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Physical, chemical and microbiological characterization of cupuassu seed during fermentation

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Abstract: The cupuassu's seeds are similar to cocoa, being possible to apply fermentative techniques used to obtain nibs and cupulate. The aim of this work to study the physicochemical transformations during the cupuassu´s seeds fermentation. For this purpose, frozen seeds from a pulp processing industry were fermented in three batches of 20 kg for seven days at room temperature. During the fermentation process, seed mass temperature, dimensions, density and composition of the kernels, color indexes (L*, C* and H*), microbiological analyzis (mesophiles, molds and yeasts), cut-proof, physicochemical analyzis (pH, acidity and glucose reducing sugars and non sucrose reduction) and centesimal composition (ashes, lipids, proteins and carbohydrates) were analyzed from triplicates. The results were expressed as dry basis. During the fermentations, the great temperatures were reached. The maximus were 43,44 and 47 ºC in three fermentations. It was observed during the fermentation process: seed darkening (L*) and color intensity reduction (Chroma), pH increase, sugar reduction and acidity reduction. The ash, lipid and protein contents were not significantly influenced by the fermentation time. According to the cut test, the kernels of 3rd fermentation, whose maximum temperature was higher, were classified as type 2 and the others as type 3. The values of apparent density, dimensions, mass and composition of seeds demonstrated that during the fermentation there was a decrease in volume with a higher proportion of cotyledon compared to testa. There was an increase in counts of total mesophiles aerobic microorganisms, molds and yeasts, during the fermentative process.
Keywords: *Theobroma grandiflorum*, byproduct, cupuaçu, residue.

Introduction

The commercial interest in cupuassu is due to the refined sensory appeal of its pulp, however, efforts have been made in the search for possible foods and applications for the seed. As a byproduct of manufacturing pulp, the seed is rich in protein (9 to 12%) and fat (57 to 61%), therefore is a source of protein and other potential nutritional components (Carvalho et al., 2006). In addition to the high fat content in the seed, its digestibility, chemical and sensory characteristics are similar to cocoa butter (Azevedo et al., 2003). Therefore, cupuassu seed has been carefully studied with a view to its use due to its nutritional value, since it is rich in fatty acids (oleic and stearic acid) and dietary fiber in addition to its functional properties (Carvalho et al., 2008; Cohen et al., 2003).

The cultivation of cupuassu has significant economic value for the Amazon region, where it is native, the pulp being its main product that represents a yield of 24 to 44% and is marketed in fresh and frozen form. Its seeds correspond to 20% of the total mass of the fruit (Lopes et al., 2003; Cohen & Jackix, 2005) and can become the object of research in the food and pharmaceutical industries, and can be used in the development of a product similar to chocolate called cupulate®, in addition to cakes, cookies, cereal bars, breads and ice cream (Cohen and Jackix, 2005; Vilalba et al., 2004).

The use of cupuassu seed as a cocoa substitute aims to guarantee the quality of the cocoa-dependent product in the off-season, to provide the addition of new characteristics to it, in addition to promoting a reduction in the price of the final product (Medeiros and Lannes, 1999). Unfortunately, the quality of the final product is still insufficient and inferior compared to cocoa products, perhaps because of the low aroma potential of the raw material or because the processing used for cocoa seeds is not suitable for *T. grandiflorum* or *T. bicolor* seeds, requiring that the procedures adapt to the biochemical and morphological characteristics of the different seeds (Reisdorff, 2004).

The industrialization process of cupuassu seeds includes the stages of fermentation, drying and roasting and occurs in a similar way to that of cocoa seeds, due to the botanical and chemical similarities between these products (Carvalho et al., 2008). All stages are fundamental for the appearance of a typical and pleasant aroma (Reisdorff, 2004). Fermentation is the stage in which the seeds, already removed from the fruit, suffer the attack of microorganisms present in the environment, initiating countless physical-chemical and biochemical reactions responsible for the development of precursor compounds of chocolate flavor and aroma (Lopes et al., 2003). In the fermentation stage, the germinative power of the seeds is lost. The fermentation process, when done more mildly, accelerates germination. When the process is prolonged, the embryo dies, and the seed is called almond (Vilalba, 2003). The appearance of cupulate® precursors is dependent on the temperature increase that occurs in the seed during fermentation. Fermentation occurs in the pulp that surrounds the almonds and occurs in the first three days leading to the death of the embryo, after which the healing takes place inside the seeds, characterized by enzymatic reactions (Jorge, 2011).

