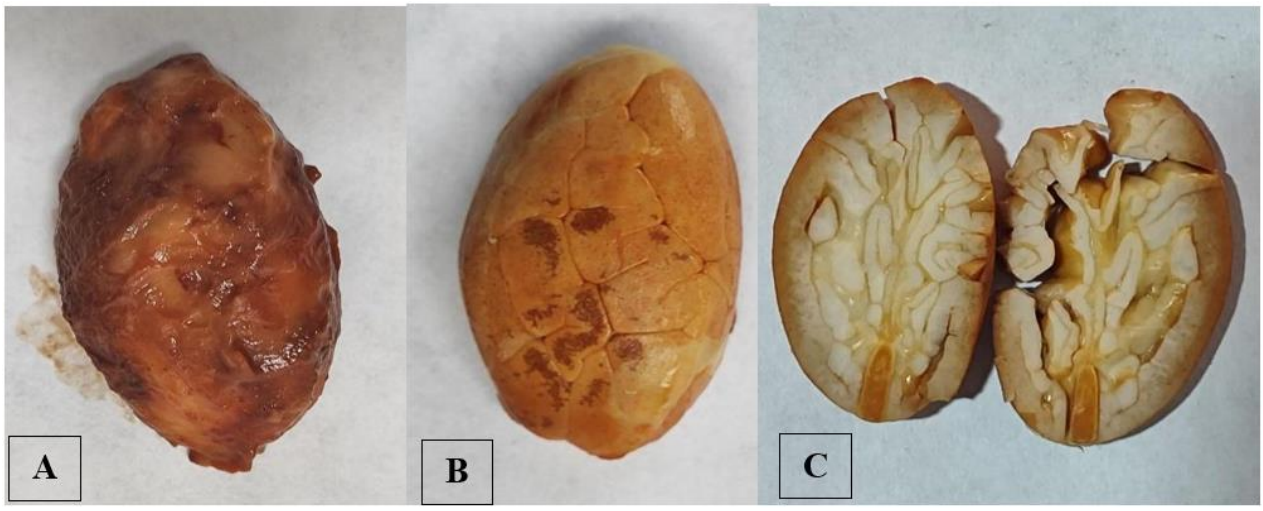


**Figure 5.** A Criollo cocoa bean; A. with mucilage, B without mucilage, and C transversely cut at the 3 days.

After 72 hours (3 days), as alcohol forms through sugar fermentation and both temperature and pH rise, acetic acid bacteria begin to predominate. These bacteria utilize the alcohol to carry out fermentation under aerobic conditions (in the presence of oxygen).

Acetic acid penetrates the seed coat and reacts with internal compounds; particularly polyphenols, which are characterized by their violet color and their astringent, bitter taste. This process is responsible for the opening of channels inside the bean cotyledons.

This second fermentation increases the concentration of acetic acid, resulting in the characteristic vinegar-like odor.

At 4 days (96 hours) of fermentation, the preparation protocol included the addition of mucilage and a portion of acetic acid bacteria. Although there are color differences in the external appearance of the beans at this time (Figure 6A and B), with the brown color becoming more prominent, there is not a notable difference in the external structure of the bean (Figure 6C) compared with day 3 (Figure 5C).

