Statistical evaluation of camu-camu pulp pasteurization using a composed rotational experimental plan

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ABSTRACT

Camu-camu (*Myrciaria dubia*) is a small fruit native of Amazon. It has been widely sprouded around the world as the fruit that presents the highest known ascorbic acid content. This fact arouses the attention of specific markets like cosmetics, pharmacy and food, especially for the drink industry, where it may be used as a natural ingredient enriched in vitamin C. The fruit is perishable and requires fast processing after harvesting which makes the frozen pulp the most usual way of commercialization in the main producers' countries like Peru and Brazil. The objective of this work was to study the influence of temperature and time of pulp pasteurization on the reduction of initial microbial counting. In order to evaluate the different binomials and their interactions a central composite rotary design (CCRD) was used, having temperature and time as independent variables (X_1 e X_2) with the coded values at five levels (-1.41, -1, 0, +1, +1.41) and the true values the usually ones of a fast pasteurization (HTST) for pulp fruit. The main answers were mesophylic bacteria (Y_1) and mold and yeast (Y_2) counting. The results for both parameters at 95% significance suggest that the models could not be considered as predictive as the variance analysis was not satisfactory. Other significance levels were evaluated and it was not possible to adjust a model. The, an evaluation of tendency and percentage of colony reduction has been performed. It was observed that the 90ºC/60s or 95ºC/45s essays could be satisfactory adopted for camu camu pulp pasteurization taking account the microorganisms destruction efficiency and energy cost.

Keywords: Myrciaria dubia; experimental design; pulp; thermal treatment; microbiology

INTRODUCTION

Camu-camu tree (*Myrciaria dubia* H.B.K. Mc Vaugh) is a wild plant found in the border of rivers and lakes of Amazon estuary, presenting a large natural distribution from East in Atlantic Ocean in Pará state, Brazil to West close to Andes, in Peru and to North in High Orinoco, in Venezuela. The plant has the ability of well adapting to different edaphoclimatic conditions. Even being natural in flood areas, it has been positively cultivated in land, which is essential for the culture expansion [1,2].

The main camu-camu producer and exporter is Peru. In Brazil, fruit production is still incipient although it has been growing. Fruit pulp is the main added value product in these countries, being exported for some Asian countries, Europe and USA, for pharmaceutical and cosmetics industry, as well as for the food industry being usually used for mixed drinks with a functional appeal, due to its high vitamin C content.

In scientific literature it is reported a large variation in vitamin C content depending of the fruits origin (agronomical and genetic conditions), harvest conditions and processing among other factors. The contents varies from 900 to 6000 mg of ascorbic acid/100 g of pulp, being the higher source for this vitamin [3;4;5].

Pasteurization is a traditional process for food conservation that uses heat (temperatures below 100^{o}C) for destroying heat sensitive microorganisms and inactivating certain enzymes, increasing shelf-life while minimizing the effects of heat on nutritive value and sensory characteristics of the product [6].

This kind of heat treatment is very much used in fruit pulp, which combined with frozen storage permits the safe commercialization of one of the most common products from primary manufacture industry that is the frozen fruit pulp. In Brazil, mainly in Amazon, where there are still few industries the technological process for producing fruit pulp is simple and of easy implementation. However, due to the high diversity of native fruits, most of them having specific characteristics, the technological parameters of their processing need to be studied in order to obtain better quality products. The need of optimize products and processes, minimizing operational time and costs has driven the search for systematic experimental design. The central composite rotary design (CCRD) method consists of mathematical and statistical procedures that can be used

in the study of correlations among one or more responses (dependent variables) with many factors (independent variables). It is a statistical technique based on the employment of factorial design, introduced in the 50´and since then it has been successfully used for modeling different industrial processes [7].
Thus, the objective of this work was to investigate how the time / temperature relation can affect the microbiological analyses of camu-camu pulp and by modeling the resulting try to optimize the pasteurization process, supplying more quality and safety to the product.

MATERIALS & METHODS

Camu-camu fruits from Moju, Pará state, in the North region of Brazil, were depulped and homogenized in an equipment type TURRAX, constituting a unique batch that was used for pasteurizer feed. Pasteurization was carried out in continuous operation mode in a small system constituted of 6 m stainless steel cooling, silicon hoses, pre-heating bath, temperature controlled retention bath and peristaltic pump with flux control (Cole-parmer model KH 77913-20).
The effect of time (X_1) and temperature (X_2) was studied using a 2^2 central composite rotational design (CCRD), with three central and four axial points, which resulted in 11 essays (Table 1). The evaluated responses were the mesophilic bacteria (Y_1) and molds and yeasts (Y_2) counting, both determined according to APHA official methods [8].
The results were statistically analyzed using the software *Statistica*® 5.0. ANOVA was used to estimate the statistical parameters [7].