The fermentation process is characterized by the production of organic acids (acetic acid and lactic acid), which are particularly important for the quality of cupuassu (Figueiredo et al., 2006). During this process, microorganisms are responsible for chemical reactions that convert sugars into ethanol, a substrate used by acetic bacteria for the production of acetic acid. In addition, citric acid is metabolized to lactic acid by lactic acid bacteria, resulting in non-volatile acidity in the final product. These lactic acid bacteria may be responsible for leucine transamination and decarboxylation, resulting in the production of important compounds such as 3-methylbutanal, a substance with strong chocolate notes (Ramos et al., 2016).

The processing of cupuassu seeds aims to reduce the discards that cause environmental pollution and food waste, and can also be incorporated into the formulations of many types of food products, promoting their nutritional, sensory enrichment and the aggregation of technological quality. Although cupuassu seed products have great potential in the food industry, they are little used and there is still a lack of information in the literature regarding their physical-chemical characteristics and response to processing.

The objective of this work was to study the physical, chemical and microbiological transformations that occur during the fermentation process of cupuassu seeds previously frozen, stored and thawed, in order to verify the main reactions and changes related to this process and to evaluate the final quality of the seeds dried and fermented.

Methods

Three batches of cupuassu seeds containing residual pulp adhered to the seed coat were obtained from a pulp processing industry in Sinop, MT, Brazil, and frozen for 30 days. Each seed batch was thawed separately and subjected to fermentation by the method of Cohen and Jackix (2005) in which a wooden box adapted according to the proposed model Grimaldi (1978) was used with all its measures reduced by half. The box, which has three compartments with a shape and volume suitable for the process, was placed away from rain and sun. Initially, the seeds (20 kg) were placed in the first compartment of the box, together with chopped banana leaves, for inoculation of the microorganisms on the surface of these leaves, and covered with burlap sacks, to help retain heat generated during fermentation. The total process time was seven days, with seed turning after 48 h for the second compartment and 72 h for the third compartment of the box.

The quality of the fermentation process of cupuassu seeds was analyzed and compared by means of daily determinations of: mass temperature at different levels (surface, middle and bottom of the box), with the aid of a mercury thermometer; physical characterization (apparent density, proportion of cotyledon, testa and germ, dimensions of length, width and thickness and average mass per almond) with ten repetitions; instrumental color and microbiological analysis.

The instrumental color analysis was performed with ten repetitions using the colorimeter (Konica Minolta, model CR-400,) with direct reading of reflectance from the coordinates L * (luminosity), a * (coordinate red / green) and b * (coordinate yellow / blue), according to the cielab system, and using the D65 illuminant. The values of a * and b * were converted to the Chroma index (C*) (equation 1) and to the Angle Hue (° H) (equation 2), whose equations are presented below:

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

Equation 1

$$°\mathrm{H} = tan^{-1}\left(\frac{b*}{a*}\right)$$

Equation 2

In the microbiological evaluation, the seeds were removed daily during fermentation and submitted to total Mold and Yeast counts by standard plate counting methods and total mesophilic aerobic microorganisms by standard plate counting methods using Agar DG18 (dichloran glycerol) and PCA (plate count agar) as culture media (Silva et al., 2010).

Aliquots of approximately 200 grams of cupuassu seeds were also removed daily during fermentation, which were dried in an oven at 50 ° C with air circulation until constant weight for physical-chemical analysis and chemical composition. The analyzes were carried out in triplicates by means of determinations of: pH from the dilution of 10 g of the sample in 100 mL of distilled water with the aid of a digital pH measurement (Tecnal, HMCDB-150); titratable acidity using the titration method with 0.1 M sodium hydroxide solution; fixed mineral residue (ash) by calcination in a muffle at 550 ° C; ether extract (lipids) by the Soxhlet method with petroleum ether; protein fraction by determining the percentage of total nitrogen in the sample, according to the Kjeldahl method and multiplication by factor 6.25; glucose-reducing sugars and non-sucrose-reducing sugars were determined by Lane Eynon's titulometric method using Fehling's solutions (IAL, 2008).

After the fermentation processes were finished, the seeds of the three batches were dried in an oven at 35 ° C with air circulation until constant weight to perform the cut test, physical characterization and color analysis. The cut test followed the method proposed for cocoa, Normative Instruction No. 38, of June 23, 2008 (BRAZIL, 2008), where 100 fermented and dried almonds were randomly removed, in duplicate, which were sectioned longitudinal shape, observed for the defects present and classified between types I, II and III or out of type.

For data analysis and evaluation of the influence of fermentation time, statistical regression tests were used in the analysis of variance (one-way ANOVA) and Tukey's post-test, using a probability level below 5% (p 0,05). To evaluate the final characteristics of the fermented and dried seeds in three fermentation batches, Tukey's analysis of variance and post-test was used ($p \leq 0.05$).