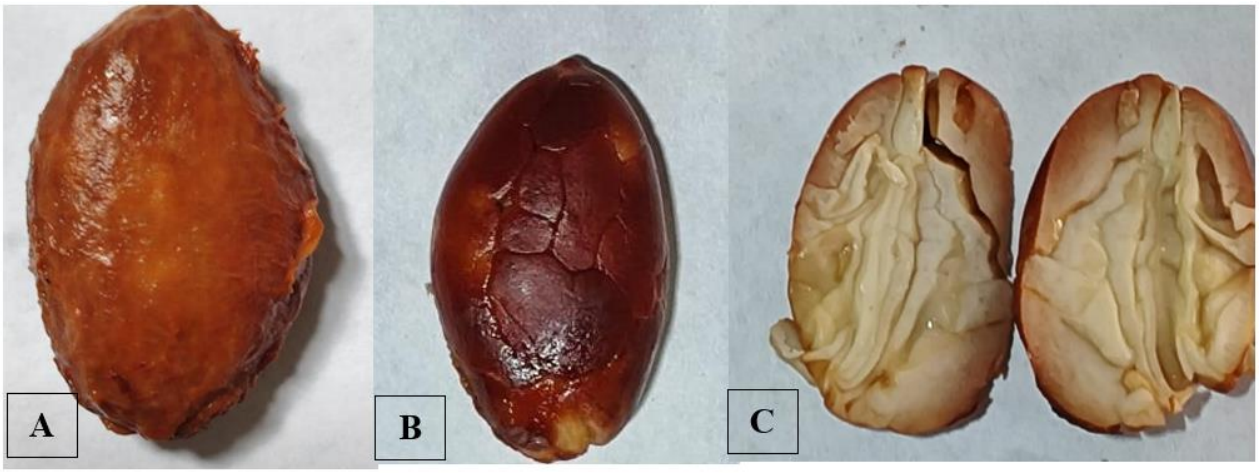


**Figure 6.** A Criollo cocoa bean; A. with mucilage, B without mucilage, and C transversely cut at the 4 days.

On day 5, the fermentation process intensified and homogenized the brown coloration in the external appearance of the cocoa beans, with slight loss of the mucilaginous layer due to dehydration. Internally, the beans retained their ivory color, and the internal grooves became more pronounced and orderly Figure 7.

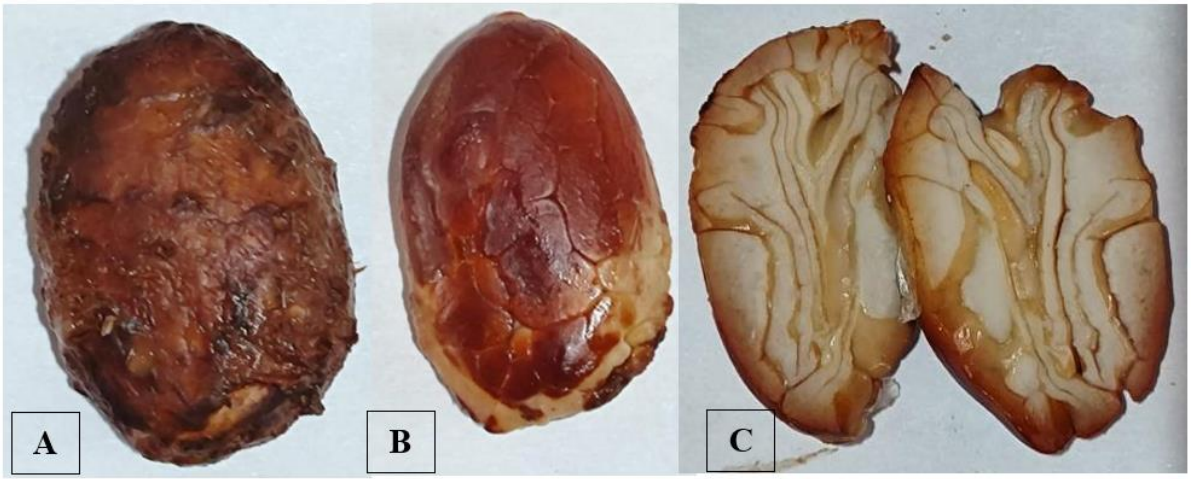


**Figure 7.** A Criollo cocoa bean; A. with mucilage, B without mucilage, and C transversely cut at the 5 days.

Figure 8 shows that the external structure of the Criollo cocoa bean is significantly dehydrated on day 6 of fermentation, displaying a reddish color after cleaning the dehydrated mucilaginous layer. The external appearance retains its ivory color. The internal grooves on the cotyledons become more prominent, outlining the well-defined internal striations that will be visible once the bean is fully dry.

