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Effect of heated fermentation and separation of cocoa juice from cocoa pulp on the quality of fermented cocoa beans

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Abstract

The fermentation of cocoa beans is a key process that shapes chocolate flavor. This study aimed to assess how various fermentation parameters influence cocoa bean quality. The main parameters of interest were the temperature of cocoa beans during fermentation, which was controlled with a heating chamber, and the presence in the fermentation substrate of cocoa juice, which can be separated from the cocoa pulp before fermentation. Aeration frequency (every day or every 2 days) and the turning start times (day 1 or day 2) were varied. Washing the fermented beans before drying was also monitored for the effecting to quality of dried cocoa beans. The temperature, pH value, bacterial count and composition of the cocoa beans were measured, and a standard cut test was performed. Results indicated that cocoa bean temperature in the fermenter was consistently 5°C higher than the heating chamber setting. The cut test confirmed that separating the cocoa juice from the pulp before fermentation gives the best bean quality, with a success rate of 86–89%. In addition, the fermented beans had higher total phenolic and total flavonoid contents, and higher antioxidant activity compared to beans that were fermented with the cocoa juice. Separating the cocoa juice from the pulp, turning the beans every day starting from day 1, and washing the beans before drying resulted in higher fermentation temperatures and lower fermentation substrate acidity, thereby reducing the acidity of the beans. These improvements in the fermentation process ensure the production of high-quality fermented cocoa beans.

Keywords: Cocoa juice, Cocoa pulp, Fermented cocoa beans, Fermenter, Heating chamber

1. Introduction

The fermentation and drying of cocoa beans are essential steps in the production of chocolate. Fermentation produces biochemical changes that improve the flavor and aroma of the cocoa beans while reducing their bitterness [1]. Well-performed fermentation is a prerequisite for the production of high-quality chocolate [2]. Drying ensures that the fermented cocoa beans reach the correct moisture content (MC) to prevent mold growth and ensure quality. However, commercial-grade dried cocoa beans must meet several requirements, including acidity and polyphenol content [3], which depend on the fermentation process.

Cocoa beans are surrounded by a pulp that consists of 82–87% moisture, 10–15% sugar, 1–3% citric acid and 1–1.5% pectin. During fermentation, the pulp drains naturally and the remaining sugars it contains are converted by the fermentation process into acetic acid. The liquid that drains from the pulp forms cocoa juice which, besides sugars, contains proteins, amino acids, vitamins (mainly vitamin C) and minerals [4]. The cocoa juice is an important source of macronutrients for microbial growth [5], but an excess of cocoa juice leads to an increase in the acidity of the fermented cocoa beans and thus to a reduction in flavor and quality [6]. The separation of cocoa juice from the pulp can help to reduce the acidity of the fermented cocoa beans and produces a useful by-product.

There are two important phases in the fermentation of cocoa beans: an anaerobic phase and an aerobic phase. The anaerobic phase lasts 48–72 h after the cocoa pod is broken open and involves the activity of naturally-occurring yeasts [7]. During the following aerobic phase of fermentation, the substrate containing the beans and pulp is turned to equalize the temperature, enhance aeration for oxygenation, boost the metabolism of acetic acid bacteria (AAB), and raise the peak fermentation temperature to speed up the fermentation process. During fermentation, the temperature of the substrate rises from 45 to 50°C and the action of microorganisms and enzymes develops the flavor of the beans [8]. The microorganisms are vital in transforming the raw cocoa beans into a product with a desirable flavor, aroma and texture, and contribute only to optimizing cocoa bean quality during the fermentation process.

