Research, Society and Development, v. 9, n. 10, e4759108742, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8742

Physicochemical and microbiological quality in the fermentation of different cocoa blends with the addition of coffee and cardamom

Qualidade físico-química e microbiológica na fermentação de diferentes blends de cacau adicionado de café e cardomomo

Calidad fisicoquímica y microbiológica en la fermentación de diferentes mezclas de cacao añadido café y cardamom

Received: 09/24/2020 | Reviewed: 10/02/2020 | Accept: 10/03/2020 | Published: 10/03/2020

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Abstract

Fermentation is an essential step in obtaining good quality almonds. Thus, the present study aimed to evaluate the fermentation process of cocoa almonds and quantify the physicochemical and microbiological quality of different cocoa blends with the addition of coffee and cardamom, aiming at the reduction of the cost of processing, the addition of value to the raw material, and technological and scientific development of the Amazon region. The experimental design used was completely randomized, arranged in a 4 x 2 x 3 factorial scheme, corresponding to four fermentation times, two fermentation, three concentrations of pulp (blends) (pure cocoa, cocoa + coffee, and cocoa + cardamom), and three replicates. The results made it possible to conclude that the temperature inside the mass measured every 48

hours after turning increased with the fermentation time, reaching its maximum on the seventh day of fermentation. The increase in the temperature inside the fermentation mass on the seventh day is due to the higher concentration of acetic acid, evidenced by the reduction in pH value and consequent reduction in °Brix. Fermentation in a greenhouse at a temperature of 40 °C enables the maintenance of stable temperature throughout the fermentation process for blends of cocoa with the addition of coffee and cardamom. It is verified that, when there is a pre-established temperature, as in a greenhouse at 40 °C, there is a decrease in the values of °Brix and soluble solids present in the final product. Temperature, pH, and sugars are determining factors for the duration of the fermentation process and quality of pure cocoa almonds and cocoa almonds with the addition of coffee and cardamom. Under the experimental conditions, fermentation time from seven days is sufficient to ensure the physicochemical and microbiological quality of cocoa blends with the addition of coffee and cardamom, but it should not exceed nine days.

Keywords: *Theobroma cacao;* Fermentation; Value aggregation; Technological innovation; Quality control.

Resumo

A fermentação é uma etapa essencial para a obtenção de amêndoas de boa qualidade. Assim, o presente trabalho teve por objetivo avaliar o processo de fermentação de amêndoas de cacau e quantificar a qualidade físico-química e microbiológica de diferentes blends cacau adicionado de café e cardamomo, visando a redução do custo de processamento, a agregação de valor a matéria prima e o desenvolvimento tecnológico e cientifico da região amazônica. O delineamento experimental utilizado foi o inteiramente casualizado, arranjados em esquema fatorial 4 x 2 x 3, constituídos por quatro tempos de fermentação, dois ambientes de fermentação, três concentrações de polpa (*blends)* (cacau puro, cacau + café e cacau + cardamomo) e três repetições. Os resultados permitiram concluir que a temperatura no interior da massa mensurada a cada 48 horas após o revolvimento apresentou aumento com o tempo de fermentação, atingindo o máximo no sétimo dia de fermentação. A elevação da temperatura do interior da massa em fermentação no sétimo dia deve-se a maior concentração de ácido acético, evidenciada pela redução no valor de pH e consequente redução do °Brix. A fermentação em estufa a temperatura de 40°C possibilita a manutenção de temperatura estável durante todo o processo fermentativo para *blends* de cacau adicionado de café e cardamomo. Verifica-se que quando há uma temperatura pré-estabelecida, conforme em estufa a 40°C, tem-se diminuição dos valores de °Brix e os sólidos solúveis presentes no produto final. A

temperatura, o pH e açucares são fatores determinantes para a duração do processo de fermentação e qualidade das amêndoas de cacau pura e acrescida de café e cardamomo. Nas condições experimentais o tempo de fermentação a partir de sete dias é suficiente para garantir qualidade físico-química e microbiológica de *blends* de cacau adicionado de café e cardamomo, não devendo ultrapassar nove dias.

Palavras-chave: *Theobroma cacao*; Fermentação; Agregação de valor; Inovação tecnológica; Controle de qualidade.

