Agriculture and Food

AIMS Agriculture and Food, 9(3): 872–886.
DOI: 10.3934/agrfood.2024047
Received: 08 May 2024
Revised: 25 June 2024
Accepted: 09 July 2024
Published: 02 September 2024

http://www.aimspress.com/journal/agriculture

Research article

Effect of fermentation parameters on the antioxidant activity of Ecuadorian cocoa (*Theobroma cacao* L.)

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Abstract: Cocoa (*Theobroma cacao* L.), indigenous to the tropical forests of the Americas, is renowned not only as the primary raw material for chocolate and its derivatives (cocoa liquor and butter) but also as a rich source of phytonutrients with beneficial health effects. Current research has elucidated that within the post-harvest process, fermentation stands as the critical stage for the formation of the principal biochemical quality markers in cocoa, known as polyphenols. These compounds contribute to the bitterness and astringency that constitute the complex flavor profile of chocolate; however, their excessive presence can be organoleptically undesirable. A high phenolic content (>10%) is associated with insufficient fermentation and certain varieties of ordinary cocoa, thereby serving as a discriminatory parameter between fine-flavor cocoa (Nacional) and bulk cocoa (CCN-51). Beyond their technological significance, these components have garnered substantial scientific interest, as polyphenol consumption is associated with potential protective effects against the development of non-communicable chronic diseases (including diabetes, cancer, and atherosclerosis), attributable to their potent antioxidant properties. In this context, the objective of this study was to evaluate the impact of fermentation time on the antioxidant capacity (AC) and total polyphenol content (TPC) in the principal Ecuadorian cocoa varieties (i.e., CCN-51 clone and Nacional). Pilot-scale fermentation experiments demonstrated significant variations in antioxidant capacity (CCN-51 clone: 785.61 to 1852.78 and Nacional: 564.32 to 1428.60 µmol TE/g) and total polyphenol content (CCN-51 clone: 52.92 to 162.82; Nacional: 40.55 to 157.50 mg gallic acid/g). Both parameters decreased markedly throughout the process, with the CCN-51 clone exhibiting greater retention.

Keywords: fermentation; kernel; antioxidants; cocoa beans; CCN-51; Nacional

1. Introduction

Nowadays, there's a growing interest in researching the effects of processing on the functional properties of cocoa [1]. This interest stems from recognizing that this product is a functional food, owing to its natural antioxidant content, particularly polyphenols.

Cocoa (*Theobroma cacao* L.) is a plant cultivated in tropical regions, with its seeds or cocoa beans being its main product. These beans are used as raw material for the production of chocolates, cocoa butter, and other derivatives. The chemical composition of the seeds is mainly influenced by post-harvest management (fermentation and drying) and by genetic factors, agronomic practices, soil type, and climatic conditions. During the fermentation process, a series of biochemical reactions occur, leading to changes in the color of the beans from violet to brown and the formation of flavor and aroma precursors that impart special characteristics to high-quality chocolates [2,25].

Similarly, during fermentation, bioactive compounds decrease due to oxidation and condensation reactions leading to the formation of high molecular weight polymers (mostly insoluble tannins), as well as leaching. Consequently, as the processing time progresses, the antioxidant capacity decreases [16]. However, most producers lack standardized processes, technical support, and adequate control, resulting in cocoa beans with bitter flavors, high acidity, and astringency, along with physicochemical properties unsuitable for commercialization [8].

The antioxidant compound contents of cocoa are influenced by both genotype and fermentation. Fermented, dried, and defatted cocoa beans typically exhibit total polyphenol values ranging from 40 to 140 mg gallic acid equivalent per gram. In contrast, non-fermented beans can vary in this content from 150 to 200 mg gallic acid equivalent per gram [4,16]. In Ecuador, the most cultivated cocoa varieties are Nacional and CCN-51. The native or Nacional variety is prized for its unique flavor and aroma, whereas the hybrid CCN-51 is known for its high yield and disease resistance [5]. In this context, fermentation becomes crucial, as improper handling of this process can lead to losses of polyphenols, which are responsible for antioxidant activity and confer beneficial effects on consumers' health.

Furthermore, the color change during cocoa fermentation serves as an indicator that biochemical processes are underway within the beans. This alteration is also crucial for the subsequent drying and roasting phases. In their fresh state, cocoa beans contain anthocyanidin pigments in purple hues, specifically 3-β-D-galactosyl- and 3-α-L arabinosylcyanidins. These substances are broken down by glycosidases throughout this process, resulting in a fainter purple color, a phenomenon known as cotyledon clearing [19].

