

Note. Quality evaluation of cupuaçu (*Theobroma grandiflorum*) purée after pasteurization and during storage

Nota. Calidad del puré de cupuaçu (*Theobroma grandiflorum*) después de la pasterización y durante su almacenamiento

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Cupuaçu (*Theobroma grandiflorum*) is an Amazonian fruit with a pleasant aroma and flavor, which offers a great economic potential. Purée of cupuaçu pulp was pasteurized (70 and 90 °C), and stored for half a year at 18 and 38 °C. The pasteurization was effective in microbial and enzyme inactivation, and stabilization of the purée. Peroxidase enzyme was inactivated (polyphenoloxidase was not detected in raw cupuaçu), some cupuaçu 'fresh notes' were lost, and the color changed slightly during pasteurization at both temperatures. Since, except for color, no significant quality differences were detected between samples processed at 70 °C and 90 °C, the pasteurization at 90 °C is recommended. The parameters that changed most during storage were the color, flavor, aroma and sugars. The purée got darker, especially at 38 °C, and the non-reducing sugars were converted into reducing sugars, while the total sugars remained constant. The final quality was determined by the storage temperature and not by the pasteurization temperature. The total color difference, TCD*, was well modeled by first-order reversible kinetics.

Keywords: cupuaçu, purée, *Theobroma grandiflorum*, color, pasteurization, storage, quality

Cupuaçu (*Theobroma grandiflorum*) es una fruta del Amazonas con un agradable aroma y sabor que ofrece un gran potencial económico. En este estudio se ha evaluado la calidad del puré de pulpa de cupuaçu pasteurizado (70 y 90 °C) y almacenado durante seis meses (18 y 38 °C). La pasteurización fue efectiva en la inactivación de microorganismos y de enzimas, y en la estabilización del puré. Durante la pasterización se desactivó la enzima peroxidasa, no se detectó polifenoloxidasa en el cupuaçu crudo, se perdieron algunas de las características sensoriales de frescura, y el color cambió ligeramente durante la pasteurización a 70 °C y 90 °C. No se encontraron diferencias significativas entre la calidad de las muestras procesadas a 70 °C o 90 °C, por lo tanto se recomienda la pasteurización a 90 °C. Durante el almacenamiento los parámetros que más cambiaron fueron el color, aroma/sabor y azúcares. El puré se pardeó especialmente a 38 °C, y los azúcares no reductores se transformaron en azúcares reductores, mientras que los azúcares totales permanecieron constantes. La calidad total final se determinó por la temperatura de almacenamiento y no por la temperatura de pasteurización. La diferencia en el color total, TCD* se ajustó a una reacción de cinética de primer orden reversible.

Palabras clave: pasteurización, almacenamiento, puré, calidad, *Theobroma grandiflorum*, cupuaçu

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INTRODUCTION

Cupuaçu (*Theobroma grandiflorum*) is an Amazonian fruit with a high nutritional value and good organoleptic properties (Venturieri, 1993). Due to its characteristic pleasant flavor and aroma, the cupuaçu is well known in this region and offers great economic potential both in Brazil and other countries. The pulp (40% of the fruit mass) is used for the production of nectar, ice cream, milkshake, spirit, jam, yogurt, chocolate fillings, sweet

desserts and other products. The fresh fruit is only available during four months of the year (January to May).

The preservation of the cupuaçu pulp at low temperature has previously been investigated (Oliveira, 1981; Miranda, 1989). However, the transformation of the fruit pulp in a commercial stable product at ambient temperature would offer more potential due to low cost of handling and storage. Oliveira (1981) processed the pulp at 100 °C for 15 min and evaluated the quality of the product during storage at 28 °C for 150 days. A pasteurization by hot-filling was suggested by Silva and Silva (1997) due to the low pH (~3.4) of the fruit (Venturieri, 1993). The aim of the pasteurization process is the destruction of microorganisms and/or inactivation of enzymes that might cause deterioration of the pulp (Gaze and Betts, 1992) during storage, although during the pasteurization process some quality properties can be lost. The color, flavor and aroma associated with the freshness of cupuaçu are very important quality attributes because they are immediately perceived by the consumer. Other physico-chemical properties, such as pH, acidity, soluble solids and sugars, can be monitored to assess the quality of the product.