Resumen

La fermentación es un paso esencial para obtener almendras de buena calidad. Así, el presente trabajo tuvo como objetivo evaluar el proceso de fermentación de las almendras de cacao y cuantificar la calidad fisicoquímica y microbiológica de las diferentes mezclas de cacao añadido café y cardamomo, con el objetivo de reducir el coste de procesamiento, añadiendo valor a la materia prima y al desarrollo tecnológico y científico de la región amazónica. El diseño experimental utilizado fue completamente aleatorizado, dispuesto en un esquema factorial 4 x 2 x 3, que consta de cuatro tiempos de fermentación, dos ambientes de fermentación, tres concentraciones de pulpa (mezclas) (cacao puro, cacao + café y cacao + cardamomo) y tres repeticiones. Los resultados permitieron concluir que la temperatura dentro de la masa medida cada 48 horas después de la rotación aumentó con el tiempo de fermentación, alcanzando el máximo en el séptimo día de fermentación. El aumento de la temperatura dentro de la masa de fermentación en el séptimo día se debe a la mayor concentración de ácido acético, evidenciada por la reducción del valor del pH y la consiguiente reducción de la brix. La fermentación en horno a una temperatura de 40oC permite el mantenimiento de una temperatura estable durante todo el proceso de fermentación para mezclas de cacao añadido café y cardamomo. Se verifica que cuando hay una temperatura preestablecida, según en un horno a 40oC, hay una disminución en los valores de brix y sólidos solubles presentes en el producto final. La temperatura, el pH y los azúcares son factores determinantes para la duración del proceso de fermentación y la calidad de las almendras de cacao puro más el café y el cardamomo. En las condiciones experimentales el tiempo de fermentación a partir de siete días es suficiente para garantizar la calidad fisicoquímica y microbiológica de las mezclas de cacao añadido café y cardamomo, no superior a nueve días.

Palabras clave: *Theobroma cacao*; Fermentación; Agregación de valores; Innovación tecnológica; Control de calidad.

1. Introduction

Cocoa (Theobroma cacao) is a typical fruit of the region of the Amazon basin, with a hot and humid climate, originating from rain forest regions of Tropical America, where, to this day, it is found in the wild, from Peru to Mexico. The interest in the cultivation of this species is in the use of its seeds (almonds) for the production of cocoa butter and chocolate (Alves & Bragagnolo, 2002). And, for the chocolate to have a pleasant aroma and flavor it is necessary not only that cocoa has good quality, but also that there is control of pH, fermentation time, fermentation temperature, and population of microorganisms present in the fermentation process.

Cocoa fermentation is a spontaneous microbiological process, in which microorganisms metabolize the sugars present in the pulp for ethanol and this is subsequently oxidized to acetic acid through an exothermic reaction. Acetic acid and ethanol penetrate the seed and, in combination with the action of heat, eliminate the germination capacity of the embryo by breaking the cell walls of the seed. These changes induce biochemical reactions within the almond, generating the chemical precursors of chocolate flavor and color (Lopez& Dimick, 1995). The generation of flavor precursors is an important result of fermentation. Poorly fermented almonds or unfermented seeds have a brown-violet or greyish wood color, which remains in chocolate and develops an apparent chocolate flavor (Beckett, 1994). Thus, the fermentation process is considered important to reduce acidity, astringency, and bitterness in cocoa seeds, being fundamental in the formation of reducing sugars and amino acids, which are the precursors of the Maillard reaction during roasting (Huang & Barringer, 2010).

The time required for seed fermentation is variable, depending on the genetic material. For the occurrence of reactions that lead to the formation of the main precursors of chocolate flavor, the seeds of cocoa of the Forastero group, the predominant type in the world, including Brazil, should generally be fermented for periods longer than five days (Beckett, 1994).

In Brazil, the most used method for fermentation is “fermentation troughs”, which are boxes constructed of wood, with removable partitions and drains at the bottom, so that it is possible to collect cocoa honey and enable ventilation. Both in this method and the other ones, pH and temperature should always be evaluated inside the mass so that it is possible to control the quality of the final product since it is objective to achieve a uniform fermentation and good quality for almonds, as it is characterized as a prerequisite for obtaining and marketing chocolates with different attributes.

However, in response to the demand of the consumer market of chocolates, cocoa producers are seeking a raw material of high sensory quality and the elaboration of differentiated flavors. For this, the correct management of cocoa is necessary during its processing, especially in the fermentation stage, in which flavor and aroma precursors develop. In this perspective and because of the need to obtain cocoa almonds with different flavors and aromas, the fermentation of cocoa seeds with mixtures of both fruit pulp and condiments, eliminating costs with logistics and acquisition, emerged in the cocoa-producing region of Rondonia, Brazil.

In view of the above, the present study aimed to evaluate the fermentation of cocoa almonds and quantify the physicochemical and microbiological quality of different cocoa blends with the addition of coffee and cardamom, aiming at the reduction of the cost of processing, the addition of value to the raw material, and technological and scientific development of the Amazon region.