Yeasts begin the fermentation process by breaking down sugars in the cocoa pulp, to produce ethanol. The conversion of ethanol into acetic acid by AAB during the aerobic phase shaped the flavor of the beans. The cocoa bean fermentation process is heavily impacted by temperature conditions, with the optimal range for microorganisms to perform fermentation being 42–45°C. Therefore, the use of controlled heating to maintain this temperature throughout the fermentation process is essential. Among the most important changes that cocoa beans undergo during fermentation is the degradation of polyphenols, which play an active role in determining the color and bitter, astringent taste of chocolate [9,10]. The pH value and acidity of the substrate during fermentation determines the quality of the cocoa beans after fermentation [11]. The presence of certain conditions that can cause off-flavors can be revealed by an assay known as the cut test, which also indicates the degree of fermentation, which decides the final flavor and quality of the beans. The cut test procedure of the International Standards Organization requires that beans be opened or cut lengthwise down the middle to expose the maximum surface of the cotyledon so that a thorough evaluation of bean quality can be made [12].

The aim of this study was to investigate the fermentation parameters affecting the quality of fermented cocoa beans. The temperature of cocoa beans in the fermenter was controlled with a heating chamber to recreate the optimal temperature profile of natural fermentation. Fermentation was also performed without controlled heating. The varied parameters of fermentation were the separation of cocoa juice from cocoa pulp before fermentation, the timing and frequency of turning cocoa beans in the fermenter, and the washing of fermented beans before drying. The standard cut test was performed to evaluate the quality of dried beans, and the temperature, pH, bacterial count and composition of the cocoa beans were measured.

2. Materials and methods

2.1 Preparation of cocoa beans

Cocoa pods were harvested in Songkhla Province, Thailand. The pods were cut open and the beans extracted manually. The selected cocoa beans were cloudy white to light brown in color, exhibiting a white fuzz with cocoa nectar on the outside.

2.2 Fermentation process and monitoring

A fermenter with a 2-liter capacity was filled with 1 kg cocoa beans and tightly sealed to create an anaerobic environment. The fermenter was initially left at room temperature for one day and then heated with a temperature-controlled heating chamber to maintain a temperature of 40–50°C for 7 days. A temperature gradient was programmed into the heating chamber that held a temperature of 45°C on day 1, a temperature of 50°C on days 2 and 3, 45°C on day 4, and 40°C on days 5 and 6**.** These setpoint temperatures were consistent with those obtained from natural cocoa bean fermentation. The variables studied included the separation of the cocoa juice from the cocoa pulp before fermentation, the frequency of substrate turning (every day or every 2 days), the starting time of turning during fermentation (on day 1 or day 2) and the washing of the fermented cocoa beans before drying. The experimental treatments are shown in Table 1. The temperature and pH of the substrate were measured throughout the fermentation process. Cocoa beans contain embryos capable of germinating into new plants. Metabolic processes that occur during fermentation increase the internal temperature and organic acids in the beans, which result in the death of the embryo and determine the completion of fermentation, which is assessed by the cut test. Embryo death serves as a critical marker for fermentation completion, indicating that the process has significantly altered the biochemical properties of the beans, to produce the desired flavor and quality for chocolate. The pH of the substrate and beans was measured with a pH meter, while the temperature in the center of the fermenting pulp was measured with a digital thermometer. The colony count of bacteria in the fermentation substrate was performed at the Division of Biological Sciences, Faculty of Science, Prince of Songkla University, Hat Yai Campus, Songkla, Thailand.

Table 1 Experimental design for the fermentation of cocoa beans using a temperature-controlled system.

Experiment	Cocoa juice separated before fermentation	Turning start time	Frequency of turning the beans	Washing fermented cocoa beans before drying
1	With cocoa juice	Day 1	Every 2 days	Unwashed beans
2	With cocoa juice	Day 2	Every day	Unwashed beans
3	With cocoa juice	Day 2	Every 2 days	Unwashed beans
4	With cocoa juice	Day 2	Every 2 days	Washed beans
5	Without cocoa juice	Day 1	Every 2 days	Unwashed beans
6	Without cocoa juice	Day 2	Every day	Unwashed beans
7	Without cocoa juice	Day 2	Every 2 days	Unwashed beans
8	Without cocoa juice	Day 2	Every 2 days	Washed beans

2.3 Cocoa bean acidity (pH)

Samples of the dried, fermented cocoa beans from all experiments were peeled and ground into nibs. Five grams of ground nibs mixed with 45 mL of hot distilled water were passed through Whatman No. 4 filter paper and the filtrate was cooled to 20–25°C. The pH of the filtrate was measured using a pH meter (Schott Instruments, Mainz, Germany) [13].