Although some research to find adequate preservation methods for this fruit has been done, there is a lack of systematization about the effect of treatment and storage conditions on the final quality and safety of the cupuaçu pulp.

The main objectives of this work were to evaluate the quality changes in canned cupuaçu purée subjected to a pasteurization process and stored for half a year.

MATERIAL AND METHODS

Samples

Cupuaçu fruit (*T. grandiflorum*) Redondo variety was purchased from a local market in Belém, Brazil. The pulp was manually extracted, frozen and stored at -20 °C. The frozen pulp (~5 kg), in 1 kg bags, was air shipped, using dry ice, to Portugal. The pulp was defrosted overnight at ambient temperature before the experiments. The purée was obtained by blending (Moulinex, turbomix2) the pulp just before the experiment. A total of 350 thermal death time (TDT) cans with 6 cm diameter and 0.8 cm height (American National Can 208 × 006) were filled with approximately 12 g of purée and manually seamed (Lanico-Maschinenbau seamer, Braunschweig, Germany).

Pasteurization and storage

Half of the cans was pasteurized by immersion in a water bath (Braun, ThermomixB) at 70 °C for 5 min and the other half was pasteurized using a water bath at 90 °C for 5 min. Batches of only five cans were used during the pasteurization to avoid slight temperature de-

cline in the water baths. The come up time (CUT) was measured (three replicates) in both cases. After 5 min, the cans were placed immediately in an ice slush for rapid cooling. The canned purée was stored for 26 weeks at 18 °C (Abalab 4500 Fitoclima, Portugal) and 38 °C (WTB Binder, Germany). The 38 °C storage was performed in order to study the worst case scenario that could occur, for example, in a tropical country. Another storage test using vacuum packed cupuaçu purée in transparent plastic bags (pasteurized at 90 °C for 5 min) was also carried out at 18 °C and 38 °C, to investigate any difference in the color behavior of cupuaçu stored in bags rather than in cans.

Quality analysis

Quality analysis was conducted before (non heated) and immediately after the pasteurization (0 weeks), and during storage until the end of the experiment (0, 1, 2, 4, 8, 17 and 26 weeks). Quality was measured in terms of microorganisms (total, yeasts and molds), enzymes (polyphenoloxidase and peroxidase), sensory evaluation (aspect/flavor/aroma) and physico-chemical parameters (pH, total acidity, soluble solids, reducing/non-reducing sugars, color). Ten cans were required to run all the mentioned analyses except for time 0 and 26 weeks in which sensory tests were performed.

Total microorganism counts were performed at 25 °C using PCA (plate count agar) medium after three days incubation according to the Portuguese Standard NP 1995 (IPQ, 1982). Molds and yeasts were counted in Cooke Rose Bengal medium after five days incubation at 25 °C according the Portuguese Standard NP 3277-1 (IPQ, 1987a).

Concerning enzymes, presence/absence tests for polyphenoloxidase (PPO) and peroxidase (PEROX) were performed. A positive control was used in these tests. A catechol solution 0.07 M in 0.05 M buffer (pH = 7) in the presence of PPO enzyme turns brown (Bolin *et al.*, 1977; Galeazzi and Sgarbieri, 1981). PEROX determination followed the Portuguese standard procedure (IPQ, 1983) in which a solution of 2% (mass/volume) of guaiacol (2-methoxyphenol) prepared with recently distilled and sterilized water and a drop of H₂O₂ (10 volumes) changes to a pink-salmon color if the enzyme is present.