Therefore, within the cocoa production domain, fermentation exerts a direct impact on the quality of the beans, primarily used in chocolate manufacturing and serving as a crucial factor for product innovation. Hence, it's vital to precisely identify the optimal conditions and timing of the fermentation process, as well as comprehend its effect on bioactive compounds, aiming to add value and unlock new market opportunities.

2. Materials and methods

2.1. Cocoa beans

The cocoa beans utilized in the fermentation trials were sourced from the Nacional and CCN51 varieties, originating from the Lita community, situated 95 km northwest of Ibarra in Ecuador. This region boasts an altitude of 571 meters above sea level and experiences an optimal climate (ranging from 22 °C to 38 °C) conducive to cocoa production, yielding high-quality beans.

The Nacional variety is highly regarded by producers due to its short fermentation time, approximately 4 days. It stands out for its unique characteristics of fruity and floral aromas, along with hints of spices, nutty flavor, fruits, spices, and other subtle notes. Consequently, it is sought after in the production of refined chocolate, appealing to consumers [18]. On the other hand, the CCN51 hybrid requires a longer fermentation time compared to the Nacional variety. Originating from Ecuador, it was developed through the cloning of Criollo and Forastero varieties. Its distinctive features include higher yield and resistance to pests and diseases. Furthermore, its elevated fat and pulp contents in comparison to the Nacional variety result in increased demand within specific market niches for large-scale chocolate production and the development of new cocoa derivatives [10].

2.2. Sample preparation

The corn harvesting was carried out based on genotype, with the Nacional variety exhibiting a yellow hue, whereas the CCN-51 hybrid is characterized by its orange coloration [22]. Subsequently, the cocoa pods were cut open, and the seeds were extracted, followed by a meticulous selection process to eliminate any raw material contaminated with insects, mold, or diseases that could compromise subsequent processes. Ultimately, the beans underwent a fermentation process within wooden boxes.

2.3. Fermentation preparation and drying

The fermentation was carried out in greenhouse settings using wooden boxes measuring 40 x 40 x 45 cm, each equipped with approximately 12 with 1cm-sized holes on both the bottom and sides to facilitate pulp drainage. A total mass of 30 kg per box was processed. Before fermentation, cocoa beans underwent a pre-conditioning phase (pre-drying or despulping) of 24 hours and 8 hours for the CCN-51 variety the Nacional variety, respectively. This process diminished the excessive mucilaginous pulp content, intending to reduce moisture and excessive acidity levels in the kernels. Consequently, this pre-treatment resulted in beans exhibiting an enhanced organoleptic profile [9]. Subsequently, the seeds were placed in the boxes and subjected to a 7-day fermentation period within the greenhouse. Throughout this time frame, the ambient air recorded an average temperature of 28 °C and an average relative humidity of 92%.

Furthermore, to prevent insect proliferation and ensure the alcoholic phase during the initial 48 hours, the grains were covered with a layer of banana leaves before the first turning. Turnings are performed every 24 hours to enhance oxygenation and ensure homogeneity in grain mass fermentation.

2.4. Sample collection and data processing

The grain samples from the fermentation treatments were collected initially and then at 72, 120, and 168 hours. Cocoa seeds were gathered from the surface, middle, and bottom of the crates, mixed, and dried via lyophilization to facilitate subsequent analysis.

