



## Quality Assessment During the Fermentation of Cocoa Beans: Effects of Partial Mucilage Removal

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**ABSTRACT:** Fermentation of cocoa beans is the most important process contributing to the flavor in chocolate and other related products. The present study aimed to investigate the fermentation at a laboratory scale of cocoa beans with and without 10% w/w mucilage removal (whole beans). The physicochemical properties and microorganism development were monitored for six days of continuous fermentation (sampling was conducted every 24 hours). The results indicated the effects of partial mucilage removal of cocoa beans before the fermentation, in which the temperature, pH, and mucilage content (with/without mucilage removal) were recorded as 36.5 °C/38.6 °C, 3.44/3.31, and 18.41%/21.84%, respectively at the final day. Besides, the density of microorganisms (yeast-mold, lactic acid bacteria, and acetic acid bacteria) of cocoa beans with partial mucilage removal was higher than whole cocoa beans due to the increased aeration of the beans with mucilage removal, creating favorable conditions for the growth of microorganisms. After the fermentation, several physicochemical properties of the two cocoa bean types were compared, which demonstrated the more favorable quality of the cocoa beans with partial mucilage removal compared to the whole cocoa beans for the fermentation, e.g., lower seed shell content (14.1% vs. 17.8%), lower total acid (1.67% vs. 2.77%), and pH of around 5.0.

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Cocoa is a perennial industrial plant suited to tropical climates (Everaert et al., 2020). Cocoa beans are used as raw materials to create products, e.g., cocoa powder, chocolate, cocoa butter, etc. (Keen, 2001, Dias et al., 2007). Cocoa is rich in polyphenols and contains nutrients, such as carbohydrates, fat, protein, vitamins, and minerals (Sorrenti et al., 2020, Manzano et al., 2021). Cocoa helps to prevent intestinal diseases and reduce the risk of colon cancer (Martín et al., 2016, Latif, 2013). Typically, the flavanols in cocoa and chocolate assist in increasing blood circulation as well as reducing the risk of cardiovascular disease. The antioxidants in cocoa beans absorbed into the human body are consumed slowly, causing insulin to be secreted regularly to stabilize blood sugar levels

(Chen et al., 2022). Some studies have shown that dark chocolate or cocoa could lower blood pressure and decrease fatigue (Darand et al., 2021, Coe et al., 2022). The quality of final cocoa beans is related to important factors, including varieties, cultivation, and especially processing techniques (fermentation and drying) (Perez et al., 2021, Chagas Junior et al., 2021). Proper fermentation techniques are essential to obtaining qualified cocoa beans (Guzmán-Alvarez, 2021). Cocoa mucilage is an important substrate metabolized during fermentation by a chain of bacteria and fungi. Changes in the mucilage layer can affect acid production by yeast and acetic acid bacteria. Therefore, the mucilage membrane layer plays an essential role in influencing the quality of cocoa beans

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after fermentation. The effect of mixing and fermentation time on the microbiota of cocoa beans was also mentioned in the report of Hamdouche et al. (2019). Romanens et al. (2018) compared the quality of 1 kg laboratory-scale and 300 kg farm-scale fermented cocoa beans in Honduras, showing that the quality was relatively similar. Cocoa trees were introduced to Vietnam quite early, but today cocoa is still a new crop here (Thanh et al., 2019). However, according to the analysis of many experts, Vietnam has many development opportunities to compete in the global cocoa market (Nguyen, 2019, Everaert et al., 2020). A few domestic studies refer to fermentation in cocoa beans. Specifically, in 2018, the densities of lactic acid bacteria and acetic acid bacteria involved in cocoa fermentation were isolated (Hue et al., 2018). In addition, strains of acetic acid were isolated after five days of fermentation at 37°C (Huynh et al., 2019). With more and more products on the market using commercial cocoa beans, it is of great significance to understand the effect of the amount of mucilage membrane on the fermentation process and the physical quality of cocoa beans commercially available in Vietnam. The objective of the present study was to investigate the cocoa fermentation process at an experimental scale to compare two types of cocoa beans: with and without partial mucilage removal (whole beans). Changes in terms of physicochemical properties (temperature, pH, and mucilage content) and the microorganism density were recorded during the fermentation.

## MATERIALS AND METHODS

**Cocoa material and fermentation:** Trinitario cocoa was purchased from Tien Giang Province, Vietnam. The ripe yellow fruits were selected for the fermentation survey. Cocoa beans were separated from the pods and placed in vats for fermentation. Cocoa beans were continuously fermented for six days, mixed once a day, and sampled every 24 hours.

The temperature of the incubator, the pH, the content of mucilage, and the density of microorganisms were determined. After the fermentation, the cocoa beans were dried to a moisture content of 7.5% before sensory and physicochemical analyses (pH and total acid).