Regarding sensory evaluation, overall quality assessment (aspect/flavor/aroma) was considered only at the beginning and at end of storage. A triangular test (Meilgaard *et al.*, 1987) was performed immediately after the pasteurization in Portugal (17 panelists) and also in Brazil with 18 judges already familiar with the product to investigate if cupuaçu purée pasteurized at 70 and 90 °C was different. After 26 weeks of storage the sensory quality was evaluated and differences due to pasteurization temperature (70 and 90 °C) and storage temperature (18 and 38 °C) were assessed with simple difference tests.

pH was measured with a pH meter (Crison, microPH 2001; Spain). Total acidity was determined by titration with NaOH (0.1 N) and expressed in terms of citric acid according to the Portuguese Standard NP 1421 method (IPQ, 1977). Soluble solids were determined by difference between total dry residue (obtained after 4 h at 70 °C and 50 mm Hg) and insoluble dry residue according to Portuguese Standard NP 784 (IPQ, 1970). Total/reducing/non-reducing sugars were determined using the Luff-Schoorl technique described in the Portuguese Standard NP 1420 (IPQ, 1987b).

Color was measured by the CIE $L^*C^*h^{\circ}a^*b^*$ color coordinates in a tristimulus colorimeter (Minolta Chroma Meter, CR 300), and by the total color difference parameter (TCD*). This parameter quantified the overall color difference of a given sample ($L^*a^*b^*$) when compared to a reference sample (original non heated purée - $L_o^*a_o^*b_o^*$) (Minolta, 1994):

$$TCD^* = \sqrt{(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2} \quad (1)$$

The colorimeter beam diameter was 8 mm, the three response detectors were set at 0° viewing angle, and a CIE standard illuminant C with diffuse illumination was used. A white calibration plate was used for calibration ($L^* = 98.15$, $C^* = 1.92$, $h^{\circ} = 93.8$, $a^* = -0.13$, $b^* = 1.92$). Each sample of purée (at least 1 cm depth and 2 cm diameter) was placed in a clear glass Petri dish for color measurements. Measurements were made in duplicate.

Data analysis and kinetics modeling

The effects of pasteurization at 70 and 90 °C (T_p = pasteurization temperature) were evaluated by comparing the quality parameters of non-heated and of heated cupuaçu samples. Following this, the effects of four treatments ($T_p - T_s$: 70 °C-18 °C, 90 °C-18 °C, 70 °C-38 °C, 90 °C-38 °C; T_s = storage temperature) and storage

time in all parameters measured were investigated by a two-factor (without replication) analysis of variance (Microsoft Excel 8.0). The TCD* color parameter was modeled during storage using the statistical program Stata 3.0 (Computing Resource Center, 1992).

RESULTS AND DISCUSSION

Pasteurization effect on quality

The pasteurization for 5 minutes was adequate for the inactivation of microorganisms and enzymes. The cans were very thin and, therefore, the purée in the can center reached the required temperature (70 or 90 °C) after approximately 220 s; after this the cans were maintained for 80 s at that temperature. An identical CUT was obtained when using plastic bags instead of cans. In addition to sensorial changes, the pasteurization significantly affected the total microorganisms, yeasts and molds, the peroxidase activity and the color (Table 1).

The original microbial load of fresh cupuaçu was very low ($\sim 10^1$ cfu/g) due to the freezing and careful handling preceding the experiment. Microorganisms were reduced to levels $< 1 \times 10^1$ cfu/g after pasteurization at both temperatures. The enzyme PPO was not detected either in fresh or pasteurized cupuaçu purée. The PEROX detected in the non-heated original cupuaçu was inactivated at both temperatures of pasteurization. A similar result was obtained by Yen and Lin (1996) when heating guava purée at 88-90 °C for 24 s.

Usually, the sensory quality of fresh fruit is superior when compared with pasteurized fruit. However, the pasteurized cupuaçu purée retained a substantial part of the original properties of the fruit and remained acceptable. No differences (1% confidence) were detected in the flavor/aroma between the purée pasteurized at 70 and 90 °C from the triangular test performed im-

Table 1. Quality changes in cupuaçu purée induced by pasteurization at 70 or 90 °C and 26 weeks storage at 18 or 38 °C.

Tabla 1. Cambios en el puré de cupuaçu inducidos por la pasterización a 70 o 90 °C y 26 semanas de almacenamiento a 18 o 38 °C.