2. Methodology

The experiment was conducted at the Food Agroindustry of the Federal Institute of Education, Science and Technology of Rondônia, Campus of Colorado do Oeste, in the municipality of Colorado do Oeste, RO, Brazil, whose geographic coordinates are 13° 06' S and 60° 29' W, with an average altitude of 407 meters.

The experimental design used was completely randomized, arranged in a 4 x 2 x 3 factorial scheme, consisting of four fermentation times, two fermentation environments (ambient temperature [simulation in a greenhouse turned off to avoid wind interference] and greenhouse at 40 °C), three concentrations of pulp (blends) (pure cocoa, cocoa + coffee, and cocoa + cardamom), and three replicates, totaling 72 experimental units.

The Forastero cocoa fruits used were acquired in a property in the rural area of the municipality of Colorado do Oeste, with a sampled amount of 1,000 fruits. Initially, a total of 50 random cocoa fruits were selected for analysis of fruit weight, peel color by the Minolta Colorimeter method, longitudinal and transverse diameters of the fruit, pulp pH, and °Brix. Subsequently, the fruits were washed in running water and cut to extract the pulp, which was transferred to a properly sanitized container and distributed in the different fermentation boxes. The fermentation boxes had a capacity for five liters of pulp and were made in a square shape. The corners were rounded using pieces of wood, and the edges were drilled with 30 equidistant and symmetrical holes.

The total yield of the fruits was 85 liters of pulp, and 4.72 liters of pulp were used in each replicate. After equally distributing the mucilage in all fermentative boxes, the third factor was added, namely blends of pure cocoa, cocoa + 30 grams of coffee grains, and cocoa + 15 grams of ground cardamom, subjected to two fermentation environments (greenhouse at a temperature of 0 °C with ambient temperature [simulation in a greenhouse turned off to avoid wind interference] and greenhouse at a temperature of 40 °C), with three replicates each treatment.

In the first 48 hours, there was no turning of the mass or opening of the greenhouses, to allow fermentation to occur, and after this period a wooden spoon was used to properly turn the masses every 48 hours in the morning, and the temperature inside the mass with measured with a laser thermometer. Pulp pH and °Brix analyses were performed every 48 hours, and the ratio for the analyses was 1:1.

ºBrix was evaluated using a benchtop refractometer, with three replicates for each sample, following the mean of the results. The pH was determined using a digital potentiometer, properly calibrated with pH buffer solutions 7.0 and 4.0 (Analytical standards of the Institute Adolfo Lutz, 2005).

Microbiological analyses were total bacterial count, also known as standard plate count (SPC). The bacteria present in almonds, and that are viable, grow to the point of being visible to the naked eye, being called colonies. Thus, it is possible to count how many colonies grew and, depending on the volume of the sample, the SPC is determined and expressed in colony-forming units per mL of sample (CFU/mL) is determined. Therefore, colonies, not bacteria, are counted in the SPC method. It is known that most of the time, a colony is formed by several bacteria, so the samples were analyzed by counting on plates. The almonds were diluted in test tubes with distilled water, and 1.0 mL of the dilution was pipetted into Petri dishes and sown in Plate Counting Agar (PCA). After homogenization, the plates were incubated at 37 ºC for 48 hours. The reading of the plate was performed with a colony counter, Quebec model, and the number of colony-forming units (CFU) was calculated according to the dilution used. In addition, there was the quantification of Total Coliforms and Coliforms at 45 °C, where total coliforms were quantified using the most probable number (MPN) technique. The presumptive test was performed with the inoculation of sample aliquots ranging from four to six series of three test tubes, containing inverted Durhan tubes and lauryl sulfate tryptose (LST) broth, incubated at 37 °C for 24-48 hours. Both results for microbiological analysis were absent.

The fermentation process remained in evaluation for seven days and then the almonds were exposed on a structure with a screen, made with 1x1 cm mesh, and stored in a gardening greenhouse for the drying process, which lasted for 7 days. After the complete drying of the almonds, the quality analysis test was carried out.