The experiment was conducted based on comparing cocoa beans without (whole beans) and with 10% w/w mucilage removal.

**Moisture content:** The present study's analytical results were expressed as dried weight by determining moisture content. Briefly, 10(±0.0001) g of the sample was weighed and ground into a porcelain cup. Then, the sample was put in the oven and dried at 105°C until the constant mass. The moisture content of the sample (X) was calculated using the formula:

$$X = \frac{m_1 - m_2}{m_1 - m} \times 100$$

X: moisture content (%); m: mass of porcelain cup (g); m<sub>1</sub>: mass of cup and sample before drying (g); m<sub>2</sub>: mass of cup and sample after drying (g).

**Fermentation temperature:** The portable thermometer was used to monitor the temperature of the fermented cocoa mass every 24 hours by inserting the thermometer stick straight into the bean block. Samples were measured once at three diagonal points of the fermenter, at a depth of 10 cm.

**pH:** 10(±0.0001) g of the ground sample was weighed into a 150-mL beaker. Then, 90 mL of boiling water was slowly added, and the mixture was stirred until no cluster was observed.

The mixture was filtered through Whatman filter paper No. 1. The pH was measured immediately by pH electrode (Mettler Toledo Seven Excellence, Germany), which was pre-calibrated using pH of 4, 7, and 10 standard buffer solutions (NIST scale).

**Mucilage content:** The content of the mucilage membrane was calculated according to the formula:

$$CN (\%) = \frac{M_1 - M_2}{M_1} \times 100$$

CN (%): the content of mucilage membrane in the grain; M<sub>1</sub>: sample weight before removing the mucilage membrane layer; M<sub>2</sub>: sample weight after removing the mucilage membrane layer.

**Density of microorganisms:** 30(±0.0001) g of sample was used to evaluate the density of microorganisms, i.e., the total number of yeast cells-molds, lactic acid bacteria, and acetic bacteria.

**Total acid:** 150 mL of distilled water was added to 10(±0.0001) g of the sample. The mixture was placed in a water bath at 80°C for 15 min. The solution was then cooled to room temperature and transferred to a 250-mL volumetric flask. The solution was made up to the calibration mark by distilled water, shaken well, and allowed to be settled before being filtered through Whatman filter paper No. 1. After that, 25 mL of the solution was pipetted into a conical flask, added three drops of 0.1% phenolphthalein, and titrated against 0.100 M NaOH solution. The total acid was calculated according to the formula:

$$\text{TA (\%)} = \frac{V \times K \times V_2 \times 100}{V_1 \times m}$$

TA: total acid (%); V: volume of sodium hydroxide 0.100 M (mL); V<sub>1</sub>: volume of solution pipetted for the titration (mL); V<sub>2</sub>: volume of volumetric flask (mL); K: corresponding acid coefficient (citric acid: K = 0.0064); m: sample weight (g).

**Number of seed and seed shell percentage:** The sample was thoroughly mixed and quadrupled, taking two portions at right angles so that one portion fitted only about 300 beans. The actual number of whole beans after removing the flat and broken seeds was weighed. The number of seeds was calculated as follows:

$$\text{Number of seeds} = \frac{\text{Number of whole bean (g)}}{\text{Mass of bean (g)}} \times 100$$

The seed shell percentage was determined as follows:

$$\text{Seed shell} = \frac{\text{Mass of coat bean (g)}}{\text{Number of whole bean (g)}} \times 100$$

**Defective cocoa beans:** The degree of fermentation greatly affects the taste and quality of cocoa. Defective beans can cause undesired taste and indicate the degree of fermentation. The cocoa beans were split in half along the length of the beans, and the number of brown, purplish, purple, gray callus and moldy beans was determined.

**Statistical analysis:** Experiments were performed with three replicates. The average and standard deviation values were calculated. The SPSS Version 20 software was employed for the t-test to compare the mean difference between surveyed results at a 5% significance level. The charts were processed by Microsoft Excel software.

## RESULTS AND DISCUSSION

Temperature and pH play an important role in the existence and growth of microorganisms. Especially in cocoa fermentation, the fermentation process with the development of different microorganisms, the temperature at the beginning as well as during the fermentation process has a certain effect on fermentation and grain quality after fermentation.

**Changes in temperature of cocoa beans during fermentation:** Figure 1 shows the change in temperature of cocoa beans during fermentation. Cocoa beans with 10% w/w mucilage removal had a higher temperature than non-mucilage removal beans for the first three days. This was the period when the properties of beans were incubated strongly changed due to the conversion of sugar in the mucilage membrane into ethanol, then it became acid. The energy generated in this process causes the particle temperature to rise.