Results and discussion

The temperature measurements at different points of the fermenting mass (surface, middle and bottom) allowed to observe significant differences as a function of time and to verify that the average values varied from 25 to 41 ° C, 25 to 41 ° C and 24 to 37 ° C for the surface, middle and bottom regions of the fermentation compartment, respectively, independent of fermentation. The maximum values reached in any of the regions during fermentations 1, 2 and 3 were 43 ° C, 44 ° C and 47 ° C, respectively, obtained on the 4th or 5th day (Figure 1A).

The average temperatures reached a level of 40 ° C. Thus, it is below the level between 45 ° C and 50 ° C mentioned by Cohen and Jackix (2005) and Vasconcelos (1999) as suitable for the development of the fermentation process. efficiently. This range was not reached in these experiments, perhaps due to the small scales conducted (20 kg / lot), the amount of pulp adhered to the seeds and inadequate general climatic conditions. By the different values obtained in the surface, middle and bottom points, it is possible to observe the importance of the seed turning throughout the process to guarantee a homogeneous fermentation.

According to Vasconcelos (1999), it is believed that the use of pulped seeds favors the aeration of the dough and, therefore, accelerates the temperature increase in the first hours of the process. On the other hand, Criollo et al. (2010) and Campos et al. (2020) obtained better responses in the quality of the fermentation process by including a certain proportion of pulp together with the seeds. Souza et al. (2016) found that the temperature of cupuassu seeds during fermentation continued to increase until the third day when it exceeded 40ºC. From the fourth day on, a reduction process started, reaching less than 30ºC on the seventh day. These authors found that the maximum temperature was reached the day before in the present work, it is believed that this is due to the fact that the initial temperature is higher and the addition of 30% sugar syrup at a concentration of 1%, by weight of the batch, may have contributed to speed up the fermentation process which results in an increase in temperature.

In the apparent density values, there was a significant effect of the fermentation time in its reduction with values that ranged from 0.9358 to 0.8302 g mL^{-1} (Figure 1B). The dimensions of width and thickness increased slightly during fermentation, while the length did not vary. The variations found were 25.64 to 26.79 mm, 20.78 to 21.53 mm and 10.98 to 11.79 mm for length, width and thickness, respectively (Figure 1C).

The decrease in the volume of the seeds is justified by the fermentation process mediated by the microorganisms that consume the pulp residues present in the seed, in addition to the loss of liquids existing in the fermentation. The values of the seed composition (Figure 1D) indicate that there was death of the cupuassu seed and triggering reactions resulting from it in the almond (Vasconcelos, 1999), confirmed by the results of apparent density, dimensions and average mass per almond. In addition, in the three fermentations the 40 ° C required for the death of the embryo was reached (Criollo et al., 2013).

There was a significant decrease in the average mass per almond during fermentation with an average variation of 5.54 to 4.97 g (Figure 1D). They found an average of 6.67g for seeds before fermentation. This difference is justified by the loss of moisture and transformations inherent to the fermentation process (Robayo, 2010).

As for the composition of the seed, it was found that the largest proportion is cotyledon, followed by the testa and germ. During fermentation there were significant effects in the reduction of the proportion of testa (from 42.22 to 28.12%) with an increase in the proportion of cotyledon (56.91 to 70.78%) and a slight increase in the proportion of germ (0.87 1.10%) (Figure 1 E).

The fermentative process influenced some instrumental color parameters, with a significant reduction in the values of L * and Chroma and with no effect on the values of hue. The variations in the values of L and Chroma and hue were from 47.77 to 39.62, 22.61 to 16.78 and 46.46 to 53.38, respectively (Figure 1 F).