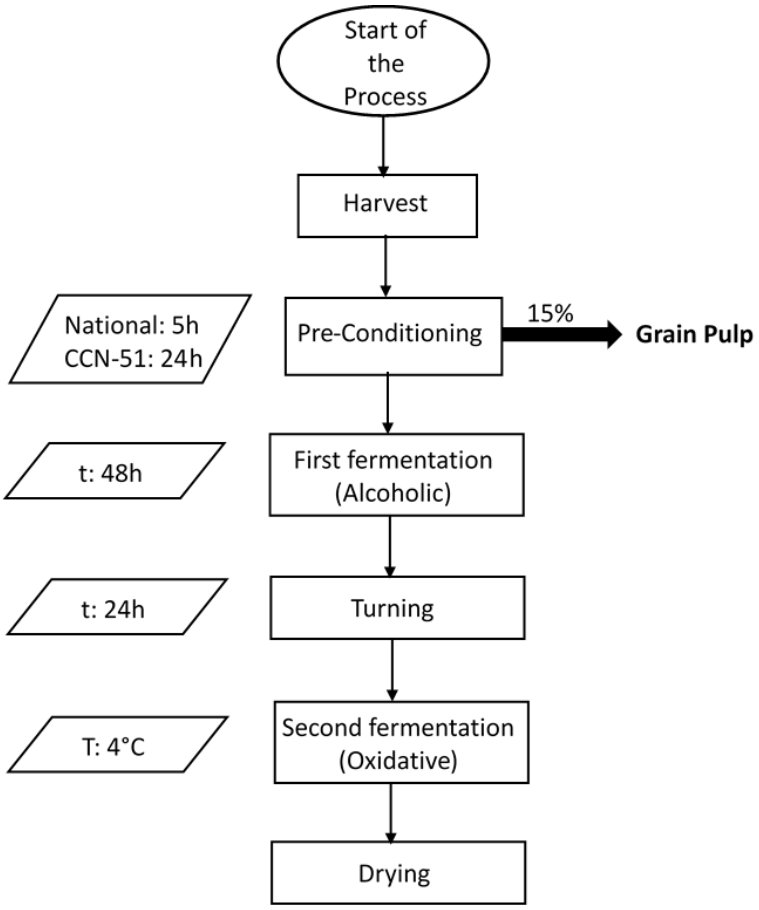

Figure 1. Fermentation process diagram.

2.5. Fermentation percentage

After fermentation, 50 grains from each test batch were randomly collected and subjected to the cut kernel test. This test was conducted using a longitudinal section to assess grain quality based on cotyledon staining and fermentation degree. The cut test results are expressed in percentage [6].

We utilized it to define the official standard for cocoa beans ISO 2451, considering its quality requirements, sampling, presentation, marking, and labeling as aspects of product classification. The cut test involves counting 100 kernels of beans. These 100 seeds are halved longitudinally and inspected. Separate counts are made for the number of beans that are defective due to being moldy, slatey, damaged by insects, and germinated. The results for each type of defect are expressed as a percentage of the 100 beans examined. The quantity of defective beans revealed in the cut test gives manufacturers an indication of the flavor characteristics of the beans.

2.6. Color

We proceeded to longitudinally cut the kernel grain samples to measure color using the NR60CP colorimeter with d/8° digital viewing illumination. We recorded the L*, a*, and b* values, from which we then calculated the chroma (C*), hue angle (h*), and color difference (ΔE), as per Equations 1, 2, and 3, respectively [7]. The hue angle (h*) expresses the relative amount of reddishness and yellowness in a sample, depicted on a 360° grid where 0° is red-blue, 90° is yellow, 180° is green, and 270° is blue. The chroma (C*ab) represents the color intensity and is measured as the distance from the origin of the coordinates.

$$h = arctg(b*/a*) \tag{1}$$

$$C* = \sqrt{a^2 + b^2} \tag{2}$$

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{3}$$

h: hue angle (Hue)
C*: chromaticity (Chroma)
ΔE: Total color difference
L*: Brightness 0 (black) a 100 (white)
a±*: + (red) to – (green)
b±*: + (yellow) to – (blue)

2.7. Temperature

The temperature during the cocoa fermentation process is a pivotal factor in determining the speed and efficiency of fermentation. Temperature readings were taken every 24 hours by inserting a thermometer into the fermentation box until the process was completed.

2.8. Solubles solids

Soluble solids comprise sugars, acids, salts, and other water-soluble compounds present in the cell juices of the fruit. Using a refractometer, the sugar content of cocoa pulp was measured for both Nacional and CCN-51 clones.

2.9. Functional properties of cocoa beans

2.9.1. Total polyphenols

The total content of polyphenols was determined using the colorimetric method with Folin-Ciocalteu reagent [21]. In a test tube, 1 mL of the extract is placed, followed by the addition of 6 mL of distilled water and 1 mL of Folin & Ciocalteu reagent. After 3 minutes, 2 mL of 20% sodium carbonate solution is added, followed by immediate agitation using a vortex, and then heated in a water bath at 40 °C for 2 minutes. The absorbance of the resulting blue-colored solution is measured at 765

nm using a spectrophotometer. Quantification is performed by interpolation on a calibration curve generated using gallic acid standards ranging from 0 to 100 ppm ($y = 0.013x + 0.0084$, $r^2 = 0.9917$), and the results are expressed in milligrams of gallic acid per gram of defatted dry sample [1].

