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Article

Clarification effects in a functional copoazú (*Theobroma grandiflorum*) beverage

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Abstract: Copoazú is an Amazonic tropical fruit with outstanding sensory characteristics. Composition analysis showed that the pulp has a high content of carbohydrates, fiber, protein, potassium and magnesium and has low sodium content. These characteristics made Copoazú pulp an interesting food matrix to innovate and incorporate bioactive compounds in order to obtain functional drinks. Nevertheless, pulp also contains a starch fraction and some compounds that can be involved in deterioration reactions such as haze formation and non-enzymatic browning, therefore is a need for clarification treatments. This study evaluates the copoazú clarified juice processing by means of unit operations such as centrifugation, enzymatic treatment with pectolytic and amylolitic enzymes and flocculation with gelatin. The resulting beverage was assessed by pH, soluble solids, soluble sugar (sucrose, fructose and glucose) and organic acids content (malic, citric, ascorbic, succinic and oxalic acid) and sensory acceptation. Every stage of the clarification process changes pulp sugars and acids composition, affecting consumer acceptance.

Keywords: Amazonic fruit; beverage quality; fruit processing; juice composition; *Theobroma* sp.; sensorial quality

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1. Introduction

Theobroma genus is typically tropical, distributed across occidental hemisphere between 18°N y 15°S. Around nine to twelve *Theobroma* species are native from Amazonia which has been assigned to the genetic distribution center. This genus have a high economic potential for regional and worldwide sustainable use [1].

T. cacao is the main specie due to its wide use in the chocolate industry. Wild species found in Amazonia as copoazú (*Thebroma grandiflorum*), maraco (*Theobroma bicolor*) and macambillo (*Theobroma subincanum*) are consider promissory because all the fruit parts (hard rind, pulp and seeds) have different potential uses as traditional derivates and innovated products in cosmetic and food industries. The *Theobroma* market is growing up due to their exotic characters [1].

Among this species, copoazú stands out because its high yielding aromatic and creamy pulp used to produce a variety of food products as juices, nectars, smoothies, jams, ice cream, sweets and even pies and cookies. Copoazú pulp has a high content of carbohydrates, fiber, protein, potassium and magnesium, and low sodium content [2].

Copoazú pulp also contains some high molecular weight carbohydrates such as highly esterified homogalacturonan that represents 2% of dried pulp and high amylose starch that represents 13% of dry defatted pulp, with a 71% of amylose [3-5]. This pulp fraction may have potential uses but in the case of juice can be involved in deterioration reactions such as haze formation and non-enzymatic browning.

As copoazú pulp products are interesting food matrixes to innovate and incorporate bioactive compounds in order to produce functional beverages, there is a need to evaluate different clarification methods. This study evaluates the changes in composition and consumer acceptance of copoazú clarified juice subjected to different unit processing operations.

2. Experimental Section

2.1. Clarified Copoazú juice processing

Fresh full-ripe copoazú fruits were obtained from Colombian Amazonia and processed in a pilot scale to obtain clarified copoazú juice as described in the following flow diagram (Fig. 1).

Copoazú pulp was obtained according to standardized process [6]. Briefly, the fruits were washed with tape water and crushed to open the hard rind, extracting pulp-seeds mass with spoons. Pulp-seeds mass was scalded (72 °C, 3 min) and processed in a 200 kg/h vertical pulping machine with 0,5 mm sieves (COMEK S.A, Colombia). The obtained pulp was pasteurized (90°C, 2 min), packed and refrigerated prior to juice separation. Juice was separated from pulp by centrifugation at 14000 rpm and 4°C for 20 min with a Sigma 3 -18KS centrifuge.

The juice was clarified with a pectolitic (Novozym ® 33095 – poligalacturonase, Novozymes ®) and an amylolitic (Amylase ™ AG 300 L, Novozymes ®) enzyme mix, (100 ppm each, 43°C, 60 min). At the end of the treatment, enzymes were inactivated by heating (90°C, 5 min).

The last treatment consisted in addition of 0,15 g/L gelatin with 5 min agitation followed by a12 h settling and final separation of flocculation product (centrifugation 14000 rpm at 4°C for 30 min).

2.3. Instrumental analysis

Pulp, juice and clarified juice were instrumentally analyzed assessing pH, soluble solids (°Brix), sugars content and organic acids content.

pH and soluble solids were determined using portable instruments in the process plant (Hanna pH-meter and Atago refractometer), allowing the samples reach room temperature (18-20 °C) before measurements.

As adequate balance between these volatile metabolites determines fruit palatability, sucrose, fructose, glucose, malic acid, succinic acid, citric acid and oxalic acid contents were assessed by means of HPLC, by comparison with areas and retention times of a calibration curve obtained for each metabolite with certified standards [7].