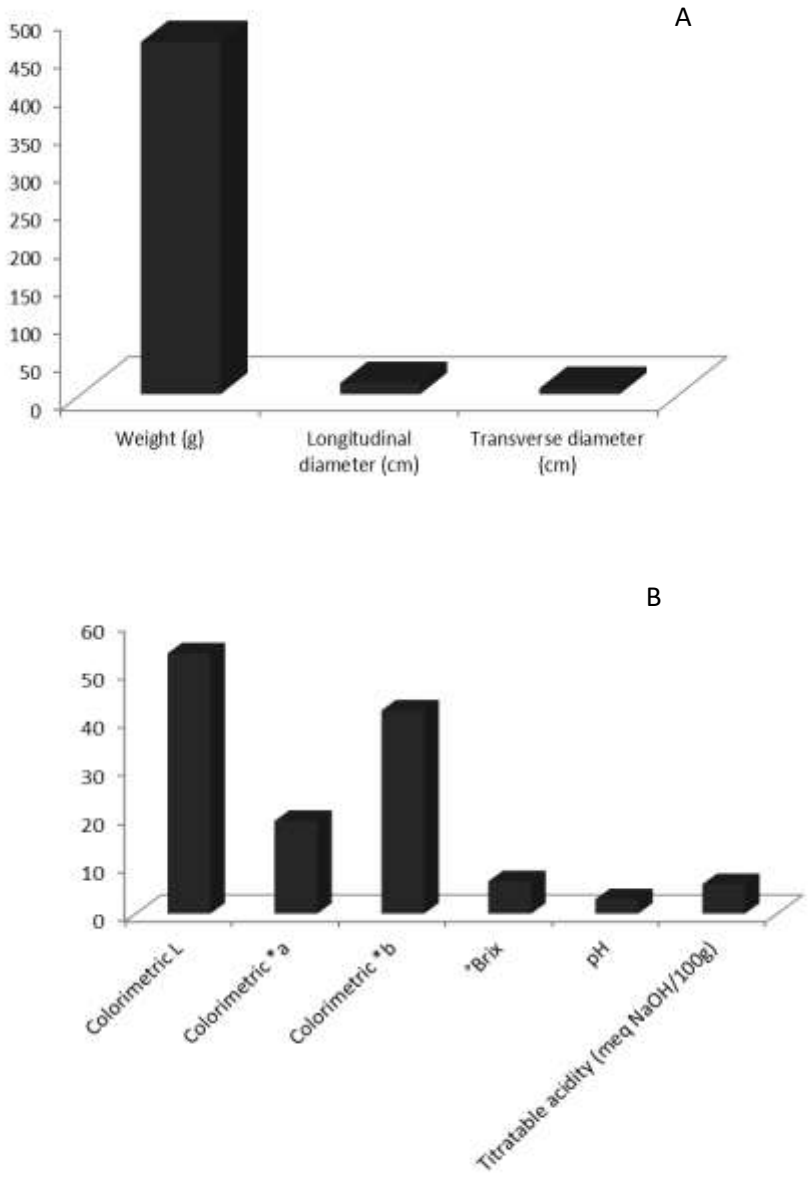
The data were subjected to the normality test (Shapiro-Wilk), and then the physicochemical results were subjected to analysis of variance, and the differences between means were compared by the Tukey test at a 5% significance level, using the statistical program Sisvar.

3. Results and Discussion

The results showed significant effects ($p \leq 0.05$) of the double interaction between fermentation time x fermentation environment for the temperature inside the mass measured every 48 hours and °Brix (Table 1); double interaction between fermentation time x different cacao blends for the temperature inside the mass measured every 48 hours (Table 2) and double interaction between fermentation environment x different cacao blends for °Brix (Table 2); while the other results did not show a significant effect of interactions and are presented independently for fermentation time, fermentation environment and blends (Figure 2 and Figure 3).

The analyses of weight and size of the *Theobroma cacao* fruits are a factor that has great variability, due to the maturation time, type of cross, environmental factors, etc. The sampled fruits showed an average of 14.84 cm in longitudinal diameter and 8.12 cm in transverse diameter, with an average weight of 463.65 g (Figure 1A). Brito and Silva (1983), studying the seasonal growth of the cocoa fruit, observed that the dimensional growth, considering length and diameter, follows a sigmoid curve and that the growth in length is initially more prominent than in diameter but, from 10 cm, the growth in diameter becomes relatively larger than that of the longitudinal diameter, which is associated with large internal changes of the tissues, such as rapid embryo development and the presence of cotyledonal material. The mucilaginous material that covers cocoa seeds can account for 35% to 37% of the weight of fresh seed and is largely lost in the form of liquid drained from the cocoa mass during the fermentation process. This liquid, called cocoa honey, is transparent, rich in fermentable sugars, and is considered to characterize the weight of cocoa fruits.

Figure 1. Characterization of cocoa fruits in terms of weight, longitudinal diameter, and transverse diameter (A) before pulp extraction and characterization of cocoa pulp for colorimetry L, *a and *b, °Brix, pH, and titratable acidity (B) before the fermentation process.



Source: Authors.