2.9.2. Antioxidant activity

The method used to measure antioxidant capacity was ABTS+. Here's how it was done: 200µL of appropriately diluted samples were placed in a test tube with pH 7 phosphate buffer. Then, 3800µL of stabilized ABTS+ working solution was added, and the mixture was vortexed for 30 seconds. It was left to rest in darkness for 45 minutes. After this incubation period, the final absorbance of each sample was measured in triplicate at a wavelength of 734nm [10]. The quantification was carried out through interpolation on a calibration curve constructed using a Trolox standard at concentrations ranging from 0 to 700 µmol of Trolox per liter ($y = 0.0012x + 0.1073$, $r^2 = 0.9995$). The results were expressed in µmol of Trolox per gram of de-fatted sample on a dry weight basis.

2.10. Statistic analysis

The data were analyzed using a 2^3 factorial model (3 factors and 2 levels) to determine significant differences between the samples at different time points. Using InfoStat software, normality and homogeneity were assessed through Shapiro-Wilks and Fisher tests, respectively. For parametric data, we employed analysis of variance (ANOVA), whereas for non-parametric data, the Kruskal-Wallis method was utilized. Additionally, Tukey's 5% tests and the significant mean difference (SMD) were conducted for treatment and factor comparisons, respectively. Furthermore, adjusted means and standard error analysis were carried out using the LSD Fisher method ($\alpha = 0.05$) for both general and mixed models.

3. Results and discussion

3.1. Total polyphenols

The results of total polyphenol content expressed in mg gallic acid/g of defatted cocoa (Dry basis) in cocoa samples from the Lita community with different fermentation times are depicted in Figure 2.

The CCN-51 and Nacional cocoa varieties exhibited a decrease in total polyphenol content at the end of the fermentation process of 67.50% and 74.25%, respectively. However, starting from the third day, a decrease of 38% in CCN-51 and 17% in Nacional was recorded compared to the seventh day.

According to the study conducted by Pallares Pallares et al. [8], it was observed that the CCN-51 clone experienced a 50.10% reduction in its polyphenol content during the fermentation process. Additionally, Cortez [11] assessed the behavior of bioactive compounds in two cocoa varieties, demonstrating a significant decrease in this variable during fermentative development [3,12].

On the other hand, Onomo et al. [13] conducted a 6-day fermentation and achieved a final concentration of 142.51 mg gallic acid/g, a value that exceeds the one obtained in the current study. These values may vary due to agronomic practices, the geographical location of the cultivation, and the grain fermentation process [14,15].

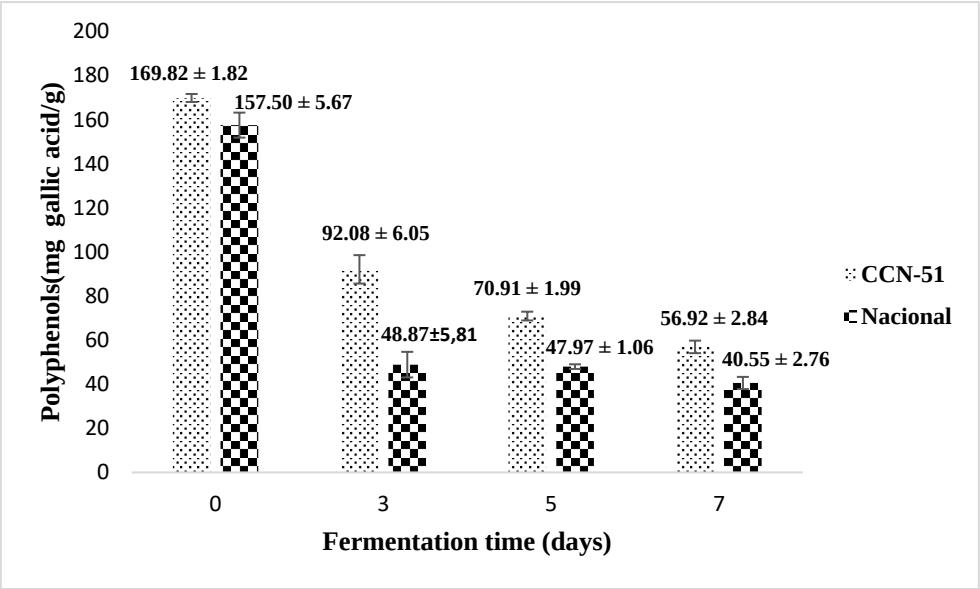

Figure 2. Polyphenol content during the fermentation time.