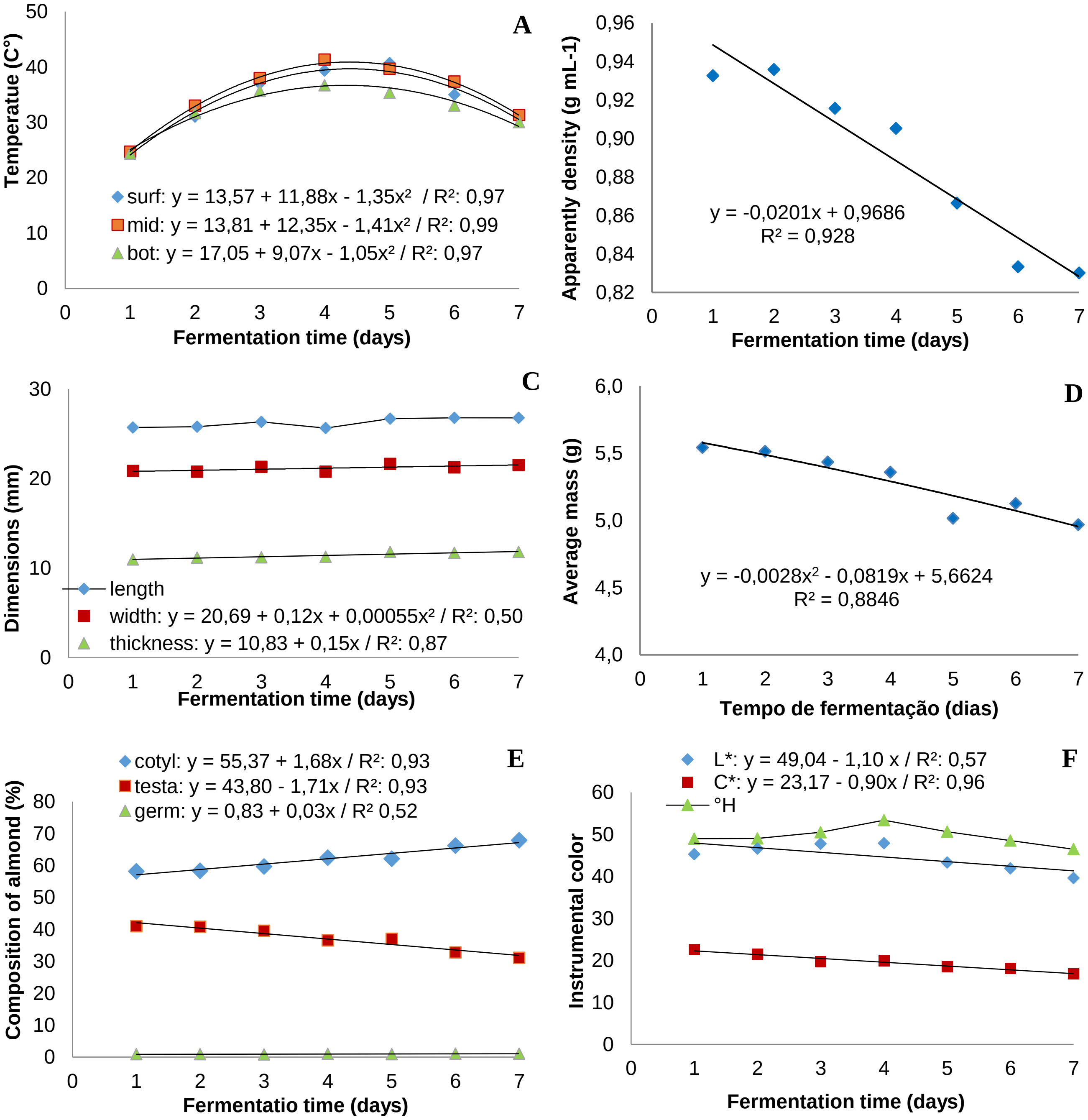

Figure 1. Physical parameters measured during the fermentation process of cupuassu seeds from industrial pulping. Surf: surface, mid: middle, bot: botton, cotyl: cotyledon.

It was found that from the first to the fourth day the almonds became lighter, and that from the fourth to the seventh day they darkened, as can be seen by the reduction in the Luminosity (L *) values (from 47.77 to 39.62). According to the Chroma index, it can be said that the almonds had a reduction in color intensity / saturation, while the Hue angle did not change significantly, indicating that there was no change in seed tonality, maintaining the corresponding values between the red and yellow tones, or that is, that make up the dark brown color, characteristic of products with cocoa / cupuassu derivatives. Souza et al. (2016) showed a reduction in L * values during the fermentation process, from 38.65 to 25.66, without however showing significant changes in the values of a * and b * that were used for the calculations of C * and ° H in the present job.

The pH and acidity values obtained (Figure 4A and 4B) differed significantly as a function of time, varying from 4.27 to 5.70 and 32.69 to 7.66 Meq NaOH 100g^{-1}, respectively. It is worth mentioning that these data do not exactly represent the values present in the wet cotyledon throughout the fermentation process, since these results are expressed on a dry basis.

There was a significant reduction in acidity with concomitant and a significant increase in pH values during fermentation. Such behavior is expected, since during the anaerobic conditions of the fermentation the microorganisms consume acids present internally and on the seed surface. This fact is important because for the positive transformations of aroma and flavor to occur, it is important that there is a loss of acidity from citric, lactic, oxalic and succinic acids (Pugliese, 2010). Lopes (2000) and Carvalho (2004) determined 11.72 meq NaOH 100 g-1 and 11.79 meq NaOH 100 g^{-1}, and Campos et al. (2020) detected variations from 15.4 to 1.7 meq NaOH 100 g^{-1} from time 0 to 108 hours of fermentation in several treatments tested. All of these values are below the values found in the initial fermentation period of the present work. Such differences can be justified by the base used, which in this work opted for dry basis. In contrast, Sousa et al. (2016) found that the acidity was significantly lower in the first days, rising during the fermentation process. Such difference in behavior may be due to the fact that these authors added sugary solution in the fermentation process and / or because they did not express the results on a dry basis, thus, the loss of moisture and water activity that occurred during the fermentation may have concentrated the acids present. These same authors point out that several factors can interfere with acidity, such as variety, fruit maturation, harvest time, planting place, conduction of the fermentation process and the amount of pulp around the cocoa seed for its fermentation.

