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Optimization Scale Pasteurization of Baobab Juice Using Response Surface Methodology (RSM)

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Abstract

The *Adansonia digitata* L. known as Baobab is the only species present in West Africa and grows wild. All parts of the plant are used by humans. In Senegal, baobab pulp is widely consumed; it is used as raw material in small and medium enterprises (SMEs) for making juices, concentrates, jams, powder. Drinks or juices from baobab are highly appreciated by consumers; however rapid fermentation can happen even after pasteurization that can shorten their shelf-life. A Doehlert experimental design was used to find a good scale of heat treatment ensuring a proper conservation of baobab juice. Results of the experimental design showed that a heat treatment of 80˚C for 10 min gives a baobab juice with good sanitary quality that meets the international standards.

Keywords

1. Introduction

The baobab (*Adansonia digitata* L.) is the unique species of the genus *Adansonia* that grows in West Africa [1]. This tree grows wild in Senegal [2]. The Senegalese Institute of Agricultural Research (ISRA) has defined two main areas producing baobab fruits near the cities of Kaolack and Tambacounda [3]. More dispersed populations of baobab can be found in the regions of Thies, Louga,

Matam and Saint Louis [4].

Baobab has an immense nutritional potential because it contains macro and micronutrients essential for health [5]. The fruit is consumed in various forms in Africa, particularly in Senegal [6] [7]. All parts of the plant are used in food, cosmetics, handicrafts, traditional medicine [8] [9]. Baobab fruits are rich in dietary fiber, minerals, especially vitamin C [1] [10] and are an important raw material for the fruit processing sector in Senegal. Overall, baobab fruit pulp and seeds contain significant amounts of nutritionally essential minerals but the amounts vary significantly among the different areas [11]. Also, there is a well-developed international trade for *A. digitata* pulp and oil in South Africa and Malawi, where it is mainly traded for use in the food and cosmetic industries [12] [13]. Processing was carried out traditionally by small and medium enterprises (SMEs) or economic interest group (EIG). Several products are obtained: drinks, concentrates, powders, jams. However, during storage fermentation of the products can occur even in pasteurized products, showing a weak thermal inhibition of microbial and enzyme activity. In SMEs, because of the lack of good hygiene practices, small scale processors generally over-pasteurize their products to ensure the shelf-life. This results in products with very low nutritional quality, because main nutrients such as vitamin C are not thermo-resistant. Using Experimental design can help optimize the heat treatments. The Doehlert experimental design is very useful because it minimizes the number of experimentations, while maintaining a good precision. This experimental design plan distributes the values on a hexagonal surface, which allows giving more strength to one of the variables (horizontal). In our case it was the temperature.

The purpose of this study was to provide a pasteurization level (time/temperature) which will reduce the biological activities in the product while keeping its biochemical and nutritional characteristics. For this, the response surface methodology was used, based on a two-factor Doehlert design [14].

2. Materials and Methods

2.1. Physical and Chemical Analyses

Some physico-chemical parameters were measured on stages of the manufacture of baobab juice. All reagents used were of analytical grade and were purchased from Sigma (L'Isle d'Abeau, France). Titratable acidity, pH and dry matter were measured using standard methods [15]. Total titratable acidity was determined with a TitroLine easy titrator (Schott Instruments, Mainz, Germany), using 0.1 N NaOH solution. The pH value was determined using a calibrated pH meter and dry matter was determined by drying a 5 g sample at 105°C up to constant weight (24 h minimum). Total soluble solids (TSS) content was measured with an Abbe refractometer (Atago, Tokyo, Japan). The total phenolic content was determined with Folin-Ciocalteu reagent, according to the method optimized by

[16]. Total and reducing sugars were determined using the Luff-Schoorl method [17]. Vitamin C (ascorbic acid) was assessed by high-performance liquid chromatography (HPLC) [18].

2.2. Preparation of Baobab Juice

The results of diagnostic methods for the production of baobab juice of a Senegalese company (Figure 1) allowed preparing a standard juice with of water/fruit juice 1/6 (v/v) sweetened by addition of sugar.