Notes: Mean ± standar desviation (n = 2).

Additionally, it was identified that the CCN-51 clone recorded higher polyphenol values compared to the Nacional variety. Statistical analysis confirmed the presence of significant differences between these varieties ($p<0.05$). These findings align with those of Borja et al. [16], who reported that the CCN-51 variety exhibited a higher polyphenol content compared to other varieties, registering a value of 95.41 ± 2.50 mg of gallic acid/g. Furthermore, the significant differences between genotypes are attributed to the degree of crop domestication [17]. Moreover, it has been demonstrated that variety is another important factor in determining the biochemical composition of cocoa [18,19].

Moreover, the findings of this research fall within the range of polyphenols reported by various studies, where the amount of polyphenols in dried, defatted fermented cocoa seeds generally ranges between 40 and 140 mg gallic acid/g, and for non-fermented beans between 150 and 200 mg gallic acid/g [4,12]. Similarly, these results are comparable to those obtained by Samaniego et al. [21], who recorded polyphenol contents in fermented beans of the Nacional variety ranging from 33.55 ± 5.74 to 71.66 ± 3.94 mg gallic acid/g. However, it should be noted that differences are influenced by the cultivation zone and other factors mentioned earlier.

Given that the phenolic content and mucilage percentage [10] of the two varieties differ significantly, the fermentation times necessarily vary for each cultivar. Consequently, Nacional cocoa reaches the optimal fermentation index more rapidly. This phenomenon is attributable to the enzymatic activity of sugar-degrading enzymes (invertase and polygalacturonase) and polyphenol oxidation enzymes (polyphenol oxidase), which require a higher degradation rate in CCN-51 cocoa [34]. Specifically, CCN-51 presents with 5.30% mucilaginous pulp and a phenolic content of 169.82 mg GAE/g, in contrast to Nacional cocoa, which exhibits 3.50% mucilage pulp and 157.50 mg GAE/g of phenolic compounds.

During the fermentation process, polyphenols undergo oxidation catalyzed by the enzyme polyphenol oxidase, resulting in a diminution of phenolic content. Extant literature [24] posits that a reduction in polyphenols ranging from 35 to 59% occurs between the third and seventh day of cocoa

fermentation. Nonetheless, it is widely accepted that the optimal fermentation state is achieved when the polyphenol loss approximates 50 %.

3.1.1. Antioxidant activity

The results obtained for the antioxidant capacity expressed in μm Trolox/g of defatted cocoa (Dry basis) in each of the treatments are presented in Figure 3.