Three replicates were taken for each sample (pulp, juice and clarified juice) and frozen at -20°C before analysis. Metabolites were extracted weighing 100 mg of each replicate and adding 3 mL of H2SO4 – water 5 mM acid solution, with 10 min agitation in a vortex equipment, followed by centrifugation (10000 rpm, 45 min). Supernatant was taken and filtered with 0,45µm PTFE membranes prior to injection.

Acid solution was preferred for extraction because low pH levels assure extract stability by slowing oxidative reactions of quantified acids [8]

For metabolite quantification, separation system consisted in an Aminex® HPX-87H columm (Bio-Rad Laboratories®, California – USA) as stationary phase and H2SO4 5 mM solution as mobile phase at a 45°C temperature. Diode array detectors and ultraviolet (214 nm) detectors were used to determinate acids content and refractive index detector for sugars, all simultaneously [7, 9].

2.3. Sensory analysis

Pulp, juice and clarified juice were evaluated by 20 consumers, male and female between 24 and 50 years, all from Sinchi institute staff. For each sample the consumers pointed flavor and texture in a hedonic scale from 1 to 5, were 1 indicates extreme dislike, 3 neither preference or rejection, and 5 extreme like.

2.4. Statistical treatment

Instrumental analysis data were subjected to a two-way analysis of variance, means were separated by an LSD test at P=0.05 for the mean factor (copoazú fruit products; pulp, juice or clarified juice). Sensory analysis results were subjected to one-way analysis of variance and, if significant, means separated by Kruskal-Wallis non parametric test with multiple comparison at α=0.05.

3. Results and Discussion

3.1. Production of clarified copoazú juice

Yield for clarified juice production from fresh whole fruits (Figure 1) was around 18%. As previous works had showed, the economic potential for this process is linked to by-products [2] and therefore they were indicated in the flow diagram (Figure 1).

3.2. Instrumental analysis

Ascorbic and oxalic acid contents were below detection limit for all samples. pH values and sugar contents (Table 1) were similar to those previously reported for this fruit [10], being sucrose the predominant sugar.

Every stage of the process changes quality traits in the Copoazú food matrix (Table 1). In the juice separation stage sugar and acid concentration increased. The fiber pellet was mainly formed by high molecular weight carbohydrates such as starch. Pulp water was the main solvent for these low molecular weight metabolites and therefore explaining a lower pH and higher soluble solids for juice and clarified juice.

Enzymatic and flocculation treatments in the clarification stage noticeably affected the juice composition by diminishing soluble solids in the clarified juice, which was explained by a decrease in sucrose, glucose and fructose sugars contents.

The clarification treatment increased some organic acids in the clarified juice (citric and succinic acid concentration). Malic acid concentration increased during juice separation but decreased again as a result of clarification. This behavior on organic acids and sugar composition were related to sensory characteristics of the Copoazú products (Table 2).

3.3. Sensory analysis

A loss of juice consumer acceptance was mainly associated to flavor losses (Table 2), which were in part due to the higher sugar content and the higher concentration of malic acid (Table 1). As the clarified juice showed lower sugar content and no detectable levels of malic acids (Table 1), no significant difference in flavor acceptance was detected between pulp and clarified juice.

Texture was affected by all process stages. As consumers were asked to evaluate a beverage, pulp consistency was thick and disgusting for consumers. Juice and clarified juice texture consumer acceptance are not significantly different from each other, as evaluators for clarified juice had a high variation in the responses. Nevertheless, palatability increased due to processing from juice to clarified juice.

4. Conclusions

Enzymatic and flocculation treatment applied in the copoazú juice clarification process caused a noticeable change in the juice sugars and organic acids composition which was in part the reason of an improvement of the scores imparted by consumers from copoazú juice to clarified juice.

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Conflict of Interest

"The authors declare no conflict of interest".

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Figure 1. Flow diagram for clarified Copoazú juice pilot-scale processing. Dashed boxes represent stage yields and by-products.