Quality parameter	Before storage			After 26 weeks storage			
	Non-heated	$T_p = 70^\circ\text{C}$	$T_p = 90^\circ\text{C}$	$T_p = 70^\circ\text{C}$ $T_s = 18^\circ\text{C}$	$T_p = 90^\circ\text{C}$ $T_s = 18^\circ\text{C}$	$T_p = 70^\circ\text{C}$ $T_s = 38^\circ\text{C}$	$T_p = 90^\circ\text{C}$ $T_s = 38^\circ\text{C}$
Microorganisms (cfu/g)	2×10^1	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
Yeasts (cfu/g)	1×10^1	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
Molds (cfu/g)	4×10^1	$< 1 \times 10^1$	$< 1 \times 10^1$	3×10^1	1×10^1	$< 1 \times 10^1$	1×10^1
pH	3.3	3.3	3.4	3.4	3.3	3.6	3.5
Total acidity (g citric acid/100g pulp)	2.3	2.2	2.3	1.8	1.9	1.7	1.7
Soluble solids (%)	8.7	8.9	9.8	10.2	10.8	11.4	11.2
Reducing sugars (%)	2	2	2	8	5	8	7
Non reducing sugars (%)	6	6	6	0	2	0	1
Total color difference (TCD*)	0.0	2.2	4.6	6.6	6.7	10.7	9.4

diately after pasteurization with the Brazilian and Portuguese sensory panels.

There were no significant changes provoked by the pasteurization at 70 or 90 °C except for color. The color of the samples changed slightly and TCD* increased. According to the Drlange (1994) color difference classification scale (Silva and Silva, 1999), distinct color ($1.5 < \text{TCD}^* < 3.0$) at 70 °C and a very distinct color ($3.0 < \text{TCD}^* < 6.0$) at 90 °C were obtained in the cupuaçu purée samples.

Since no significant sensory quality differences between pasteurization at 70 or 90 °C were noticed, a temperature of 90 °C might be recommended to assure microbial safety in case of higher initial contamination.

Storage effect on quality

After pasteurization and a long storage, the color got darker, some more changes occurred in the flavor/aroma and the non-reducing sugars were converted into reducing sugars. The browning of the purée was probably non-enzymatic but due to Maillard and/or caramelization reactions (Fennema, 1976). The purée maintained good quality when stored at 18 °C and acceptable quality when stored at 38 °C.

The total microorganism counts did not exceed 6×10^1 cfu/g (in almost all the cases $< 1 \times 10^1$ cfu/g) during the storage at 18 or 38 °C. Concerning yeasts, all the counts were $\leq 1 \times 10^1$ cfu/g and the maximum mold count was 7×10^1 cfu/g, although for most part of the analysis it was $\leq 1 \times 10^1$ cfu/g. PPO and PEROX were not detected during the storage.

No sensory differences were observed between the two pasteurization processes (70 and 90 °C) after 26 weeks storage at 18 or 38 °C. Some differences were observed in the color/flavor/aroma of the purée stored at 38 °C compared with the purée stored at 18 °C. Overall, the purée stored for 26 weeks at 18 °C retained a substantial part of the organoleptic quality of the pasteurized fruit.

Time factor had a significant (5%) effect in all the parameters concerning physico-chemical quality and the treatment was significant for all parameters except for total acidity and soluble solids (Table 1). A slight decrease in total acidity and an increase in soluble solids were observed with time. The same results were observed with peach pulp (Askar *et al.*, 1996). After six months no differences were observed in terms of total acidity and soluble solids between 18 and 38 °C.

Although the non-reducing sugars (NRS) were converted into reducing sugars (RS), the total sugars remained approximately constant (8%) throughout the whole experiment (Figure 1). When heating at 70 or 90 °C for 5 min and stored at 18 or 38 °C for 26 weeks, the NRS decreased from 6% to 0%, and the RS increased