The temperature of non- mucilage removal beans was the highest after 4 days of fermentation (40.3°C), and beans with 10% mucilage removal reached their maximum after 3 days of fermentation (41.2°C). Both results were lower than the temperature of the fermented beans in the Dominican Republic, 51°C after two days (Gálvez et al., 2007), and the temperature of the fermented beans in Can Tho Province (Vietnam) at 44.7°C (Tung et al., 2006). The lower fermentation temperature of cocoa in the experiment than in other studies could be explained by the smaller mass of fermented beans and the different mixing processes.

After reaching the maximum temperature, both types of cocoa beans began to decrease slightly towards the end of the fermentation process because most of the sugar in the mucilage membrane layer was converted to ethanol. As shown in Figure 1, the temperature drop was observed in 2 days and 1 day for cocoa beans with 10% w/w mucilage removal and whole bean, respectively.

This showed that the partial removal of mucilage membrane would enhance the process of converting alcohol into acid in cocoa beans. On the last day of incubation, the temperature of the two beans increased slightly because the acids formed began to penetrate the cocoa bean kernel, hydrolyzing the protein to amino acids (Oracz and Nebesny, 2019). The temperature variation trend during fermentation in the present study was similar to other publications (Tung et al., 2006, Samagaci et al., 2014).

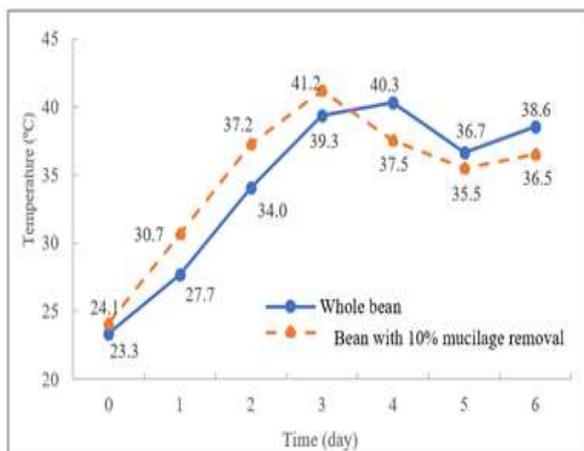


Fig 1: Changes in temperature of cocoa beans during fermentation

*pH changes during fermentation:* The changes in pH value during fermentation of cocoa beans were because sugars in the cocoa mucilage were converted to lactic acid and ethanol to acetic acid, which then diffused into the nucleus (Apriyanto et al., 2019). The pH of cocoa beans gradually decreased from the beginning of fermentation, indicating an acidic reaction occurred during fermentation. The results presented in Figure 3 show that in the first two days, the pH value dropped from 4.34 to 2.94 (without mucilage removal) and from 4.33 to 2.91 (beans with 10% w/w mucilage removal). After two first days, the pH increased slightly to 3.31 and 3.44 for whole beans and partial mucilage removal, respectively. The obtained pH values during the fermentation in the current study were lower than that of the Dominican Republic cocoa beans, pH of 4.48 (Gálvez et al. 2007).

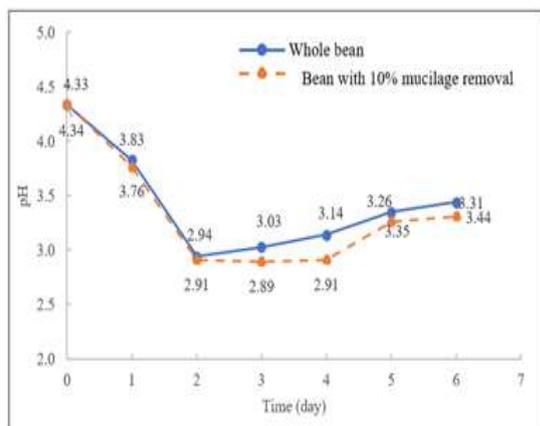


Fig 2: Changes in pH of cocoa beans during fermentation

*The changes in mucilage content during fermentation:* The reduced mucilage content increases the aeration of the bean mass, creating favorable conditions for the growth of microorganisms, thus leading to a difference in bean quality after fermentation and drying

(Guzmán-Alvarez, 2021). Figure 3 shows that the mucilage content decreased sharply and steadily from day 1 to day 3, i.e., 40.20-29.27% (whole bean) and 30.48-21.63% (with 10% w/w mucilage removal). Because the mucilage was partially removed before the fermentation, in the first 3 days, the decrease of mucilage content was basically due to the strong hydrolysis and oxidation reactions. Meanwhile, the reduction rate of mucilage content for the whole bean was high, which might be primarily due to the loss of steam.