2.4 Cut test measurements

The cut test is the standard method for assessing the quality of beans after fermentation and for determining the success of fermentation for further processing [14]. A total of 100 beans per experiment were cut lengthwise down the center to expose the maximum cut surface of the cotyledons according to the method described by Ogundare et al [15]. Both halves of each bean were visually examined in full daylight and, using a standard colorchart, categorized according to the color of the cross-section — fully brown, partially purple-brown, fully purple and slate. The percentage of each color category was calculated to determine the Cut Test Score (CTS) as shown in Equation 1.

$$CTS = (1 \times A_0) + (0.5 \times A_1) + (0 \times A_2) \quad (1)$$

where A_0 = fully brown beans, A_1 = partially purple-brown beans and A_2 = fully purple and slate beans.

2.5 Preparation of cocoa extracts

Five grams of ground nibs were soaked in 50 mL of distilled water for 30 min at room temperature with frequent stirring before centrifugation at 4,000 rpm for 5 min. The extract solution was kept at 4°C for analysis.

2.6 Determination of phenolic contents

The total phenolic contents of individual cocoa extracts were determined using the Folin–Ciocalteu method. In brief, 0.5 mL of extract solution was mixed with 9.5 mL of distilled water and 0.5 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 2.0 mL of Na_2CO_3 (10%) were added to the mixture and incubated for 2 h. The absorbance was measured with a UV spectrophotometer at 730 nm against a blank without extract. The results were expressed as milligrams of gallic acid equivalents per gram of cocoa bean (mg GAE/g).

2.7 Determination of flavonoid contents

The flavonoid content of cocoa extracts was determined using a colorimetric method [16]. An aliquot of 0.5 mL of extract solution was mixed with 0.15 mL of 5% $NaNO_2$ and incubated for 5 min. After adding 0.15 mL of 10% (w/v) $AlCl_3$ and incubating for 5 min, 1 mL of 1M NaOH and 3 mL of distilled water were added. The mixture was then incubated at room temperature for 30 min and the absorbance was measured at 510 nm and compared to a blank. The results data were expressed as mg/g quercetin equivalents (mg QE/g) of cocoa bean.

2.8 DPPH radical scavenging activity

The antioxidant activity of cocoa extracts was measured by evaluating free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [17]. In brief, 2 mL of extract solution were added to 2 mL of 0.1 mM DPPH solution. The mixtures were set aside in a dark room for 30 min, and then the absorbance was measured at 517 nm against a blank containing an equal amount of DPPH and ethanol. The percentage of DPPH scavenging was calculated using the following equation (Equation 2):

$$\% \text{ scavenging of DPPH} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

where A_0 = absorbance of the control and A_1 = absorbance of the test extracts.

2.9 Statistical analysis

Each experiment was performed in triplicate. All data were analyzed using ANOVA and Duncan's new multiple range test to distinguish means and differences. Statistical analysis was considered significant at $p<0.05$ using SPSS version 12.0.

3. Results and Discussion

3.1 Effect of cocoa bean fermentation with heating chamber

The fermentation of cocoa beans, both with and without a heating chamber, was performed over a period of 7 days and the results were compared. The temperature of cocoa beans in the fermenter without heating was found to remain nearly constant at 30–33°C, indicating that the fermentation was not successful. With heating, the temperature of the cocoa beans gradually increased during fermentation, peaking at 55°C on day 3, which exceeded the temperature of the heating chamber (50°C) by 5°C (Figure 1). During this period, microorganisms initiated an exothermic reaction in which sugar was converted into ethanol [18]. Consequently, the temperature in the fermenter was about 5°C above the set temperature in the heating chamber for a period of 3 days. Subsequently, the temperature decreased towards the end of fermentation on days 6 and 7. The decrease in temperature was attributed to the consumption by microorganisms of all the available nutrients in the cocoa bean pulp, reducing the amount of heat generated by the metabolism of sugar and oxidation of ethanol [19].