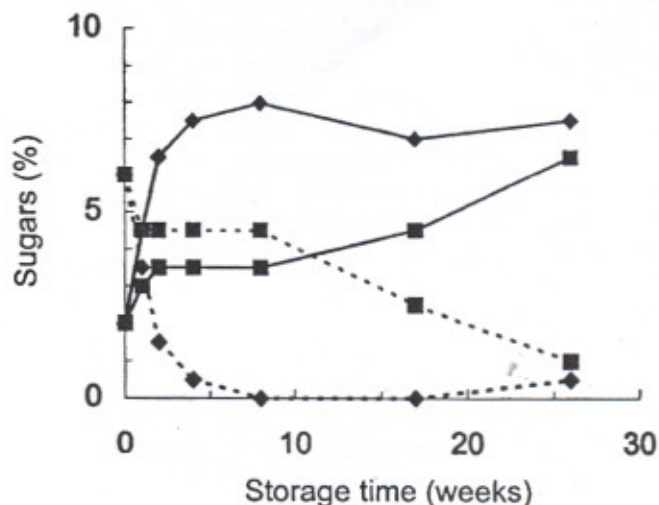


Figure 1. Sugar changes in cupuaçu purée during six months storage: (■) 18 °C storage; (◆) 38 °C storage. (—) Reducing sugars. (---) Non-reducing sugars.

Figura 1. Cambios en los azúcares del puré de cupuaçu durante los 6 meses de almacenamiento a 18 °C (■) y 38 °C (◆). Azúcares reductores (—). Azúcares no reductores (---).

from 2% to 8% (Table 1). This change was more rapid at 38 °C (four weeks) than at 18 °C (>26 weeks). Kennedy *et al.* (1990) also observed that the inversion of sucrose was enhanced at low pH (<4) and at higher storage temperature. Sucrose inversion was also observed in orange juice (Kaanane *et al.*, 1988; Trifirò *et al.*, 1995). Babsky *et al.* (1986) reported non-enzymatic browning, sucrose inversion and total acidity decrease in apple juice concentrate stored for 111 days at 37 °C. At 38 °C the sugar inversion reaction with RS formation followed a first order reversible model (Villota and Hawkes, 1992):

$$\frac{X - X_f}{X_o - X_f} = e^{-k \times \text{time}} \quad (2)$$

where: X = RS or NRS (%), X_f and X_o are the final/equilibrium (after some weeks) and initial (0-heated) values of RS or NRS (%), time is the storage time (weeks) and k the reaction rate (weeks^{-1}). The value of k at 38 °C was 0.7 weeks^{-1} . At 18 °C this model was not adequate.

Finally, the TCD* continued to increase after pasteurization from approximately two weeks (at 38 °C) to five weeks (at 18 °C) of storage; after this period it remained constant until the end of storage (Figure 2). At this stage the difference in color compared with the original cupuaçu was greater but still acceptable ($\text{TCD}^* < 12$) for both storage temperatures (Drlange, 1994). Peach pulp behaved in the same way (Askar *et al.*, 1996). Ibarz and Bermejo (1991) also observed few changes in the

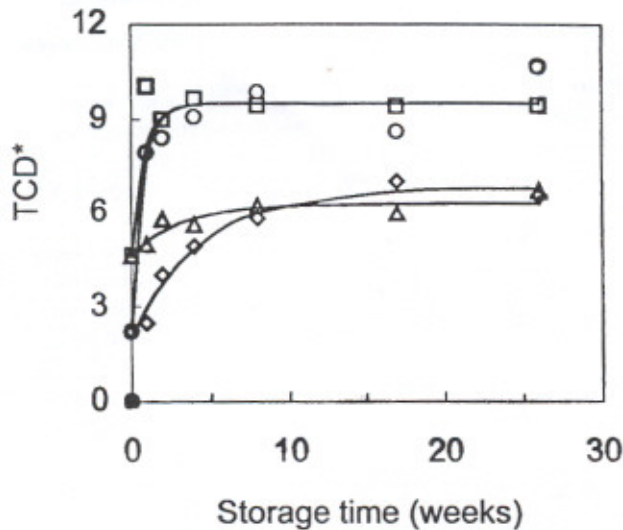


Figure 2. Color changes (TCD*) in cupuaçu purée with pasteurization and during six months storage. Experimental (symbols) versus predicted data (—). (●) non-heated sample; (◊) $T_p = 70\text{ }^\circ\text{C}$, $T_s = 18\text{ }^\circ\text{C}$; (Δ) $T_p = 90\text{ }^\circ\text{C}$, $T_s = 18\text{ }^\circ\text{C}$; (\square) $T_p = 90\text{ }^\circ\text{C}$, $T_s = 38\text{ }^\circ\text{C}$; (\circ) $T_p = 70\text{ }^\circ\text{C}$, $T_s = 38\text{ }^\circ\text{C}$.