After 5 days of fermentation, the almonds showed pH values above 5.0 (Figure 2), which according to Lopes (2003) demonstrate a significant increase in the potential for the development of precursors of chocolate flavor and aroma. Ramos et al. (2020) found that all cupuassu fermentation experiments showed progressive pH increases, and that values above 8.0 should be avoided in order to prevent the formation of undesirable compounds (off flavors).

All of these physico-chemical changes during fermentation are essential for the final quality of the product. The increase in the temperature of the bulk during fermentation is attributed to the metabolic activity of microorganisms. During the 1st day, certain yeast species that require little oxygen predominated, and the temperature is not that high. With the consumption of citric acid by the yeasts, the pH value of the pulp gradually increases, making the medium more favorable for bacteria producing lactic acid. When the pH of the pulp reaches values above 4.0, acetic acid-producing bacteria begin to predominate, reaching higher temperatures, from 45ºC to 50ºC (Dias, 1987). Thus, parameters such as temperature, pH and acidity provide fast and accurate determinations throughout the fermentation process, being important indicators of their quality.

As for the proximate composition, there were no significant differences depending on the fermentation time in the contents of ash, lipids, proteins and carbohydrates, whose average values were 3.52, 45.58, 9.95 and 41.67g.100g^{-1}, respectively.

As for the proximate composition, no significant variations in ash, lipids, proteins and carbohydrates were observed as a function of time. Vasconcelos (1999) and Lopes (2000) detected slight losses in the total protein content during fermentation. During fermentation, specific hydrolysis of proteins occurs, especially of the globulin fraction, providing the formation of peptides and amino acids (Lopes et al., 2003). Two proteolytic enzymes are involved, an aspartic endopeptidase (EC 3.4.23) and a serine-type carboxypeptidase (EC 3.4.16), whose hydrolysates participate in Maillard reactions in the presence of reducing sugars. The products of this reaction contribute essentially to the characteristic aroma of a cocoa product (Reisdorff, 2004).

The present study did not reveal a reduction in protein content during fermentation, possibly justified by the method used being nitrogen determination, that is, hydrolysis products can still be quantified as proteins. Carvalho et al. (2008) found that the protein content in cupuassu seeds decreased from 26.17% to 21.15% after the fermentation process, and to 20.12% after roasting.

Glucose-reducing and non-sucrose-reducing sugars could be detected only on the first and second day of fermentation. Vasconcelos (1999) found reduced levels of sucrose from the third day and Robayo (2010) found a reduction in the levels of reducing sugars in one of the fermentation processes. Glucose-reducing and non-sucrose-reducing sugars could be detected only on the first and second day of fermentation. Vasconcelos (1999) found reduced levels of sucrose from the third day and Robayo (2010) found a reduction in the levels of reducing sugars in one of the fermentation processes.

Initially, the hydrolysis of sucrose in glucose and fructose occurs (Lopes et al., 2003), which are intensely consumed by fermentative microorganisms, especially in the initial anaerobic phase that occurs in the first days of fermentation. At this stage of fermentation, yeasts break down the sugars of the adherent pulp into ethanol which is oxidized by bacteria to acetic acid in the subsequent aerobic phase (Reisdorff, 2004).