The heating chamber enabled the substrate in the fermenter to reach the required temperature, ensuring successful fermentation. The heating chamber also acted as an insulator, preventing heat loss as well as promoting a higher fermentation temperature [20]. This type of fermenter makes it possible to achieve a high-quality product from a small amount of cocoa beans without fail.

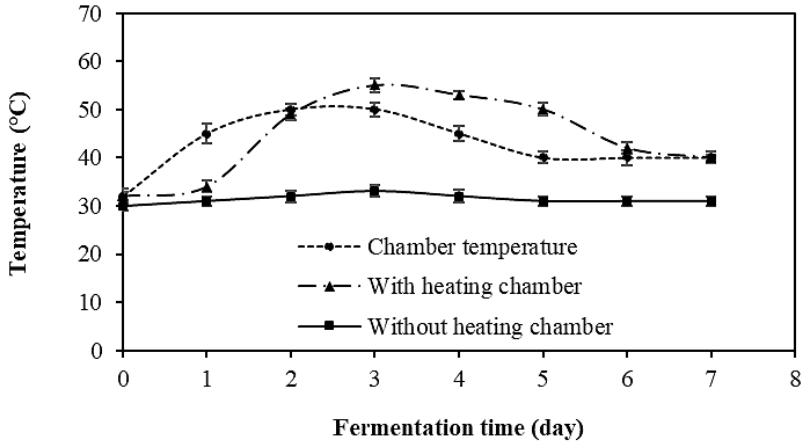

Figure 1 The temperature of cocoa bean fermentation substrate with and without controlled heating.

3.2 Effect on fermentation temperature of separating cocoa juice from cocoa pulp

The effects on fermentation of separating the cocoa juice from the cocoa pulp were investigated. A comparison was made with the fermentation of cocoa beans with the juice. The results showed significant differences ($p < 0.05$) in fermentation temperature between the experimental groups. Fermentation temperatures were higher when the cocoa juice was separated, especially temperatures on the third day (Figure 2). A higher quality, accelerated fermentation was obtained without the juice, which was attributed to the increased activity of enzymes in the cocoa beans, which effectively break down the flavor precursors at 45°C [17]. After separating the cocoa juice, there was less sugar in the substrate, which allowed for a faster decomposition of the cocoa pulp. This, in turn, accelerated ethanol production and promoted faster oxidation to acetic acid, resulting in more complete fermentation, and significantly improving the overall fermentation process. Additionally, fresh cocoa juice, which has a delicious, fruity flavor, can be obtained as a byproduct.

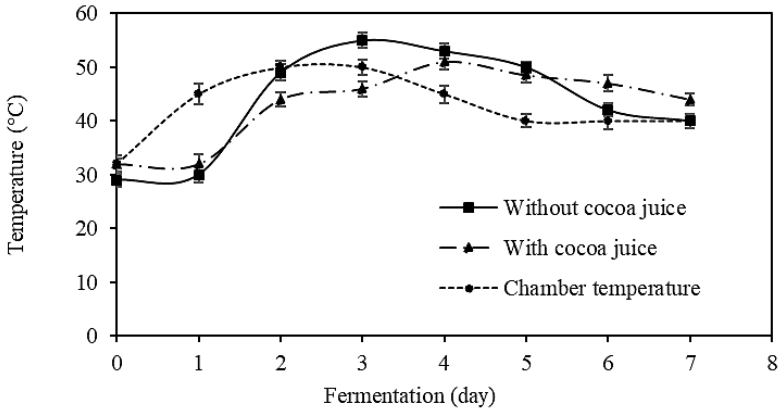

Figure 2 Effect on fermentation temperature of the separation of cocoa juice from cocoa pulp.