2.3. Drink Packaging

Cold pack was performed before pasteurization. The conditioning was carried out in glass bottles of 60 mL sealed with plastic plug. They were a reference package, the glass being impermeable to oxygen. Before filling, the bottles and caps were disinfected with bleach containing 100 ppm chlorine for 30 min, rinsed and dried. The capsule on one bottle conditioned was pierced at its center to introduce an electrode for measuring the temperature during pasteurization.

2.4. Drink Pasteurization

The bottles were fed into a pre heated water bath (polystat, Bioblock Fischer Scientific, France).
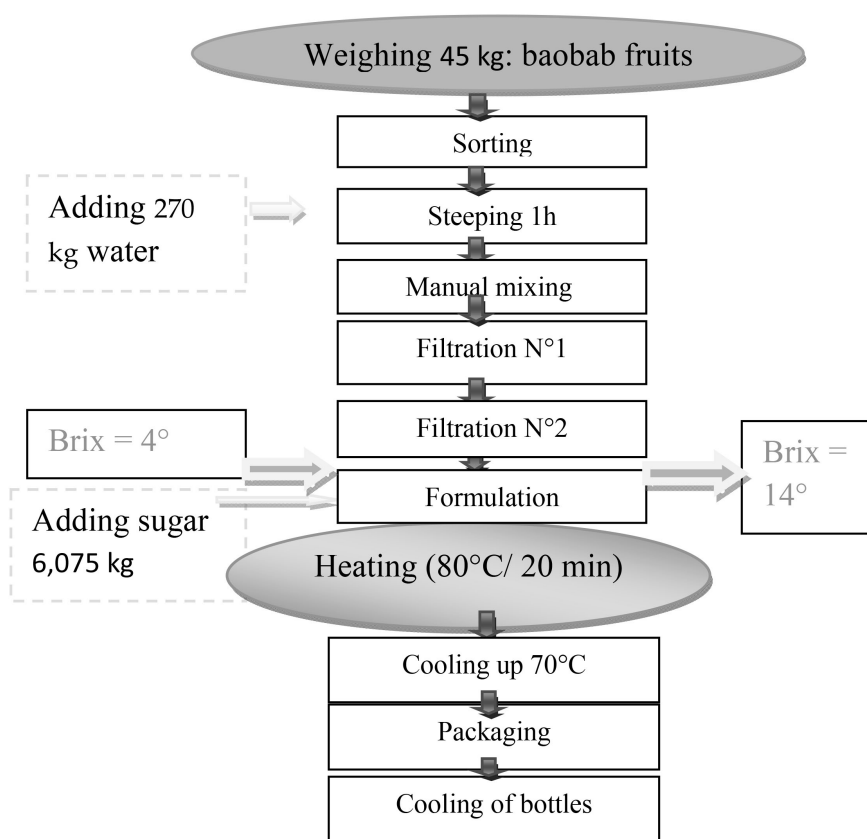

Figure 1. Process flow diagram of baobab juice of some companies in Dakar/Senegal.

A thermocouple (type K, NiCr-Ni sensor, ref. ZA 9020-FS Thermo E4) of two electrodes was used to measure the temperatures of the water and the product. The thermocouple was connected to a data logger (V2.3 ALMEMO® 2890-9, Ahlborn, Holzkirchen, Germany). After application of a scale of pasteurization, the product was cooled in ice water [1].

2.5. Microbiological Analysis of Pasteurized Products

Microbiological analyzes were performed on the pasteurized products. The main microorganisms searched were: Total Mesophilic Aerobic Flora (AMTF), yeasts and molds which were found in most the stages of baobab juice processing. Norms, culture media and incubation temperatures used for microbiological analyzes were shown in Table 1.