Table 1. Compositional rotational 2^2 plan with three central and four axial points[1]

X_1 (Temperature in Celsius)		**X_2 (Time in seconds)**	
(-1)	85	(-1)	45
(+1)	95	(-1)	45
(-1)	85	(+1)	75
(+1)	95	(+1)	75
(0)	90	(0)	60
(0)	90	(0)	60
(0)	90	(0)	60
(-1,41)	83	(0)	60
(+1,41)	97	(0)	60
(0)	90	(-1,41)	39
(0)	90	(+1,41)	81

[1]axial levels ($\propto$) determined in function of independent variables number (n=2) by equation $\propto=(2^n)^{1/4}$

RESULTS & DISCUSSION

The counting on raw camu-camu pulp, before thermal treatment, was 2.1 x 10^2 (est.) CFU/g for bacteria and 1.82 x 10^2 (est.) CFU/g for molds and yeasts. The evaluation of bacteria (Y_1) counting data has shown that for this parameter temperature was significant at both linear and quadratic models at 95% significance (Table 2) and the data were submitted to variance analysis (Table 3).

Table 2. Calculated effect and interactions for mesophilic bacteria parameter.

Category	**Effect**	**Pure Error**	**t(2)**	**p**
Mean	*18,333*	*8,333*	*2,200*	*0,158*
Temperature (Linear)	*-41,882*	*5,103*	*-8,207*	*0,014*
Temperature (Quadratic)	*28,021*	*6,073*	*4,613*	*0,043*
Time (Linear)	-17,588	5,103	-3,446	0,074
Time (Quadratic)	4,270	6,073	0,703	0,554
Temperature x time	17,500	7,216	2,424	0,136

The results may be considered regular (R^2 0.77; Fcalculated/Ftabulated = 3; lack of adjustment > 0.10) and so the generation of a predictive model was not indicated. Nevertheless, by means of trending graphics (Figure 1), it could be observed that temperature has more effect in the process. Independently of the time level tested the temperature increase causes more destruction in these microorganisms. This trend is potentialized in the range between the central point 0 (90ºC) to axial +1,41 (97ºC) from the experimental design.

Table 3. Analysis of variance (ANOVA) for mesophilic bacteria parameter.

Category	**SS**	**DF**	**MS**	**F calculated**	**F tabulated***
Regression	18461,5	2	9230,76	13,54	4,46
Resids	5452,12	8	681,51		
Lack of Fit	5035,45	6	839,24	4,02	19,3
Pure Error	416,67	2	208,33		
Total SS	23913,6	10			

* Tabulated values [9]: F regression (2;8;95); F lack of fit (6;2;95).

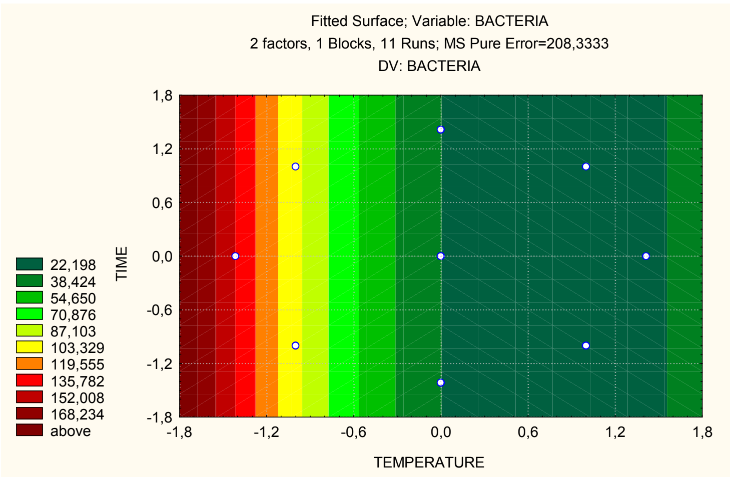

Figure 1. Contour plot for mesophilic bacteria evaluation in function of pasteurization time and temperature.

Pareto´s chart confirms the negative effect of temperature on the response. There is significant reduction (p≤0,05) on bacteria counting with increasing temperature (Figure 2).

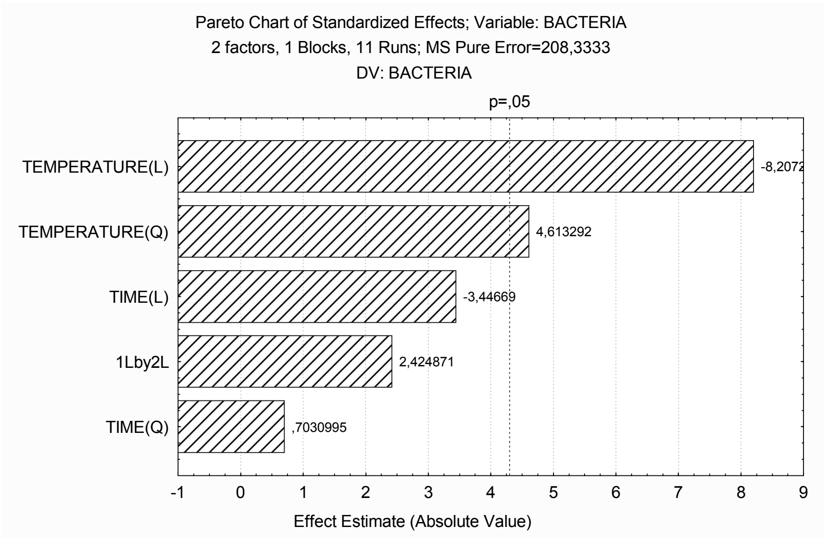

Figure 2. Pareto´s chart of time and temperature evaluation effect for mesophilic bacteria.