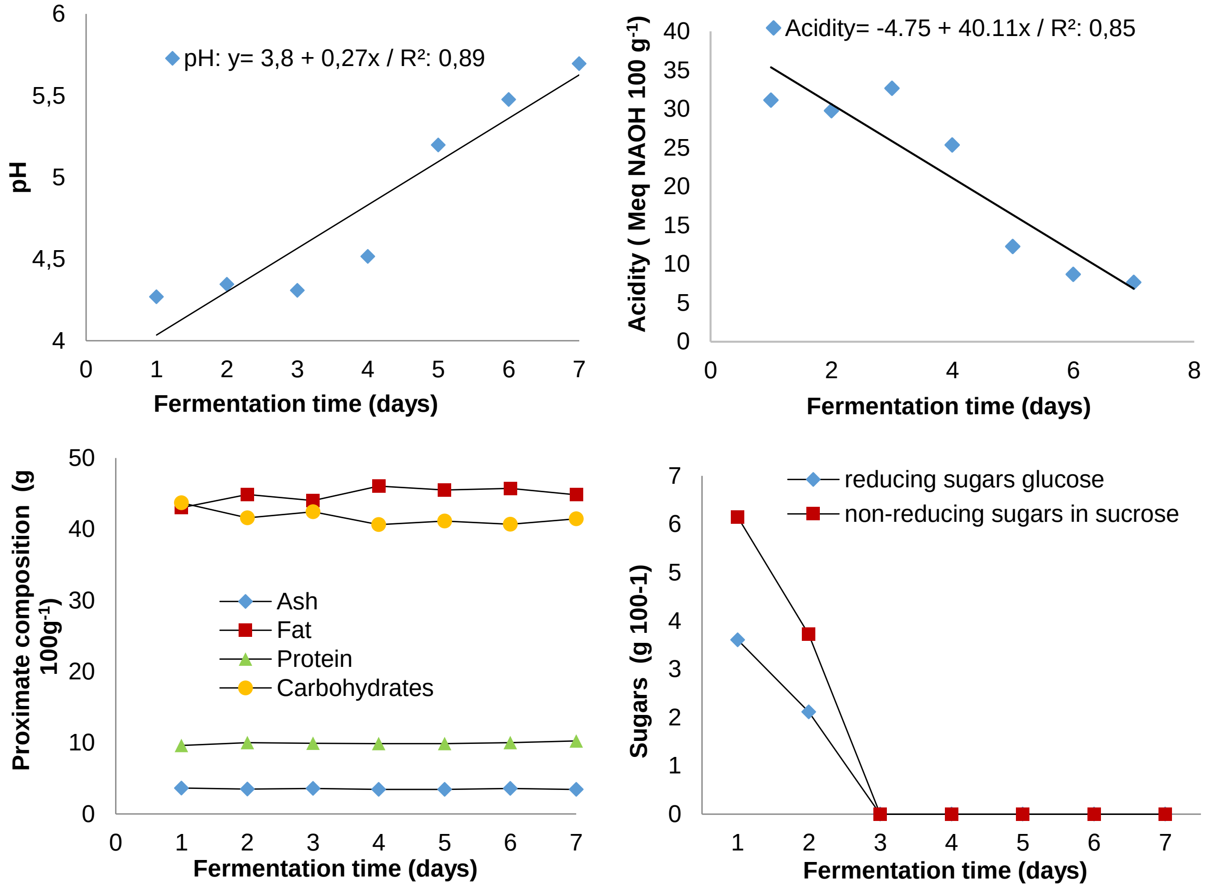

Figure 2. Chemical parameters and chemical composition (dry basis) in cupuassu almonds during fermentation. BS: dry basis.

Analysis of the microbiological profile (Figures 3A and 3B) showed the behavior of microorganisms throughout the fermentation process, in which the counts of total aerobic mesophilic microorganisms increased abruptly, especially in the first two days of fermentation, followed by a slight drop on the third day, and continue growing until the end of the fermentation period. At the start of fermentation, yeasts proliferate converting sugars to ethanol and CO_2 and also produce pectinolytic enzymes. The initial pH of the pulp, the presence of citric acid and low levels of oxygen in the fermenting mass during the first 24 hours, favor the colonization of the mass (Robayo, 2010). Over the 7 days of fermentation, variations of aerobic mesophilic microorganisms were found for lots 1, 2 and 3 from 7.6 x 10^4 to 3.10 x 10^7 UFC g^{-1} (log = 4.88 to 7.49), 1.12 x 10^4 to 1.68 x 108 UFC g^{-1} (log = 4.04 to 8.22) and 3.95 x 105 to 6.32 x 10^7 UFC g^{-1} (log = 5.60 to 7,80), respectively. The highest and lowest counts were detected in the second and first batch of fermentation, respectively.

As for molds and yeasts (Figure 3B), a behavior similar to that mesophilic microorganisms were observed, there was a proliferation and increase of this population throughout the fermentation, with a more pronounced decrease in relation to bacteria on the third day, followed by an increase in the population until the end of the fermentation. Most of the time, the highest and lowest counts were observed in the second and first batch, respectively, with inversion at the end of the fermentation process. Counts ranging from 1.70 x 10^4 to 5.00 x 10^7 UFC g^{-1} (log = 4.23 to 7.70), 1.19 x 10^4 to 2.79x 10^7 UFC g-1 (log = 4.07 to 7.17) and 1.26 x 10^5 to 1.93 x 10^7 UFC g^{-1} (log = 5.10 to 7.28), for lots 1, 2 and 3 of fermentation, respectively.