2.6. Experimental Design

Pasteurization parameters were optimized by RSM. For this, the Doehlert plan of two factors was used. The minimum and maximum factors (temperature-time) were fixed in advance. The central point was repeat three times to calculate the error of the equation of the curve. The experimental design obtained shows that the temperatures and times pasteurizers were independent variables. The, AMFT yeasts and molds were the answers from the interaction of the independent variables.

The mathematical model which corresponded to the plan was:

$$y = a_0 + a_1 T + a_{11} T^2 + a_2 Tp + a_{22} Tp^2 + a_{12} T \times Tp$$

y was the dependent variable (AMFT, yeasts and molds)

a_0 was the constant

a_1, a_{11}, a_2, a_{22}, a_{12} were coefficients that represent the linear effects, quadratic independent variables and their interaction, T was the temperature and Tp the time.

2.7. Statistical Analysis

The analysis of the experimental design and calculation of expected responses were handled by the software Statistica (Statsoft Version 7 USA), as well as the responses to confirm the validity of the plan.

3. Results

3.1. Physicochemical Characteristics

The physicochemical characteristics of the baobab juice at various stages of the manufacturing process have been reported in the Table 2.

3.2. Fitting Model

Both responses of interest were aerobic mesophilic total flora (AMTF), yeasts and molds. The results of nine trials using the Doehlert plan are shown in Table 3.

Table 1. Standards and culture media used for microbiological analyzes.

Floras	ISO standards	Culture media	Temperature and duration of incubation
AMFT	NF EN ISO 4883 [19]	PCA (Plate Count Agar)	30˚C/24h
Yeasts and molds	NF EN ISO 7954 [20]	Sabouraud with chloramphenicol agar	25˚C - 30˚C for 5 days

Table 2. Physicochemical characterization of baobab juice samples.

Parameters Samples	Number of Samples	pH	TSS	Titrable acidity (mEq/100mL)	Total sugar (g/100g)	Reducing sugar (g/100g)	Total polyphenol (g/100g)	Vitamin C (g/100g)
Raw material	5	3.55 ± 0.04	-	136.3 ± 3.77	34.63 ± 0.36	5.89 ± 0.33	3.87 ± 0.23	340.19 ± 1.5
Unsweetened juice unpasteurized	3	3.22 ± 0.01	4.00	115.65 ± 0.035	25.54 ± 0.74	1.401 ± 0.01	0.27 ± 0.001	20.00 ± 0.001
Sweet juice unpasteurized	3	3.22 ± 0.01	14 ± 0.09	94.4 ± 0.065	23.97 ± 0.16	1.47 ± 0.14	0.23 ± 0.001	13.81 ± 0.001
Sweet juice pasteurized	3	3.24 ± 0.12	14 ± 0.09	82.98 ± 0.002	18.96 ± 0.24	1.61 ± 0.14	0.19 ± 0.002	16.47 ± 0.001

Table 3. Experimental design essays and corresponding values of the microbiological analysis.

|	Temperature (˚C)	Time (min)	AMTF (CFU/mL)	Yeasts & molds (CFU/mL)
BAOBAB	40	22.,5	7545	4090
	52.5	7.35	6454	3727
	52.5	37.66	3182	3545
	65	22.5	3727	3663
	65	22.5	3818	3454
	65	22.5	4000	3454
	77.5	7.35	1727	1182
	77.5	37.66	121	145
	90	22.5	53	82

The regression from the results of the 7 experimentations (the central point is repeated 3 times) of the Doehlert design was made with the Statistica software. The regression coefficients and the different constants of the mathematical equation of the experimental model for AMFT and yeasts and molds are given in Table 4.

The statistical analysis provided by the plan and those obtained by the experiments results show a perfect match between the model and the observed data, and this was confirmed by the regression coefficient R^2 = 0.99 for the AMFT and 0.97 for the yeasts and molds.

For AMFT variable, the Pareto diagram (Figure 2(a)) showed that the linear effect of the temperature and the linear and quadratic effects of time were significant, whereas the quadratic effect of temperature was not significant on the response AMFT.

Table 4. Regression coefficients and model parameters for the two dependent variables AMFT and Yeast & Molds.