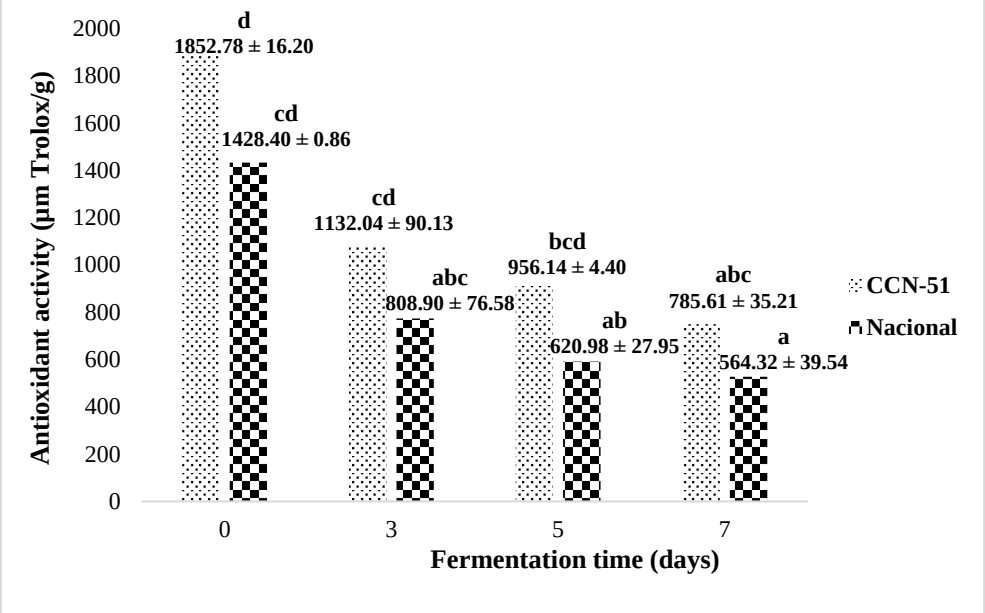

Figure 3. Antioxidant activity during the fermentation time.

Notes: Mean ± standar desviation (n = 2).

It was observed that the unfermented CCN-51 variety exhibited higher antioxidant activity compared to other treatments, followed by the Nacional variety. Additionally, fermented Nacional beans at 5 and 7 days showed significantly lower levels of antioxidant activity. Furthermore, no significant differences were determined between the antioxidant activity values of the CCN-51 variety fermented for 7 days and the Nacional variety fermented for 3 days.

The fermentation time has a significant effect on both cocoa varieties regarding the decrease in antioxidant activity by 57.60% for CCN-51 and 60.49% for Nacional during the 7-day fermentation process. Previous studies have noted a similar relationship between antioxidant activity and fermentation time, observing a decrease of 58.3% during the process [8]. Additionally, Melo [23] observed that for up to 48 hours, a higher content of bioactive compounds was retained. However, after this period, a decrease in antioxidant activity was evident.

Similarly, the results are comparable to those of Brito [18], who reported antioxidant values at the beginning of fermentation of 1296.57 ± 52.50 μm Trolox/g, while in the intermediate stage, a content of 1155.81 ± 365.23 μm Trolox/g was obtained, and at the end of the process, a value of 789.85 ± 212.58 μm Trolox/g was recorded. Likewise, this decrease during fermentation is due to the loss of a significant amount of phenolic compounds responsible for antioxidant activity, including (-)-epicatechin, (+)-catechin, and quercetin. These transformations can be influenced by the activity of the

enzyme polyphenoloxidase and the epimerization of epicatechin, caused by changes in pH during the fermentation process. Additionally, fermentation and drying can cause a loss of up to 70% of these chemical metabolites, as well as the composition of cocoa is closely linked to its variety and geographical origin [25].

In terms of variety, CCN-51 exhibited a higher antioxidant capacity compared to Nacional. This finding aligns with Borja Fajardo et al. [16], who similarly noted CCN-51's superior antioxidant capacity compared to other cocoa varieties. Furthermore, the data presented in Figure 3 surpass those obtained by Tello Alonso et al. [26], who reported values for different clones ranging from 752.10 ± 8.40 to 297.47 ± 7.10 µm Trolox/g in fresh beans and 85.7 ± 0.03 to 69 ± 0.02 µm Trolox/g in fermented beans. The variability in antioxidant activity among different genotypes is also attributed to factors such as geographic location, cultivation type, and post-harvest processing, as previously mentioned in the polyphenol study.

3.1.2. Relationship between antioxidant capacity and total polyphenol content

The correlation analysis between the variables: total polyphenol content versus antioxidant activity, recorded a highly positive correlation (R^2 = 0.94), meaning that as the polyphenol content increases, the antioxidant capacity of the grains increases proportionally (Figure 4).