Cocoa color varies with species, origin, agricultural techniques, climate, soil, and fruit maturity degree (Lopes, 2000; Mattietto, 2001; Efrain et al. 2011), ranging from green to brown, and is an important factor to indicate the degree of fruit maturity. Analysis with the

Minolta Colorimeter showed a small variation in fruit color, in which the parameter L averaged 53.83, while *a and *b averaged 19.18 and 42.03, respectively (Figure 1B). The L* coordinate represents how light or dark the sample is, with values ranging from 0 (totally black) to 100 (totally white); the a* coordinate can assume values from -80 to +100, where the extremes correspond to green and red, respectively; and the b* coordinate corresponds to the intensity from blue to yellow, which can range from -50 (totally blue) to +70 (totally yellow). Thus, it can be inferred that the fruits used in the study showed brown, green, and yellow ripening colors.

The ºBrix scale is calibrated by the number of grams of sugar contained in 100g of solution. When the refraction index of a sugar solution is measured, the reading in the percentage of °Brix must match the actual concentration of sugar in the solution. The scales in the percentage of °Brix show the percentage concentrations of soluble solids contained in a sample (solution with water). The soluble solids contained is the total of all solids dissolved in water, starting with sugar, minerals, proteins, organic acids, etc. (Paula et al. 2020). It should be noted that, as the fruit develops, there is an accumulation of sugar in the pulp, until it reaches the appropriate level for harvesting. The ideal content for the fruit to be considered of good quality, in some cases, will depend on the destination of the fruit to the processing. In this context, the pulp of the cocoa fruits had values of 6.7, 3.0, and 6.7 for °Brix, pH, and acidity, respectively (Figure 1B). According to (Chitarra & Chitarra, 2005), the content of organic acids, with few exceptions, decreases with maturation due to the respiratory process or the conversion of sugars, because this period corresponds to the one of the highest metabolic activity. Organic acids constitute excellent energy reserve of the fruit, through their oxidation in the Krebs cycle. Thus, the sugar/acid ratio increases during maturation in most fruits.

Due to the difficulties that cocoa producers encounter in controlling temperature for an efficient fermentation process and ensuring the final quality of the product, as well as adding value, the study made it possible to verify the appropriate fermentation time and temperature, as well as the aggregation of value to the product by inducing flavor with the addition of different blends of coffee and cardamom to the cocoa pulp. The mean values of the temperature inside the mass in the ninth of fermentation time were not presented, and it is appropriate to mention that the final temperature, that is, after seven days of fermentation, averaged 36 °C inside the mass. The fermentation process longer than nine days is not indicated, as there is the beginning of butyric fermentation (chemical reaction performed by anaerobic bacteria through which butyric acid is formed). Thus, the final product of

fermentation is not indicated for the production of chocolate blends, but only for the production of butter and other by-products.

The temperature inside the mass measured every 48 hours after turning increased with fermentation time, reaching the maximum on the seventh day of fermentation, on the order of 41 °C, and differing statistically from the values of the other fermentation times (Figure 2A), while the fermentation in the greenhouse at a temperature of 40 °C allowed the maintenance of stable temperature throughout the fermentation process (Figure 2B), ensuring the maintenance of the quality of the final product, and the cocoa mixture with the addition of coffee kept its temperature higher than those of the other treatments, on the order of 38.8 °C (Figure 2C).

The increase in the temperature of the fermentation mass after 48 hours of fermentation can be favored by its turning, as it provides the incorporation of oxygen, favoring the development of acetic bacteria. These microorganisms convert ethanol produced in the first hours of the fermentation process into acetic acid and water, ensuring an exothermic reaction, which generates energy, hence increasing the temperature. After the seventh day of fermentation, the temperature inside the mass decreased and stabilized on a ninth day at 36 °C. Efraim (2004) and Cruz (2012) observed that there is an increase in the temperature inside the mass until the 3rd day, the period where there is the death of the embryo, subsequently decreasing and stabilizing until the end of the fermentation process. Thus, it can be affirmed that the maximum temperature point in the fermentation process for cocoa under the studied conditions occurred on the seventh day of storage.

According to Zamalloa (1994), Dias (1998), Lopes (2000), Mattietto (2001), Efraim (2004), and Cruz (2012), good commercial cocoa fermentations should reach 45 to 50 ºC in approximately 72 hours after the start of the fermentation, remaining stable until the end of the process, and temperature fluctuations occur due to metabolic variations of microorganisms. On the first day, there is a predominant action of yeasts that require little oxygen, making the temperature not so high. From the second day until the end of fermentation, the mass was turned, causing the temperature to be close to 40 ºC.