Model	Depending variables	
	AMFT	Yeast & Molds
R^2	0.99	0.97
a_0	3847.93	3523.57
a_1	−3795.15	−2326.93
a_{11}	−24.67	−718.83
a_2	−2439	−609.5
a_{22}	−1929.2	−2028.8
a_{12}	832.97	−427.71
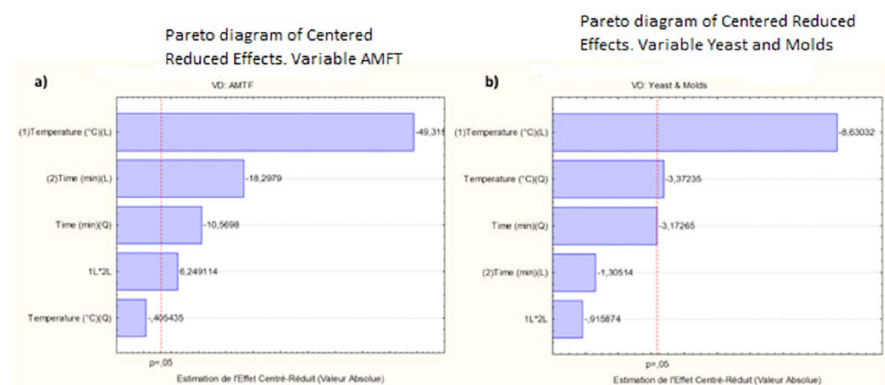
Figure 2. Pareto diagrams showing effects of pasteurization temperature and pasteurization time on (a) AMFT (b) Yeasts and Molds flora.

However, on the yeasts and molds inactivation, the Pareto diagram (**Figure 2(b)**) showed that only the linear effect of temperature was significant.

The representation in the space (2D) of the surface of the AMFT response versus time and temperature (**Figure 3**) in the frame of the extreme limits of the plan shows the strong effect of temperature/time on the total flora. by calculation, using the obtained equation, it is possible to obtain the temperature/time that permiited to approach zero AMFT.

From the spatial representations obtained (**Figure 3** and **Figure 4**), the time/temperature pairs that gave values less than or equal to 1000 are the best because they meet the standards for yeast and molds. However, for the conservation of micronutrients the best will be the use of high temperatures with short times.

4. Discussion

The results of the estimated effects and those obtained by experiments showed that the Doehlert plan chosen was perfectly adequate for this study with regression coefficients $R^2 > 0.9$.

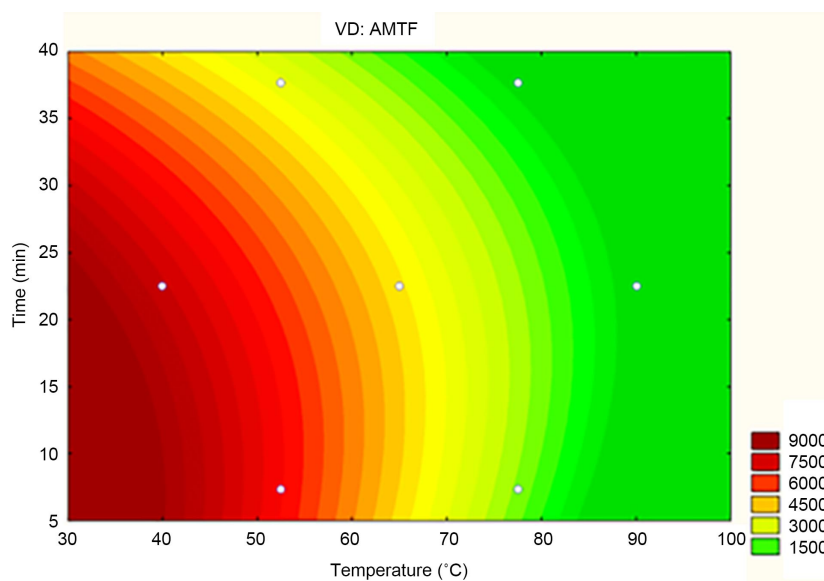The outcome of Lee *et al.* [21] [22] using an experimental model different