3.3 Effect of turning on cocoa bean pH

3.3.1 Effect of turning frequency

Whether the cocoa juice was separated or not, turning the cocoa beans every day resulted in dried beans with pH values above 5.0 whereas turning the beans every two days resulted in pH values of 4.5 (Figure 3). The difference between the results was significant ($p < 0.05$). Overall, the results showed that turning the beans every day increased bean pH during fermentation, making them less acidic. Once the fermentation environment became more aerobic, lactic acid bacteria (LAB) mediated the fermentation and converted the intermediate metabolites into lactic acids. During the final stage of fermentation, AAB oxidized the ethanol produced by yeasts and LAB into acetate, which was then further converted into carbon dioxide and water. However, acetic acid is highly volatile and seldom accumulates under aerobic conditions [21]. Therefore, turning the cocoa beans every day raised the pH of the beans more than turning the beans every 2 days.

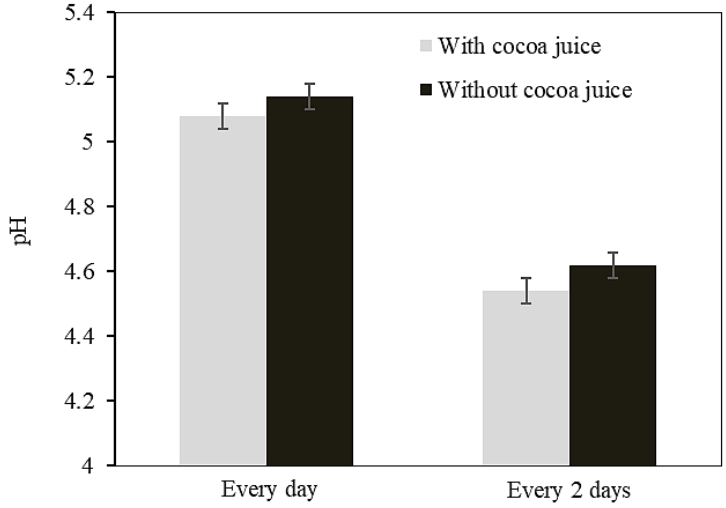

Figure 3 The effect on dried bean pH of the frequency of turning the beans.

3.3.2 Effect of turning start time on cocoa bean pH

Starting the turning of the cocoa beans on day 2 of fermentation had a significant effect ($p < 0.05$) on the pH of the dried beans, resulting in a significant increase in acidity, driven by the growing population of AAB [22]. Starting the turning of the beans on day 1 produced cocoa beans with a high pH, indicating a lower acidity more suitable for chocolate production (Figure 4). Turning the mixture encourages the settling of a sugar-rich, mucilaginous liquid from the cocoa beans at the base of the fermentation vessel, reducing the available substrate for yeast to produce ethanol for eventual conversion into acetic acid [23]. Starting to turn the beans on day 1 of fermentation reduced the stimulation of yeast growth and biodiversity whether the cocoa juice was separated or

not from the pulp. The fermentation process of cocoa beans induces microbial activity and the production of metabolites, resulting in an increase in temperature and pH values [24].

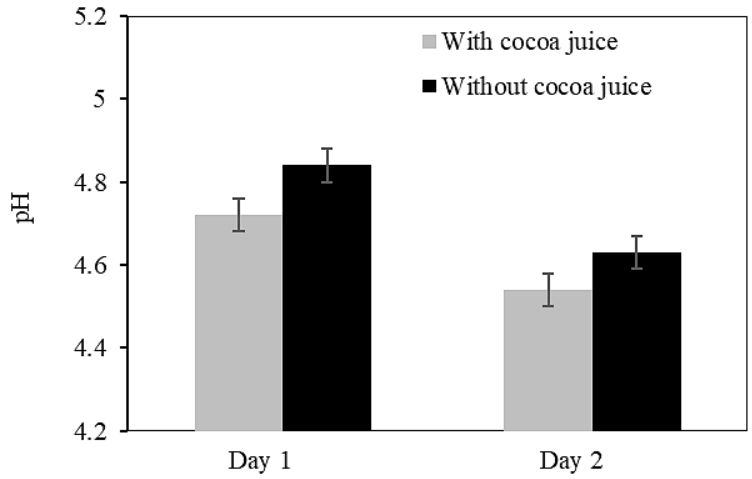

Figure 4 Effect of turning start time of cocoa beans on pH.