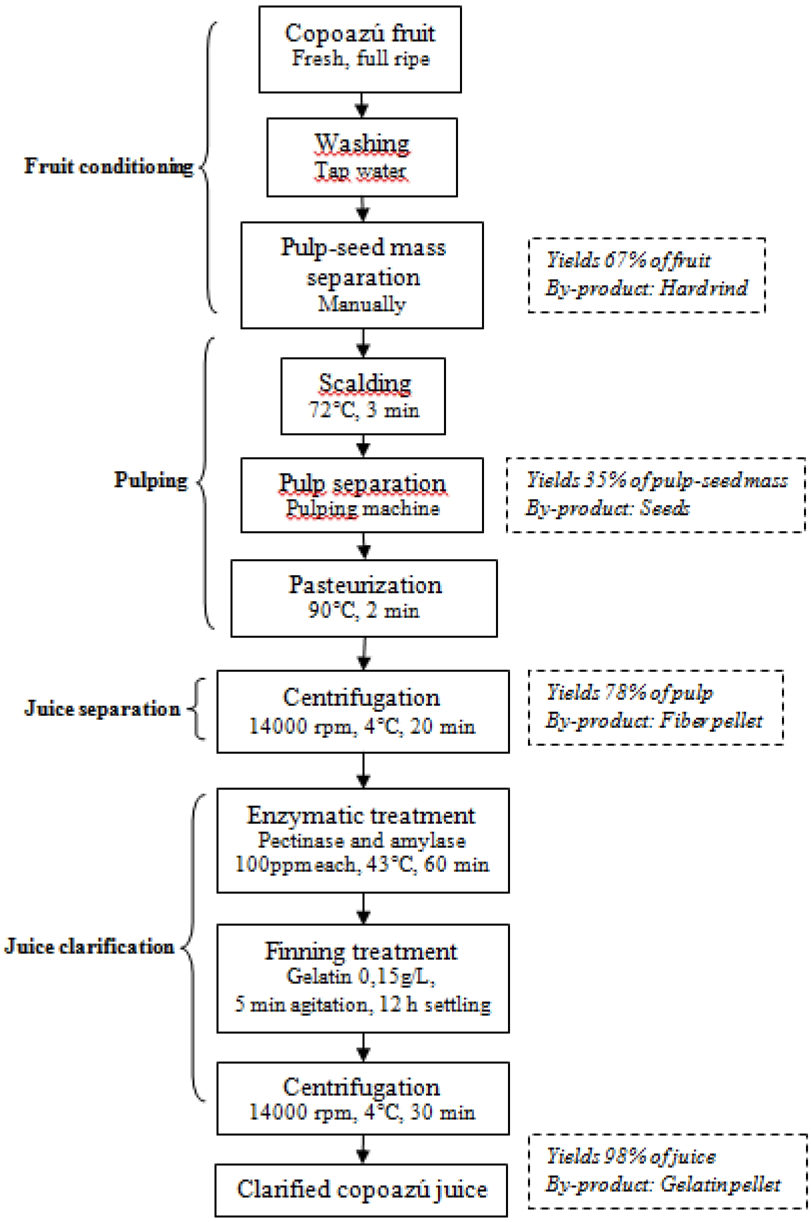

Table 1. Instrumental analysis of copoazú products (mean ± SD, n=3) during processing.

	Pulp	Juice	Clarified juice	LSD
Physicochemical traits				
pH	3.13 ± 0.15 ^{A}	2.18 ± 0.01 ^{B}	2.10 ± 0.02 ^{B}	0.18
Soluble solids (°Brix)	6.90 ± 0.56 ^{C}	39.60 ± 0.26 ^{A}	34.47 ± 0.31 ^{B}	0.80
Sugars and organic acids contents (mg/100g)				
Sucrose	52.03 ± 11.58 ^{C}	315.14 ± 10.95 ^{A}	280.73 ± 14.56 ^{B}	24.9
Glucose	28.21 ± 1.93 ^{C}	135.55 ± 4.63 ^{A}	102. 28 ± 5.72 ^{B}	8.8
Fructose	26.79 ± 1.83 ^{C}	129.21 ± 4.44 ^{A}	112.62 ± 5.59 ^{B}	8.5
Citric acid	20.33 ± 1.31 ^{C}	103.65 ± 3.23 ^{B}	117.92 ± 6.01 ^{A}	8.0
Succinic acid	nd ^{C}	9.53 ± 0.42 ^{B}	10.76 ± 0.54 ^{A}	0.8
Malic acid	nd ^{B}	38.75 ± 1.15 ^{A}	nd^{B}	1.3

Means followed by the same letter are not significantly different. (LSD test, P=0.05). nd=results below detection limit.

Table 2. Consumer preference for flavor and texture of copoazú products (mean ± variance, n=20).

	Pulp	Juice	Clarified Juice
Flavor	4.3 ± 0.3 ^{A}	3.3 ± 0.4 ^{B}	4.2 ± 0.3 ^{A}
Texture	2.5 ± 0.2 ^{B}	3.5 ± 0.3 ^{A}	4.1 ± 0.9 ^{A}

Means followed by the same letter are not significantly different according to a Kruskall-Wallis multiple comparison (α=0.05)