The ºBrix scale of the pulp with cocoa almonds on the 1st day of the test showed values of 3.38 ºBrix and, after 48 hours of fermentation, the values were reduced to an average of 2.18 ºBrix, remaining stable and not differing statistically from those found on the 3rd day of fermentation (Figure 3). It is possible to observe that the soluble solids content of the pulp with cocoa almonds is altered with the fermentation time and that soluble solids can exert influence on the specific mass, since it decreased with the increase of fermentation time,

and there were low pH values (Figure 2D). However, fermentation is an essential step in obtaining good quality almonds, due to complex biochemical reactions that cause embryo death and hydrolysis of sugars and proteins (Efrain et al. 2010). The cocoa pulp is rich in sugar, with approximately 15% monosaccharides and 84% moisture, 0.20% lipids, and 0.8% proteins. The first phase of fermentation starts very quickly and the growth of yeasts is favored, due to the sugar content of the pulp (15% of monosaccharides), pH around 3.5, and anaerobic conditions (Santos, 2013).

Another point to consider is the wooden box with a rounded shape because it facilitates the dissipation of heat and turning of the mass during fermentation, allowing in addition to the obtaining of a product with higher quality, the regional scientific and technological development.

Figure 2. The temperature inside the mass and pH values as a function of fermentation time, fermentation environment, and different cocoa blends. Means followed by the same letter in the bars do not differ by Tukey test at 5% probability level.

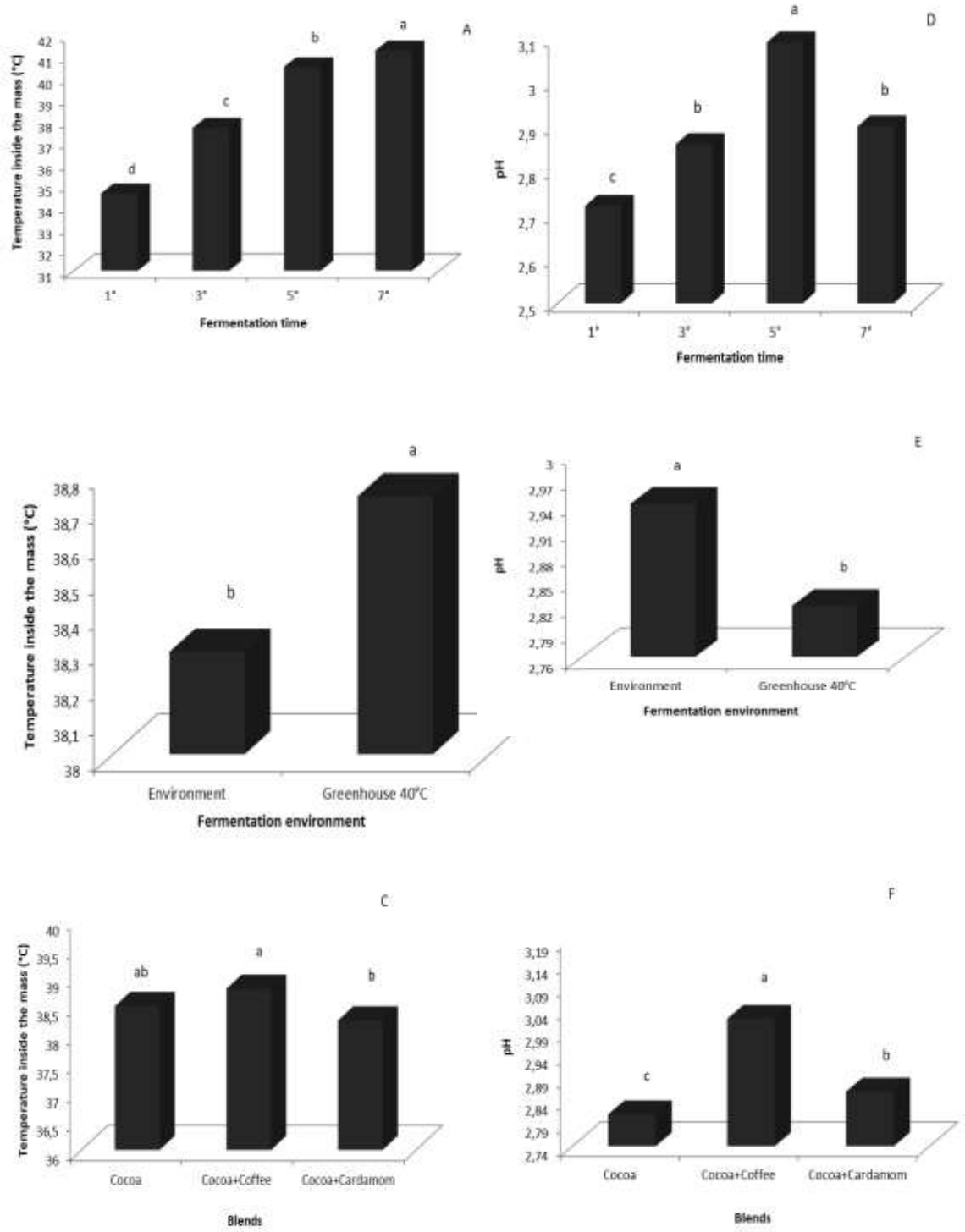
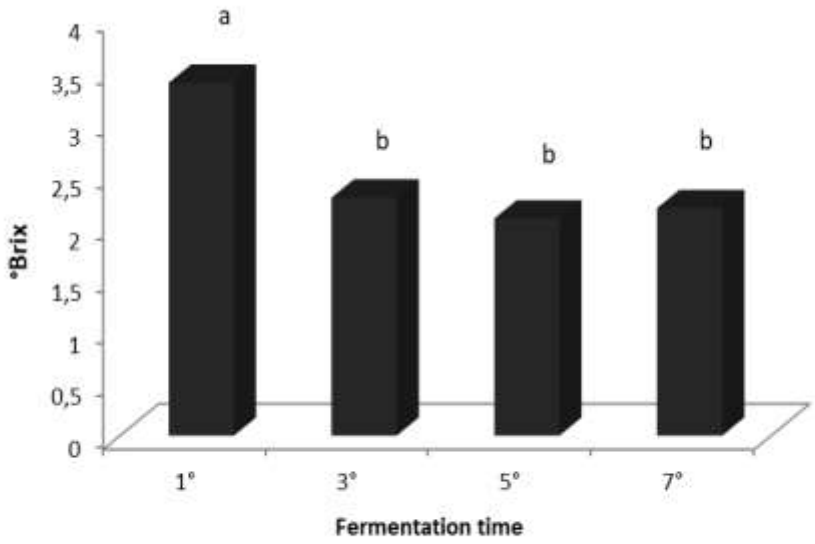