3.4 Effect of washing fermented cocoa beans on the pH of dried beans

Fermented cocoa beans that were washed before drying exhibited pH values which ranged from 4.78 to 4.86 after drying. These values were significantly higher than the pH values of dried beans that were not washed before drying, which ranged from 4.54 to 4.62 (Figure 5). Washing the fermented cocoa beans before drying reduces their acidity, increases browning during drying, and improves the flavor of the final chocolate [25]. Washing the fermented beans before drying might also remove acids. Drying involves the migration of volatile acids and the biochemical oxidation of acetic acid in the beans, resulting in an elevation of alkalinity [26].

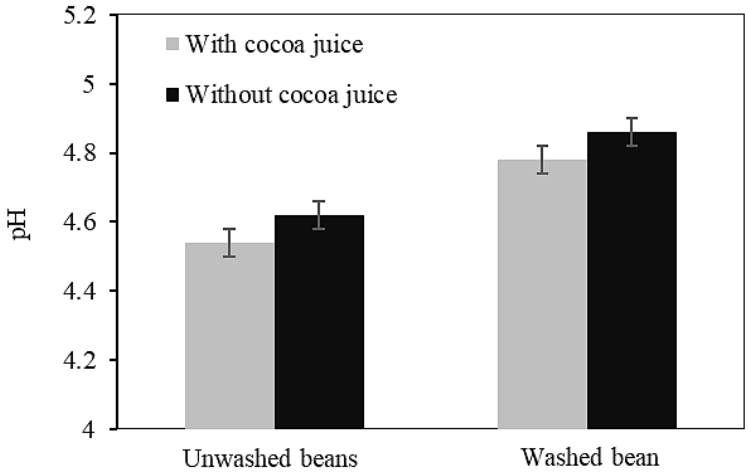

Figure 5 Effect of washing fermented cocoa beans on the pH value of dried beans.

3.5 Cut test

After 7 days of fermentation under different experimental conditions, the fermented cocoa beans were evaluated by the cut test. The results are presented in Figures 6 and 7. For treatments 5–8, cocoa beans fermented without the cocoa juice, the CTS ranged from 86% to 89%. For treatments 1–4, where were fermented with the cocoa juice, the CTS ranged from 78% to 82%. The results also show that the cocoa beans fermented without the cocoa juice before were completely brown, indicating fermented cocoa beans of the best quality. While this test may not be completely relevant for beans with low anthocyanin content, a purple interior signifies premature termination of fermentation, whereas a brown interior indicates successful completion of the process [27].

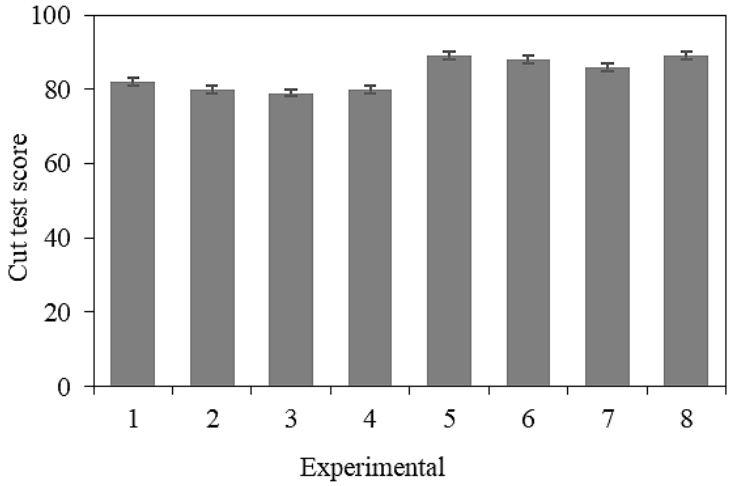

Figure 6 Determination of cocoa bean quality using cut test scores.

