Theobroma Cacao: An Evaluation of Enzyme Treatment with Pectin in the Pulping of Cocoa

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Cocoa (*Theobroma cacao L.*) in Brazil has a notable social and economic influence. However, when processed, cocoa generates byproducts that are discarded, including its pulp. This study aimed to evaluate the influence of the enzymatic treatment with pectinase on the physical-chemical properties of the extracted cocoa pulp. Untreated pulps obtained a lower yield (T1: 1.40% and T2: 4.4%) than those receiving treatment (T3: 15.62% and T4: 19.47%). Comparing the processes, the pH values remained close between 3.49 to 3.41, the soluble solids content between 16 and 19 ºBrix and the colorimetric analysis reporting L* 27.22 to 27.49; a* 11.35 to 11.59 and b* 0.26 to 0.51. The results demonstrated that the enzymatic treatment is a viable and expressive alternative in the pulping of cocoa beans, contributing mainly to the final or intermediate product desired yield.

Keywords: Cocoa Pulp. Pectinase. Agro-Industrial Residue.

Introduction

Due to its high volume, cocoa pulp became an agro-industrial residue, and the search for its applicability has been growing considerably [1]. From some studies, we observed the use of cocoa pulp for several products, such as cocoa jellies, fine and fermented beverages, alcohol, vinegar, yogurts, ice cream, juices, and even pulp in its natural form [2]. Another considerable residue in the cocoa industry is the shell of this fruit since it makes up approximately 70% of it [3].

Although it is considered a waste product, cocoa pulp is a raw material with great potential for application in the industry [1]. Its composition is formed by water (approximately 85%), sugar (12.5%), and other compounds in small quantities, such as proteins and citric acid [4]. The chemical composition of cocoa is directly related to numerous factors, and the main one is the type of cocoa.

Cocoa (*Theobroma cacao L.*) is a tropical species spontaneously found in the humid lowland forests of South and Central America [5]. It is one of the cultures capable of keeping the man in the countryside, thus having high social and economic importance in several tropical regions worldwide [6]. However, a series of byproducts need to be generated to obtain the final product to be commercialized, among them cocoa pulp and honey, which are still little used for commerce, being generally consumed *in natura* due to their perishability [7].

Knowing the characteristics of the pulp varieties currently planted in Brazil is necessary to allocate part of the pulp for producing new products. Cocoa pulp is characterized by the presence of fibers, pectin, and insoluble fibers, giving the product high viscosity with the pasty aspect of non-Newtonian fluid. These characteristics are ideal for producing alcoholic beverages such as wine and some foods such as jams, marmalades, and syrup [8]. Owing to the substantial presence of pectin, which plays an essential role in food projects due to its ability to modulate the response of humans to inhibitory effects on lipid intake and absorption in food [9], pectinase aims to perform pectin hydrolysis and clarification of fruit juices by dehydration. Pectinases are frequently used in the fruit juice industry and winemaking, with the filamentous fungus *Aspergillus niger* as the primary source [9]. Adding pectinases in juice processing reduces

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viscosity and increases the number of soluble solids in the final product [9].

Materials and Methods

Sample Selection

The cocoa (*Theobroma cacao L.*) samples were of a foreign variety and were purchased in Salvador, Bahia, Brazil. They were kept at room temperature until the moment of processing.

Pulp Extraction

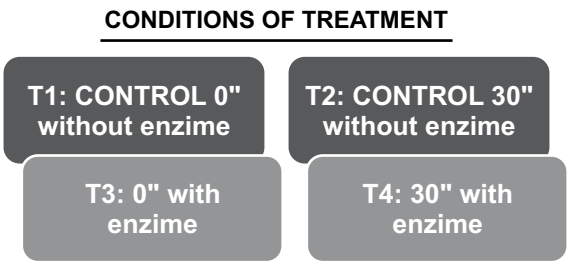
For extracting the pulp, the fully ripe pulps were first selected. While still in the shell, they were sanitized with a chlorine solution (100 ppm) for 15 minutes. After rinsing, the cocoa samples were opened, and the pulp was removed. In a beaker, 25g of pulp, 125µL of Pectinex® Ultra Tropical enzyme (Novozymes) (mixture of pectinases, cellulases, hemicellulases, and beta-glucanases), and 50mL of water were homogenized, and the enzyme action time was counted (Figure 1). For this assay, two blanks were evaluated (T1 and T2).

After the system-resting period, the yield was calculated, and the physico-chemical analyses were performed (in duplicate).

Yield

The yield consisted of the pulp released from the seeds and was calculated using the masses obtained according to Equation 1, in which SW - starting weight and FW - final weight.

Figure 1. Enzymatic treatment conditions for cocoa pulp at 0 and 30 minutes.