Figure 3. °Brix as a function of fermentation time. Means followed by the same letter in the bars do not differ by Tukey test at 5% probability level.



Source: Authors.

According to the data, the cocoa pulp is a product that, due to its low pH (ranging from 2.8 to 3.1), can be classified as of high acidity, with a statistically significant difference ($p<0.05$) in relation to the fermentation environment and cocoa blends analyzed (Figure 2E and 2F). The pH values in the fermentation process in an environment of the greenhouse at 40 °C were low and differed statistically ($p<0.05$) from the fermentation process at ambient temperature (Figure 2E), while the cocoa blends with the addition of coffee had pH values closer to 3.1 and the blends of pure cocoa and cocoa with the addition of cardamom showed even lower values (Figure 2F). This pH range, combined with the soluble solids content, favors the growth of yeasts, to the detriment of bacteria. And this fact represents an advantage when using thermal and/or fermentative processes, ensuring the microbiological quality of the product and the completion of the fermentation stage and immediate start of drying, in order to prevent the development of microorganisms and the occurrence of undesirable reactions. It should be noted that the high acidity of cocoa does not from the almond, but is rather acquired during fermentation when the tissues of the cotyledons absorb acid and other substances produced by the microorganisms involved in the process.

Thus, it is pertinent to associate the temperature increase inside the fermentation mass with a higher concentration of acetic acid (Figure 2A), evidenced by the reduction in pH value (Figure 2D) and consequent reduction in °Brix (Figure 3).

The decomposition of the double interaction between fermentation time and fermentation environment showed that the temperature inside the mass increased significantly with the fermentation time, reaching the maximum value on the seventh day of fermentation and in a greenhouse at 40 °C (Table 1), while ºBrix decreased by approximately 95% between the first and seventh days of fermentation, differing statistically from the other treatments in relation to fermentation time and environment (Table 1). It is observed that, when there is a pre-established temperature, such as the greenhouse at 40 °C, there is a decrease in the values of °Brix and soluble solids present in the final product, which characterizes the less sweet taste.

In the decomposition of the double interaction between fermentation time and different cocoa blends, the temperature inside the mass was significantly higher from the 5th day of fermentation, both for the treatment of pure cocoa and for the blend of cocoa with the addition of coffee and cardamom, not differing statistically from the 7th day of fermentation (Table 2). The temperatures inside the mass of the different blends remained between 40 °C and 41 °C between the 5th and 7th days of fermentation. The results corroborate Chávez et al., 2020, in which observed the best quality characteristics were obtained after 8 days of fermentation with the type of wooden box fermenter.The blend of cocoa with the addition of cardamom fermented in a greenhouse at 40 °C had the lowest values of °Brix, differing statistically from the other treatments (Table 2).