Figura 2. Cambios del color (TCD*) del puré de cupuaçu después de la pasteurización y el almacenamiento durante 6 meses. Datos experimentales (símbolos) frente a los predichos (—). (●) muestra non procesada; (◊) $T_p = 70\text{ }^\circ\text{C}$, $T_s = 18\text{ }^\circ\text{C}$; (Δ) $T_p = 90\text{ }^\circ\text{C}$, $T_s = 18\text{ }^\circ\text{C}$; (\square) $T_p = 90\text{ }^\circ\text{C}$, $T_s = 38\text{ }^\circ\text{C}$; (\circ) $T_p = 70\text{ }^\circ\text{C}$, $T_s = 38\text{ }^\circ\text{C}$.

color of concentrated pear juices stored at 20 °C for 200 days compared with the juice stored at 37 °C. Chan and Cavaletto (1982) observed color changes with processing and storage at ambient temperature and 38 °C for six months in papaya and guava purées. In terms of the CIE color coordinates (L, C, h, a, b), the pasteurization implied a significant decrease in L^* . In our work C^* and b^* increased significantly during storage. Differences in a^* were detected between samples stored at 18 °C and 38 °C. The same results were obtained with the cupuaçu purée packed in the plastic bags and stored at 18 °C; however at 38 °C the cupuaçu darkened much quicker and the kinetics were different. At this higher temperature, the

bags might have some permeability to O_2 and besides the non-enzymatic browning that occurred in the canned purée, other oxidative or light induced reactions could have taken place, affecting the kinetics. Overall, although the T_p (pasteurization temperature) determined the initial color of the purée, the final color after storage was determined only by the T_s (Table 1). These results were in agreement with the sensory results.

TCD modeling*

The TCD* change during storage was also well modeled with a first order reversible model ($r^2 = 0.99$). In Equation 2, $X = TCD^*$, and X_i and X_o refer to TCD_i^* and TCD_o^* , respectively. TCD_o^* depended on the T_p whereas TCD_i^* was dependent on T_s , and the color change rate, k , depended on the previous factors and the product itself (Table 2); k was higher at higher storage temperature. Figure 2 presents the predicted versus experimental data for TCD*. The browning of strawberry juice and the red color loss followed the same type of kinetics (Speers *et al.*, 1987). The reaction rate constant, k , for red color loss at 20, 30 and 45 °C was respectively 0.21, 0.66 and 0.84 week⁻¹. These values were comparable with those obtained for cupuaçu (Table 2), between 0.22 and 1.34 weeks⁻¹. Ibarz *et al.* (1989) determined that the non-enzymatic browning of pear juice followed first-order reaction kinetics. The same result was obtained with the kinetics of red color loss in orange juice (Trifiró *et al.*, 1995).

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Table 2. Estimated TCD_i^* , TCD_o^* and k for the first order reversible model (Equation 2).

Tabla 2. Valores estimados de TCD_i^* , TCD_o^* y k del modelo de primer orden reversible (Ecuación 2).

T_p (°C)	T_s (°C)	TCD_o^*	TCD_i^*	k (week ⁻¹)	Residuals range
70	18	2.0 ± 0.3	6.8 ± 0.3	0.22 ± 0.05	-0.50 - 0.32
90	18	4.6 ± 0.3	6.3 ± 0.2	0.31 ± 0.16	-0.35 - 0.36
70	38	2.3 ± 0.9	9.5 ± 0.4	1.34 ± 0.47	-0.89 - 1.18
90	38	4.6 ± 0.1	9.5 ± 0.1	1.19 ± 0.17	-0.07 - 0.21

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