The results obtained for molds and yeasts (Y_2) were similar to those observed for bacteria (Figure 3), with temperature being significant at 95%. However, for the prediction model study the results were not satisfactory (R^2 0.73; Fcalculated/Ftabulated = 0.33; lack of adjustment > 0.10), mainly due to the bad Fcalculated/Ftabulated relation, making not possible the mathematical model adjustment and the generation of trend and response surface curves. For a regression be not only significant but also useful for predictive purposes the Fcalculated/Ftabulated value must be higher than 3 [9].

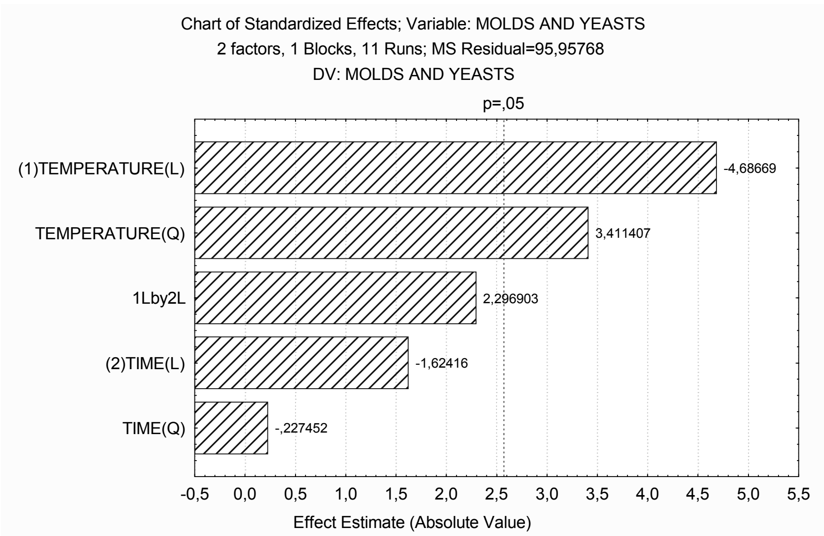

Figure 3. Pareto´s chart of time and temperature evaluation effect for molds and yeasts.

For the proposed experimental plan, microbiological results did not generate safe statistical models that would permit to evaluate other conditions processes in order to optimize pasteurization. Other significance levels were tested ($p \leq 0,10$ and $p \leq 0,30$), but as well no parameters were satisfactory for prediction of models. In addition, mathematic models with lower significance levels only must be used for more subjective responses, as in sensory analysis, for example [7]. Though, even if the results were satisfactory for prediction at $p \leq 0,10$ and $p \leq 0,30$, the models would not be recommended. On the other side, most tests provided significant reduction in the initial microbial load (Table 4), making that the pulps presented counting levels in accordance to Brazilian legislation.

Table 4. Microbial counting reduction of treated samples related to initial counting

Essays	Reduction of mesophilic bacteria (%)	Reduction of molds and yeasts (%)
1 (85ºC/45sec)	48,84	69,78
2 (95ºC/45sec)	≈100	≈100
3 (85ºC/75sec)	81,39	≈100
4 (95ºC/75sec)	≈100	≈100
5 (90ºC/60sec)	95,35	≈100
6 (90ºC/60sec)	95,35	≈100
7 (90ºC/60sec)	83,72	≈100
8 (83ºC/60sec)	27,90	61,54
9 (97ºC/60sec)	≈100	≈100
10 (90ºC/39sec)	72,09	94,50
11 (90ºC/81sec)	≈100	≈100

It can be observed that temperatures above 90ºC and time processing above 60 seconds cause important reduction values in microbial counting and that, in some treatments, it has attained 100% reduction, which represents a very good result.

CONCLUSION

Although the results have shown that the experimental responses were significant ($p \leq 0.05$), when submitted to a multiple regression analyses, with linear and quadratic effect of temperature, the F test has shown that the models were not predictive. In spite of the determination coefficients were regular (0.77 e 0.73 for mesophilic bacteria and mold and yeast respectively), the models presented low relation for regression and a significant lack of adjustment. So, the generation of mathematical models that would permit to estimate the microbial counting in function of temperature is not recommended. However, from the tendency observed in the design and considering the process costs, it is possible to suggest the binomials 90ºC/60s or 95ºC/45s as the best ones for the pasteurization of camu-camu pulp, regarding the microbiological aspects.

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