Figure 7 Cut cocoa beans from various fermentation treatments.

3.6 Change in bacterial population during fermentation

The total bacteria count during cocoa bean fermentation on days 2 and 4 are shown in Table 2. The fermentation substrate without cocoa juice showed low total bacterial counts of 9.2 x 10^6 and 74 x 10^6 CFU/g on days 2 and 4, respectively. The fermentation substrate with cocoa juice showed higher total bacterial counts of 62 x 10^6 and 100 x 10^6 CFU/g on days 2 and 4, respectively. During cocoa bean fermentation, bacteria and other microorganisms play a crucial role in transforming the raw beans, imparting the desired flavor and aroma characteristics. Initially, the fermentation process is initiated by yeasts, which ferment sugars in the cocoa pulp, producing ethanol. Later, bacteria, such as AAB metabolize ethanol into acetic acid, which contributes to the development of the cocoa flavor [28]. During fermentation, the acetic acid diffuses into the beans. Although some of it is lost during drying, most of it is retained. It is therefore crucial that the initial conditions and the fermentation and drying processes do not lead to excessive acid production. Separating the cocoa juice helps remove potential impurities and unwanted microorganisms, reducing microbial activity. Separating the cocoa juice before fermentation significantly reduced the population of microorganisms in the fermentation substrate ($P < 0.05$), indicating that when the microbial count was low, the production of acetic acid was also lower, leading to a reduction in the acidity of the cocoa beans. Therefore, this was considered a suitable condition for the development of good raw materials for chocolate production.

Table 2 Total bacterial count in fermentation substrate on days 2 and 4.

Experimental	Total Bacterial Count CFU/g (10^6)	
	Day 2	Day 4
Without cocoa juice	9.2±0.20[a]	74±0.15[a]
With cocoa juice	62±0.24[b]	100±0.12[b]

Note: Data were represented as the mean ± standard deviation. Mean values having a common letter within the same line and column are not significantly different according to Duncan's multiple range test at $p < 0.05$

3.7 Effect of cocoa juice on total phenolic content, total flavonoid content and antioxidant activity

The separation of the cocoa juice from the cocoa pulp before fermentation increased the total phenolic and flavonoid contents and the antioxidant activity of the dried cocoa beans. The results for antioxidant activity were significantly (p<0.05) associated with total phenolic and flavonoid content. The total phenolic content was 136.88±0.03 mg GAE/g beans, and the total flavonoid content was 626.24±0.12 mg QE/g beans, with an antioxidant activity of 82.9±0.15% (Table 3). The separation of the cocoa juice removes some undesirable microorganisms, reducing the microbial activity that can degrade phenolic and flavonoid compounds, which are beneficial compounds in the dried beans [29].

Table 3 Total phenolic content, total flavonoid content, and antioxidant activity of cocoa beans before and after fermentation under different conditions.

Condition	Phenolic mg GAE/g	Flavonoid mg QE/g	Antioxidant (%)
Before fermentation	52.35±0.42	50±0.25	51±0.19
Without cocoa juice	136.88±0.03	626.24±0.12	82.9±0.15
With cocoa juice	74.06±0.18	626.04±0.36	77.4±0.59

4. Conclusion

The results of this study showed that fermenting cocoa beans in a fermenter with a heating chamber keeps the beans at a temperature 5°C higher than the temperature of the chamber, leading to high-quality fermented cocoa beans. This method allows for the successful fermentation of small batches. The optimal conditions for cocoa bean fermentation were as follows: separating the cocoa juice from the pulp before fermentation, turning the beans every day, starting on day 1 and washing them before drying. In this condition, fermentation temperature was higher and acidity lower. The cut test showed an 86–89% fermentation success rate with fully brown beans. Additionally, this method enhanced the total phenolic content, flavonoid content, and antioxidant activity in dried cocoa beans, which are desired properties for chocolate production.

5. Acknowledgements

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