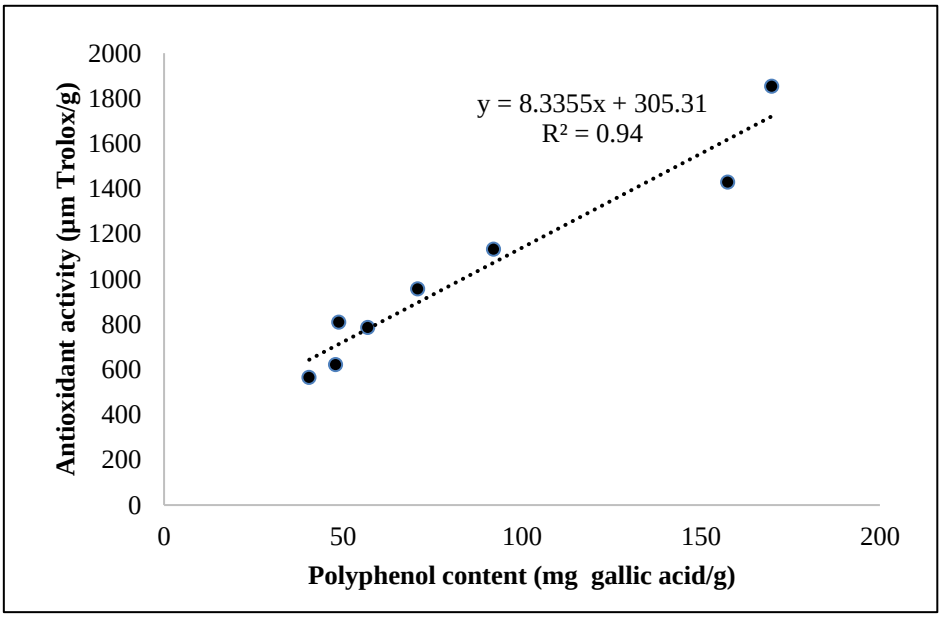

Figure 4. Correlation of polyphenols and activity antioxidant.

3.1.3. Soluble solids

In Table 1, the variables measured during the fermentation process are presented. The sugar content in degrees Brix in the CCN-51 and Nacional type varieties was 17 °Brix, indicating that both varieties share a common characteristic in terms of sweetness. This can be attributed to genetic and environmental factors that influence sugar accumulation in the pulp during development [27]. The O'Payo and Nugu cocoa varieties, originating from Nicaragua, showed similar values of 17.50 and

17.40 °Brix, respectively. These results support the similarity of sugar content in the different cocoa varieties used in the present study [28].

The pulp (mucilage) plays a crucial role in fermentation due to its high sugar content (>70%), which serves as the substrate for the proliferation of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). These microorganisms are responsible for the formation of compounds that contribute to the non-thermal aroma and flavor profile of chocolate [20].

3.1.4. Fermentation percentage

The fermentation percentage was determined through the cut test kernel (Table 1). Based on these results, the Nacional variety achieved a higher temperature and fermentation percentage compared to CCN-51. Optimal fermentation is achieved when the seed mass temperature reaches 45–48 °C within 72 hours of initiating the process [23]. Grains are considered of quality when at least 50% of them have been fermented. This percentage serves as a key benchmark for determining the achieved fermentation quality [29]. Moreover, this quality parameter may vary due to various factors such as fruit maturity, cocoa variety, storage time, fermentation container, process duration, stirring frequency, mass volume, and environmental conditions [30].

Table 1. Soluble solids and fermentation percentage.

Variety	Soluble solids (°Brix)	Fermentation percentage (7 days)
CCN-51	17	75.30
Nacional	17	79.30

Notes: Mean ± standar desviation (n = 2).

3.1.5. Temperature and color of the cocoa bean during fermentation

In Table 2, we present the results corresponding to the temperature and grain color during fermentation. As the cocoa fermentation time progresses, there is a progressive increase in temperature in both varieties. In the case of the Nacional variety, a maximum temperature of 47 °C is reached on the fifth day, followed by a decrease to 39 °C by the completion of the seven-day fermentation period. Meanwhile, CCN-51 reached its maximum temperature record on the sixth day and subsequently decreased. The temperature increase values of the fermentative mass may vary depending on the environmental conditions where the fermentation process took place [23]. Furthermore, the thermal increase is attributed to the enzymatic activity of both the cocoa's endogenous enzymes and the exogenous enzymes of the present microorganisms. Enzymes generate heat as a result of exothermic metabolic reactions [31]. Likewise, the temperature decrease is due to the reduction of the microorganism population [32]. Additionally, it is important to highlight that the maximum temperature recorded in both cocoa types was above 41 °C, which is sufficient to deactivate the germination capacity of the seeds while activating the precursors of the characteristic aroma and flavor of chocolate [33].