As for the microbiological aspects during fermentation, similar growth behaviors were observed for mesophilic microorganisms, molds and yeasts, being very pronounced in the first two days, followed by a slight drop in the microbial population on the third day and a subsequent increase until final fermentation period. The initial phase of fermentation is anaerobic, from the third day on, the seeds are revolved daily to homogenize the heat and the process becomes aerobic (Pugliese, 2013). In anaerobic glucose fermentation, large amounts of lactate and traces of acetate, ethanol, formate and 2,3-butanediol are formed, while under aerobic fermentation there is a mixture of lactate, acetate and acetoin-diacetyl (Figueiredo et al., 2006). The reduction in the yeast population was more pronounced than the mesophilic microorganisms, possibly because they metabolize citric acid causing an increase in the pH of the dough, which inhibits its growth and creates a favorable environment for lactic acid bacteria. According to Ramos et al. (2020), during the fermentation of cupuassu seeds there is a proliferation of bacteria and yeasts with a predominance of yeasts like *Pichia* and *Hanseniaspora*, and bacteria like *Acetobacter* and *Lactobacillus*. The concentration of the pulp of the cupuassu grain and its composition influence the diversity of bacteria and yeasts, with the highest concentrations of pulp (15%) along with the seeds providing a predominance of bacteria over yeasts, Criollo et al. (2010) optimized the fermentation process by using 30% pulp and adding 0.1% yeast (*Saccharomyces cerevisiae*), thus the fermentation temperature reached 44 ° C, and the proportion of fermented grains reached 56.14 % with greater development of fruit flavors.

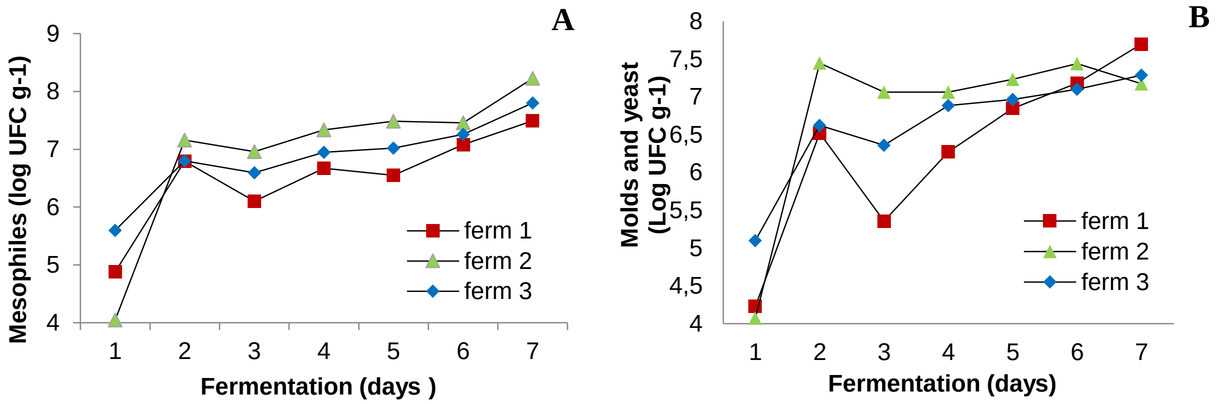

Figure 3. Count of aerobic mesophilic microorganisms and molds and yeasts in three fermentation batches. Ferm1, ferm 2 and ferm 3: lots 1, 2 and 3 of cupuassu seed fermentations.

At the beginning of fermentation, yeasts proliferate, converting sugars to ethanol and CO_2 and producing pectinolytic enzymes. The initial pH of the pulp, the presence of citric acid and low levels of oxygen in the fermenting mass during the first 24 hours, favor the colonization of the mass (Robayo, 2010). The graphs generated from the results demonstrate that the growth and predominance of a group of microorganisms together with the products of their metabolism can affect others, since on the third day there was a reduction in the population, also coinciding with the period of temperature increase in the seed mass. Fermentation 3, for example, was the one that reached the highest temperature and the best quality profile of the seeds fermented by the cut test (Table 1). Thus, we believe that the total and general counts of microorganisms cannot always be related to the fermentative quality, since lower temperatures can also provide intense microbial growth, including undesirable ones.

After the end of the fermentation, the seeds were dried and their quality was evaluated by means of the cut test (Table 1), where it can be seen that the biggest defect found was related to breaks (12.5 to 16.5%) followed by slate seeds (8 to 15.5%), moldy (4.5 to 6%) and flat (0 to 1%).

Table 1. Evaluation of the fermentative quality of cupuassu seeds by the cut test.