Equation 1

$$Yield\ (\%) = [\frac{SW\ (g) - FW\ (g)}{SW\ (g)}] * 100 \quad (1)$$

pH Determination

The pH was measured with a bench pHmenter, previously calibrated according to the manufacturer's standards, in which 10mL of the sample were analyzed following the method described by Adolfo Lutz [10].

Determination of Total Titratable Acidity

Based on the method described by Adolfo Lutz, 10mL of the sample was set aside and titrated with an aqueous solution of NaOH (0.1M). The result was expressed as % (v/v) [10].

Soluble Solids Determination

The Adolfo Lutz method was used, and the total soluble solids content was determined using a portable digital refractometer (Portable Digital Refractometer - BZW65). Appropriate calibrations of the refractometer were made using water at 20°C according to the manufacturer's instructions. An aliquot of the sample was added to the refractometer prism, and the scale read directly in Brix [10].

Colorimetric Analysis

A colorimeter (CR 10, Konica Minolta) was used to analyze the color of the cocoa pulp, selecting the CIELAB color space and using 3 repetitions per plate. In this color system, L* represents brightness (L*=0 - black and L*=100 - white), and a* and b* are the color coordinates responsible for chromaticity: (+a* = red and - a* is green, +b* is yellow and -b* is blue) [11].

Results and Discussion

The enzyme used to make the extraction of cocoa pulp aims to perform the hydrolysis of pectin, bringing benefits such as increased yield and clarification of the pulp. When comparing the physical-chemical analysis of the fruit pulp in question (Table 1) with the literature values, it was observed that the average pH of the sample had a value close to that found by Santos [8] in his test of characterization of cocoa honey for the production of jelly without added sugar.

The titratable acidity of the cocoa pulp had a value close to that of Silva and colleagues [12], who observed 1.16% in their analyses. All the results found after the analyses follow what is established by the current Brazilian legislation [13]. The soluble solids content meets the content reported in cocoa pulp by Pettipher [14], whose soluble solids concentrations ranged between 16.55 and 19.90 ºBrix. When comparing samples T1 and T2, there was an increase in yield from 1.401% to 4.413%. However, the other parameters do not indicate expressive changes; when comparing T3 and T4, there is also an increase in yield of more than 4%. Regarding yield, sample T3 (with treatment at 0 minutes) compared to T1 (no treatment at 0 minutes) yield was about 11 times higher when compared to T2 (no treatment at 30 minutes) to T4 (with treatment at 30 minutes) yield that increased about 4 times. However, pH, titratable acidity, Brix, and density, remained close. Table 2 presents the colorimetric analysis parameters of the cocoa pulp after enzymatic treatment. The L* parameter expresses the luminosity or clarity of the sample and varies from 0 to 100, so the closer to 100, the lighter the sample, and the further away, the darker. More positive a* values indicate a tendency towards red color and more negative towards green color. The more positive b* values express a higher yellow intensity, and the more negative ones have a higher blue intensity.

Therefore, the analyzed samples show higher turbidity, average L* at 27, with a reddish (average a* of 11.5) yellowish (average b* 0.3) tendency. The present study's results indicate no expressive variation in the parameters of colorimetric analysis for samples with and without enzymatic treatment.

Conclusion

We demonstrated in this study that the yield in diluted pulp submitted to enzymatic treatment was 11 times higher when compared to the treatment without enzyme. It shows that the application of the pectinex ultra tropical enzymatic complex is a good alternative for pulping cocoa seeds since this diluted pulp can serve as a basis for the elaboration of cocoa nectar, with greater use of this fruit, generating labor savings and without specific utensils/equipment. However, further studies are needed to evaluate other dosages and combinations with other enzymes and the application of other effects, such as temperature and agitation.

Table 1. Physical-chemical analyses of cocoa pulp with and without enzymatic treatment.

Analyses	Samples			
	T1	T2	T3	T4
Yield (%)	1.40±0.53	4.41±0.10	15.62±9.22	19.47±2.75
pH	3.49±0.01	3.49±0.02	3.41±0.01	3.42±1.98
Total titratable acidity (%)	0.99±0.99	0.99± 0.86	1.10±0.66	1.11±1.54
°Brix	18,70±0.01	16.55±0.01	17.40±0.01	19.90±0.01
Density (g/mL)	0.96±0.02	1.00±0.01	0.99±0.05	0.99±0.02

Source: Authorship.

Table 2. Colorimetric analysis of cocoa pulp with and without enzymatic treatment.

Parameters	Samples			
	T1	T2	T3	T4
L*	27.49±0.02	27.46± 0.05	27.33± 0.10	27.22± 0.06
a*	11.41± 0.04	11.35± 0.023	11.59± 0.03	11.56± 0.04
b*	0.26±0.02	0.29± 0.02	0.30± 0.04	0.51± 0.07

Source: Authorship.

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