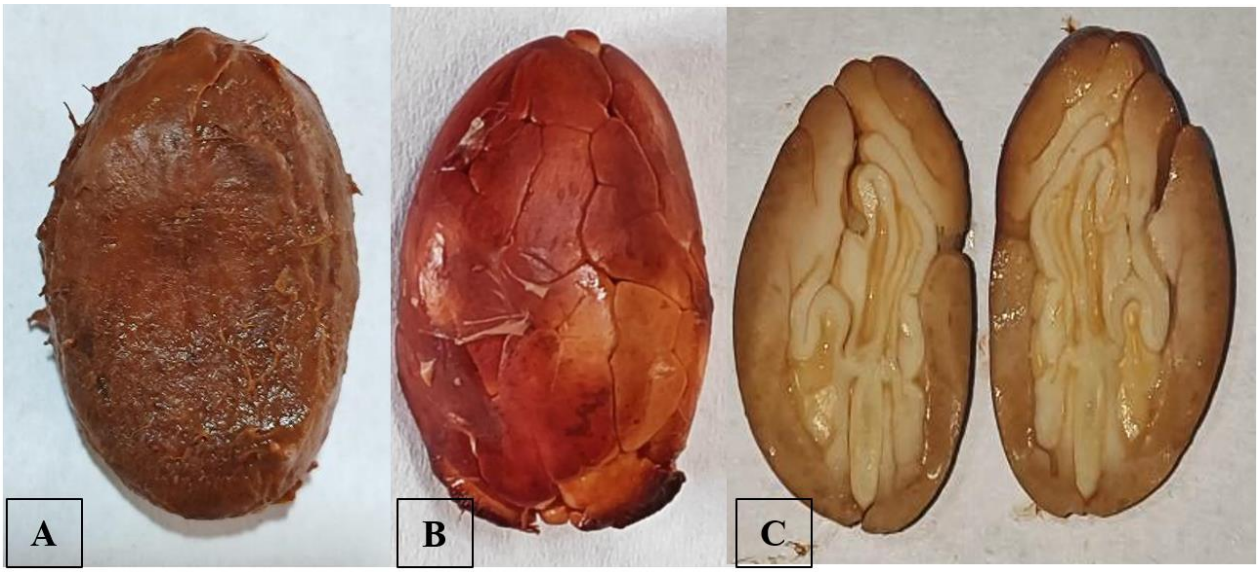


**Figure 8.** A Criollo cocoa bean; A. with mucilage, B without mucilage, and C transversely cut at the 6 days.

Once the Criollo cocoa is dry and the cutting test is applied, the cotyledons should have a cinnamon color, defined as a "light break"; The term "light break"; refers to the light brown or cinnamon color assumed by Criollo cocoa bean cotyledons once dried; it is the definitive visual indicator of optimal fermentation and drying.

Furthermore, the internal structure (kidney-like appearance), in addition to the light brown hue, must exhibit open internal veins (distinct cracks or striations). This demonstrates that the embryo died properly during fermentation.

On the seven day (Figure 9) the beans have loss the week mucilaginous layer, intensified it redness color and the internal grooves on the cotyledons look similar to those show at the 6 days in Figure 8C.