Fermentations (Repetions)	Defects in seeds (%)			
	Breaks	Slate	Moldy	Flat
1ª	14,5	15,5	6	1
2ª	12,5	12,5	4,5	0
3ª	16,5	8	4,5	0

Through the cut test (Table 1), fermented and dried seeds can be classified according to their fermentative quality. Normative Instruction nº 38, of June 23, 2008 (Brazil, 2008), for fermented cocoa beans to be classified as Type 1 (superior), there must be 0-5% of slates, 0-5% of flattened and 0-4 % of molds. Using this method to assess the pattern of fermented cupuassu almonds, almonds from the 3rd fermentation can be classified as Type 2 (5-10% slates, 5-6% flattened and 4-6% moldy) and the others as Type 3. It can be seen that there is a high amount of seeds damaged by the pulping processes of the fruit in the industry. It was also possible to verify that the fermentation process did not show the maximum efficiency, taking into account the high proportion of slate seeds. These problems are possibly not reflections solely related to the conduct of the fermentation process, but also to the quality of the seeds received by the industry. As the main objective of the region's agribusiness is to obtain pulp, machinery often ends up damaging some seeds during pulping. These damaged seeds are not viable and compromise the quality of the fermentation process.

The evaluation of the parameters of mass, dimensions, composition and instrumental color of the fermented and dried cupuassu almonds, from the three fermentative processes (Table 2). For the parameters of mass, length, width, cotyledon, forehead and germ, there were no differences depending on the different batches of fermentation processes, with average values of 2.72g, 25.96 mm, 20.78 mm, 70.20%, 29.07% and 0.73%. The thickness and color parameters (L *, C * and ° H) differed between the fermentation batches, with variations ranging from 9.86 to 11.43 mm, 36.40 to 40.61, 13.79 to 17, 08 and 45.28 to 47.81.

Pessoa et al. (2019) determined measures of length, width and height (thickness) of 24.48, 19.88 and 11.56mm, respectively. Lopes (2000) found values of 22.00, 11.95 and 7.47mm for the parameters length, width and thickness, respectively, and for average mass the value of 0.9816g. The differences compared with the values of the present study can probably be related to the use of seeds from another cupuassu genotype. The variation of the thickness parameter may be related to a low quality fermentation process of the seeds of the 1st fermentation, as observed in the cutting test (Table 1), where there was a higher average of slates and moldy seeds.

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The color comparison of the fermented and dried seeds from the three fermentation batches (Table 2) shows that the seeds of the 3rd fermentation, whose fermentation process was more efficient because these seeds showed fewer defects (Table 1), were darker (lower L *) and with a shade closer to red than yellow (° H).

The main factors that interfere in the fermentative quality are: fermentation system (baskets, trays, bags and piles), room temperature, pH and acidity of the pulp and cotyledon, time and temperature of the process and periodicity of the mass rollings (Lopes et al., 2003). Campos et al. (2020) and Robayo et al. (2010) found a positive influence on the fermentative quality of the addition of pulp (15%) and sugar solution (30%) at a concentration of 1%, respectively, mixed with the seeds. Such results indicate that these actions can provide acidic substrate of carbon source and fermentable energy for microbial fermentation to occur and promote the proliferation of beneficial microorganisms for transformation

Tabela 2. Características físicas finais das sementes fermentadas e secas oriunda dos três lotes de fermentações

Parameter	1ª fermentation	2ª fermentation	3ª fermentation
Mass (g)	2,79 ± 0,29a	2,64 ± 0,39a	2,73 ± 0,58a
Length (mm)	26,04 ± 2,02a	25,82± 2,25a	26,03 ± 2,93a
Width (mm)	20,46 ± 2,29a	20,60 ± 1,48a	21,31 ± 2,14a
Thickness (mm)	11,43 ± 2,56a	9,86 ± 1,16b	9,88 ± 2,04b
Cotyledon (%)	72,32 ± 0,06a	69,47 ± 0,14a	68,82 ± 4,04a
Forehead (%)	27,02 ± 0,22a	29,80 ± 0,09a	30,41 ± 4,12a
Germ (%)	0,68 ± 0,16a	0,73 ± 0,04a	0,78 ± 0,08a
Color (L*)	40,61 ± 4,92a	38,99 ± 4,18ab	36,40 ± 3,79b
Color (C*)	17,08 ± 3,25a	13,79 ± 4,06b	16,00 ± 4,73ab
Color (°H)	47,81 ± 8,66ab	53,30 ± 7,29a	45,28 ± 6,22b

Different letters on the same line indicate significant differences ($p<0.05$) by the Tukey test.

Conclusion

Through these results, it is clear the influence of temperature and other factors related to seed characteristics, amount of pulp adhered to the seed, etc. in the quality of the fermentation process.

The physical-chemical, microbiological, color analysis and cut test parameters serve as markers of fermentation process efficiency.

There were significant changes in temperature, density and seed composition, pH, acidity and microbial count in cupuassu seeds during the fermentation process.

The previous freezing of the seeds did not negatively influence the quality of the fermentation process, since the seeds presented all the expected responses of physical-chemical and microbiological transformations.

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