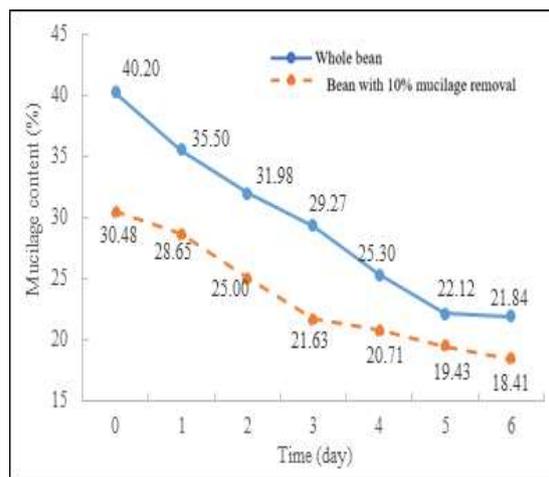


Fig 3: Change of mucilage content of cocoa mass during fermentation

*Changes in microbial density during fermentation:* Various microorganisms were present in cocoa bean fermentation, including yeasts and bacteria (Salazar et al., 2022). However, depending on the characteristics of the raw materials, the initial density and number of microorganisms can differ. Fermentation plays an important role in the production of chocolate and its products thanks to the development of characteristic odorants and flavors through the action of microorganisms (Voigt et al., 2018), including yeast (Figure 4a), lactic acid bacteria (Figure 4b), and acetic acid bacteria (Figure 4c). In the current study, the yeast-mold and acetic acid bacteria were the highest on the third fermentation day, then gradually decreased. The density of lactic acid bacteria reached the maximum value on the second fermentation day and then gradually reduced. Generally, the density of yeast-mold, lactic acid, and acetic acid of cocoa beans with partial mucilage removal was higher than whole cocoa beans. This was because the aeration of the beans with mucilage removal was more than the whole beans, creating favorable conditions for the growth of microorganisms. The obtained results were similar to the fermentation trend of Indonesia's cocoa beans (Rahayu et al., 2021).

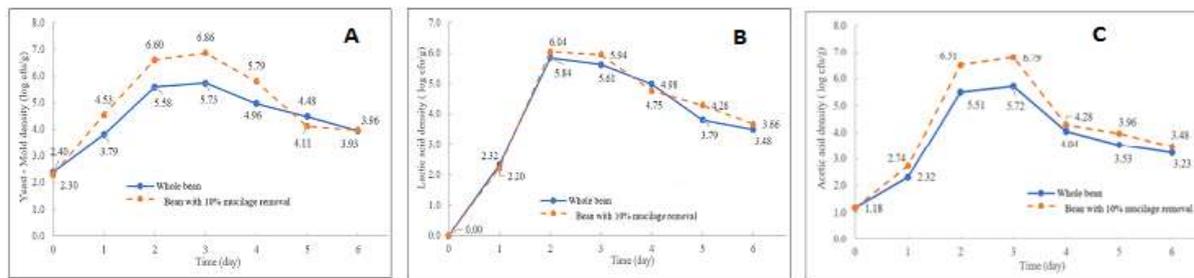


Fig 4: Density of (a) yeast-mold, (b) lactic acid bacteria, and (c) acetic acid bacteria during fermentation

*Physicochemical properties of cocoa beans after the fermentation:* After fermentation, beans with partial mucilage removal had a dark brown color, less chocolate flavor, and less sour taste than whole beans. The taste of cocoa beans is exhibited through sour and acrid taste and related to the pH of the beans, in which the higher the pH is, the lower the acidity is, resulting in the better quality of cocoa beans. Table 1 indicates that both two types of cocoa beans (with and without partial mucilage removal) met the standard grade 1A specified in Vietnam's National Standard (TCVN 7519, 2005). The low percentage of seed shell resulted in better bean quality for chocolate production, in which whole beans accounted for a higher proportion compared to cocoa beans with mucilage removal (17.8% vs. 14.1%), indicating that removing the mucilage before fermentation has an effect on the later kernel shell content. The pH and total acid value represented the acidity of the dried cocoa beans. Dry cocoa beans were considered suitable for chocolate processing, with pH values of around 5 (Apriyanto, 2016). Based on the results of the analysis of physicochemical parameters, it could be further confirmed that the more favorable quality of the cocoa beans with partial mucilage removal compared to the whole cocoa beans

Table 1: Quality comparison of two types of dried cocoa beans

	Whole beans	Beans with 10% w/w mucilage removal
Number of seed (100 g)	88	87
Humidity (%)	6.61	6.24
Seed shell (%)	17.8	14.1
pH	4.19	5.06
Total acid (%)	2.77	1.67
Classification (TCVN 7519, 2005)	1A	1A

*Conclusions:* The results show that the cocoa beans with partial mucilage removal demonstrate better behaviors in terms of metabolic reactions compared to the whole beans. The lower content of mucilage before the fermentation assists the metabolism of essential substances, such as ethanol, acetic acid, etc., promoting the biochemical reactions to form precursors of cocoa flavor (reducing sugars, amino acids, polyphenols, etc.), resulting in better cocoa bean

quality after the fermentation. For further study the experimental setup can be applied on a larger scale to obtain well-qualified products.

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