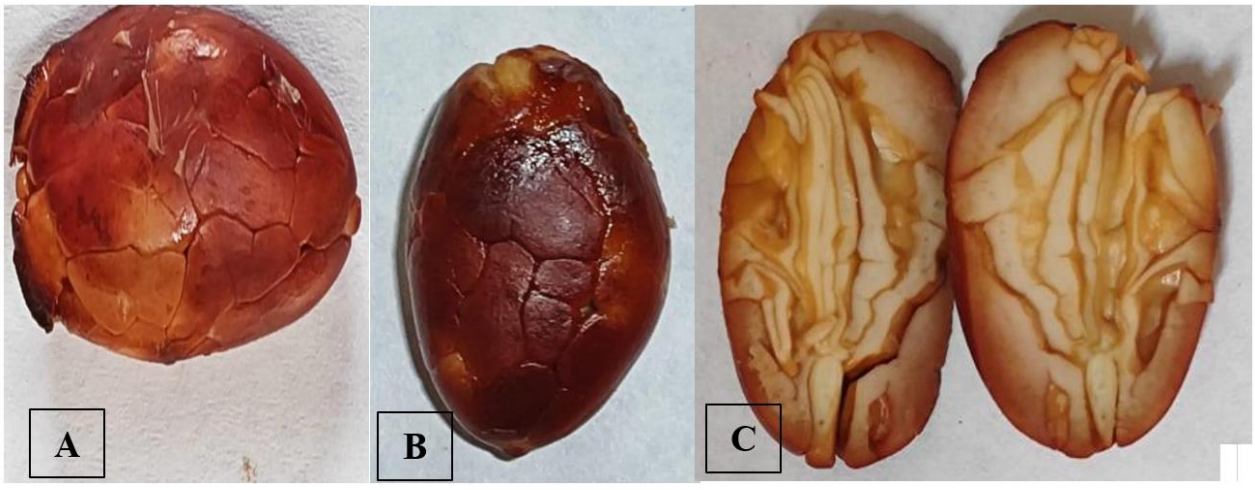


**Figure 9.** A Criollo cocoa bean; A. with mucilage, B without mucilage, and C transversely cut at the 7 days.

Figure 10 compares Criollo cocoa that is fermented (A) with cocoa that is fermented and sun dried (B). As shown in the Figure 10A, the cotyledons of Criollo cocoa exhibit a light brown color [27] that becomes darker toward the periphery, giving Criollo cacao a distinct identity that sets it apart from other types of cocoa.

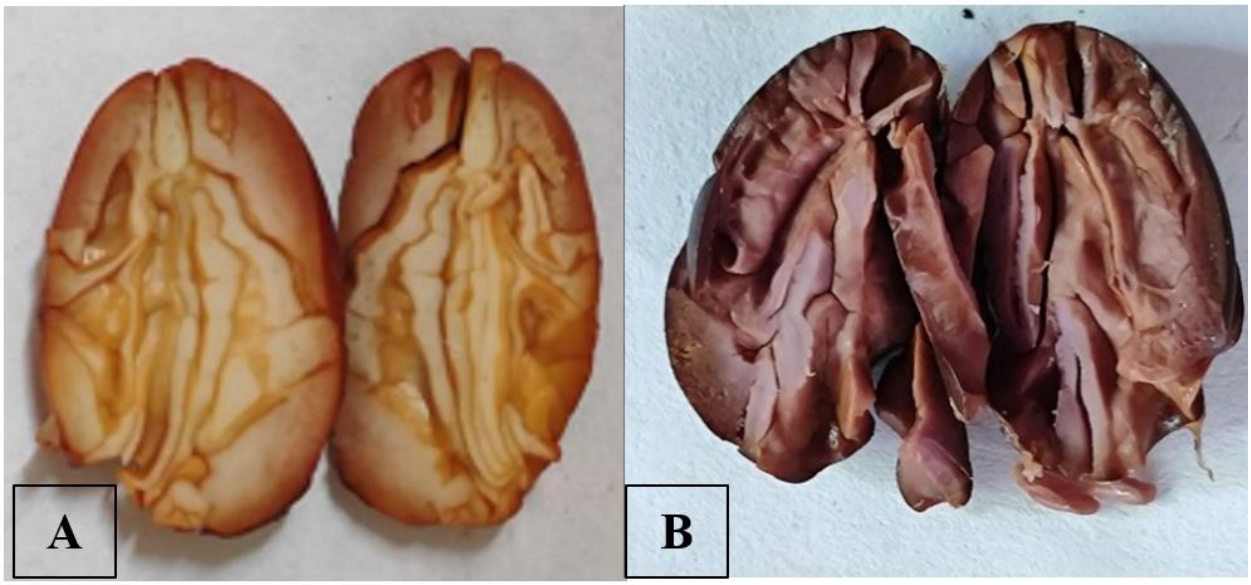


**Figure 10.** Longitudinal section of Criollo cacao: A) fermented; B) fermented and sun-dried.

## 4. CONCLUSIONS

In conclusion, in addition to chemical and biochemical changes, structural changes occur during fermentation that defines the properties of cocoa once it is fermented and dried. Furthermore, Criollo cacao exhibits an ivory internal coloration throughout the fermentation process due to the absence or low concentration of anthocyanins. Upon sun-drying, this coloration results in a light brown or cinnamon hue, giving Criollo cacao a distinct identity that sets it apart from other types of cacaos, which display a deep brown color.

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