Furthermore, during the fermentative process of cocoa beans, color was measured, wherein it was observed that luminosity (L*) significantly decreases as fermentation progresses (p-value < 0.05), as anthocyanins are destroyed by enzymatic hydrolysis, leading to whitening and subsequent darkening of the beans [34]. Similarly, the chromaticity components a* and b* exhibit a similar pattern, with a

significant decrease (p-value < 0.05), indicating that the a* value presents a less reddish color and the b* value less yellow. This behavior is also attributed to the breakdown of anthocyanins [34]. Wherein, yellow and brown colors are associated with the presence of anthocyanins in cocoa beans [25]. According to the study conducted by Chire et al. [35], no significant differences were found in color parameters such as luminosity (L*) and the components a* (red-green) and b* (yellow-blue) during the fermentation process. However, color varied due to various factors such as genotype and post-harvest processing. In the present study, significant differences in color parameters were observed, which could be attributed to these factors.

The chromatic alterations observed during fermentation are attributable to the catalytic action of polyphenol oxidase, which mediates the transformation of phenolic compounds, notably epicatechin, into quinones. This enzymatic process engenders a remarkable transition from the characteristic violet hue of fresh cocoa beans to the distinctive brown coloration associated with fermented and dried cocoa.

3.1.6. Humidity

After 7 days of fermentation, moisture content values ranging from 21.23–21.47% for CCN-51 and 22.07%–21.37% for Nacional variety were recorded (Table 2). These findings suggest that no significant changes occurred in the moisture composition of the beans throughout the fermentation process. It is worth noting that moisture measurement was conducted directly on the beans, ensuring accuracy in the results. Similarly, Efraim et al. [36], noted a decrease in moisture during the fermentation process; however, no significant differences were observed. Thus, these results support the research by indicating that moisture is not a critical factor in the cocoa fermentation stage.

4. Conclusions

The results of this research have allowed us to determine that there is a higher content of polyphenols and antioxidant activity in the unfermented cocoa beans of the CCN-51 variety compared to the Nacional variety. Furthermore, it was shown that fermentation time and variety are determining factors in antioxidant content. As fermentation time progressed, a decrease in polyphenol contents and antioxidant capacity was observed in both varieties. However, it was found that the CCN-51 variety retained a greater amount of antioxidants.

Use of AI tools declaration

The authors affirm that they have not utilized artificial intelligence (AI) tools in the creation of this article.

Acknowledgments

Authors want to recognize Jessica Guevara and Jonathan Sánchez for their support in the experimental development of this research. The authors also wish to acknowledge Mario Álvarez and Mónica Silva for the use of the facilities and equipment belonging to the project “Canje de Deuda Ecuador-Espana” (AECID—Agencia Espanola de Cooperación Internacional para el Desarrollo).

Table 2. Temperature, humidity, and colorimetry of the cocoa during the fermentation time.

Time (h)	Humidity (%)		Temperature (°C)		Colorimetry											
	CCN-51	Nacional	CCN-51	Nacional	CCN-51						Nacional					
					L*	a*	b*	°h	C*	ΔE	L*	a*	b*	°h	C*	ΔE
0	21.23 ± 0.15	22.07 ± 0.21	32.33	33.87	35.37	5.60	14.99	69.51	16.00	-	33.20	2.83	12.04	76.76	12.37	-
72	21.30 ± 0.10	21.57 ± 0.49	36.93	44.43	35.63	3.15	13.92	77.23	14.27	2.68	33.02	2.19	11.69	79.40	11.89	0.76
120	21.43 ± 0.12	21.57 ± 0.15	42.80	47.47	34.58	3.12	13.22	76.74	13.58	3.15	33.01	2.87	12.02	76.58	12.35	0.20
168	21.47± 0.06	21.37 ± 0.21	44.50	38.80	32.84	2.41	11.52	78.20	11.77	5.35	32.83	2.63	11.86	77.50	12.14	0.46

Notes: Mean ± standar desviation (n = 3).

Conflict of interest

All authors declare that they have no conflict of interest.

Author contributions

Conceptualization, M.-Q.A., I.S., M.P.N.; methodology, M.-Q.A., I.S., M.P.N. and O.Ch.M.; chemical analysis, M.-Q.A., I.S. and O.Ch.M.; statistical analysis M.-Q.A.,M.P.N.; field sampling and agronomical management, M.-Q.A., I.S., M.P.N. and O.Ch.M.; writing—original draft preparation, M.-Q.A., I.S., M.P.N.; writing—reviewand editing, M.-Q.A., I.S., M.P.N.; project administration, M.-Q.A., I.S.; funding acquisition, M.-Q.A., I.S., M.P.N. and O.Ch.M. All authors have read and agreed to the published version of the manuscript.

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