In the end, the test of *Theobroma cacao* quality classification indicated that the fermentation was of good quality, since it exceeded 70%, so it is possible to obtain results of 76% fully fermented almonds, 17% of partially fermented almonds, and 7% of unfermented almonds. CEPLAC defines values above 85% as high-quality fermentation, and values between 70 and 85% as regular and of good quality, considering fully and partially fermented almonds.

The quality control applied in cocoa processing was of paramount importance to ensure quality in all evaluated treatments of pure cocoa almond and the respective blends (cocoa + coffee and cocoa + cardamom), as the most probable number/mL (MPN/mL) of Coliforms at 35 ºC and Coliforms at 45 ºC were lower than 0.1 MPN/mL, with the absence of *Escherichia coli* and Total Bacterial Count lower than the limits established by RDC 12 of ANVISA (BRASIL, 2003). The results indicate that there was adequate hygienic-sanitary

quality throughout the processing of the fruit and storage during fermentation, demonstrating superior and excellent microbiological quality for the fermentation process (Table 3).

Table 1. Decomposition of the double interaction between fermentation time and fermentation environment for the temperature inside the mass and °Brix.

Fermentation time	Environment	
	Greenhouse (environment)	Greenhouse 40°C
	Temperature inside the mass (°C)	
1°	33.61 bC	35.62 aD
3°	36.77 bB	38.55 aC
5°	40.44 aA	40.55 aB
7°	40.33 bA	42.22 aA
Fermentation time	Environment	
	Greenhouse (environment)	Greenhouse 40°C
	°Brix	
1°	3.31 aA	3.46 aA
3°	1.97 bC	2.60 aB
5°	2.11 aC	2.06 bC
7°	2.60 aB	1.77 bD

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test at the 5% probability level.

Table 2. Decomposition of the double interaction between fermentation time and different cocoa blends and decomposition of the double interaction between fermentation environment and different cocoa blends.

Fermentation time	Different *blends*		
	Cocoa	Cocoa+Coffee	Cocoa+Cardamom
	Temperature inside the mass (°C)		
1°	35.00 cA	34.50 cA	34.33 cA
3°	36.66 bA	39.50 bB	36.83 bA
5°	41.00 aA	40.00 aA	40.50 aA
7°	41.33 aA	41.16 aA	41.33 aA
Fermentation environment	Different *blends*		
	Cocoa	Cocoa+Coffee	Cocoa+Cardamom
	°Brix		
Greenhouse (environment)	2.54 aA	2.44 aA	2.63 aA
Greenhouse 40°C	2.50 aA	2.60 aA	2.21 bB

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test at the 5% probability level.

Table 3. Microbiological analysis for quality control applied to the fermentation of different cocoa blends considering Total Coliforms (TC), Thermotolerant Coliforms (T) at 45 °C, *Escherichia coli*, Total Bacterial Count (TBC), and *Salmonella sp.*

Tratamentos	Microbiological fermentation quality control				
	(TC)	(T)	*Escherichia coli*	*TBC*	*Salmonella sp*
Cocoa	≤ 0,1	≤ 0,1	Absence	Absence	Absence
Cocoa+Coffee	≤ 0,1	≤ 0,1	Absence	Absence	Absence
Cocoa+ Cardamom	≤ 0,1	≤ 0,1	Absence	Absence	Absence

4. Considerações Finais

The temperature inside the mass measured every 48 hours after turning increased with fermentation time, reaching its maximum on the seventh day of fermentation.

The increase in the temperature inside the fermentation mass on the seventh day is due to the higher concentration of acetic acid, evidenced by the reduction in pH value and consequent reduction in °Brix.

Fermentation in a greenhouse at a temperature of 40 °C allows the maintenance of stable temperature throughout the fermentation process for blends of cocoa with the addition of coffee and cardamom.

It is verified that, when there is a pre-established temperature, as in a greenhouse at 40 °C, there is a decrease in the values of °Brix and soluble solids present in the final product.

Temperature, pH, and sugars are determining factors for the duration of the fermentation process and quality of pure cocoa almonds and cocoa almonds with the addition of coffee and cardamom.

Under the experimental conditions, fermentation time from seven days is sufficient to ensure the physicochemical and microbiological quality of cocoa blends with the addition of coffee and cardamom, but it should not exceed nine days.

Acknowledgments

The authors thank the Federal Institute of Education Science and Technology of Rondonia for providing resources to conduct the research and for granting scientific initiation scholarships.

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