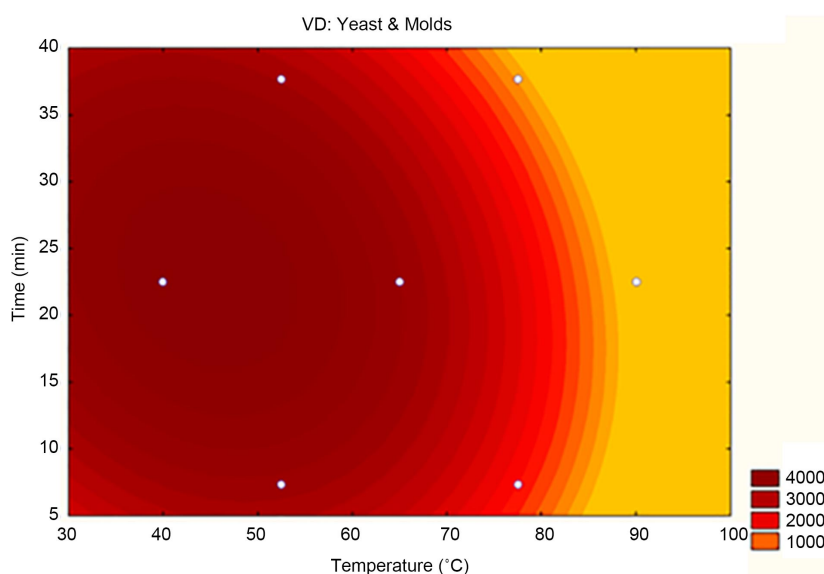**Figure 3.** Response surface plots showing effect of temperature and time of pasteurization on AMFT.

Figure 4. Response surface plots showing effect of temperature and time of pasteurization on Yeasts and Molds.

from Doehlert are confirmed by our study. For this study, the application of a pasteurization of 80°C/10 min permitted to optimize the sanitary quality of baobab juice. The work of Bull *et al.* [23] showed a limited number of yeasts, molds and acetic acid bacteria were able to grow in fruit juice at low pH (3.3 to 4) as the baobab juice and orange. This study confirms that the temperature of proposed treatment would minimize the contamination of baobab juice for different microorganisms. The level of pasteurization defined by our study was in perfect harmony with the work of. Rodriguez V.M. *et al.* [24] who showed that the functional properties of pasteurization extracts of *Aloe barbadensis Miller*

were optimal at temperatures between 65˚C and 85˚C with a time range of 15 to 35 min using Box-Behnken plan.

On one hand, Chen Y. *et al.* in their study [25] showed that high temperatures of pasteurization were the most efficient to control microbial growth, however they resulted in degradation of phenolic compounds. They also showed that the high temperature treatments have better performance than simple juice on complex viscous juice.

On the other hand Laorko *et al.* [26] showed that microfiltration, could provide better preservation of physicochemical and taste properties of juice, but especially effective on the inhibition of bacterial growth. Also, according to physicochemical and sensory tests confirmed by many types of research, juices produced by membrane filtration are of an excellent quality. Membrane fouling is a critical issue and inhibits the wider application of membranes in the fruit juice industry [27] [28].

5. Conclusions

Microbial contamination of plant products causes a lot of inconvenience to manufacturers. So, the majority of fruit and vegetable juice manufacturers indicated that there was a significant need to control microbial spoilage as part of brand protection. Thus improvement of control over microbial spoilage merits the dedication of resources and, would improve business efficiency by reducing waste. Heat pasteurization is traditionally the most used for the preservation of drinks and juices during storage. Heat treatment of 80˚C/10 min applied to baobab juice permitted to produce a good hygienic quality juice. However, the application of high temperatures can cause the destruction of some natural nutritional constituents (vitamin C, polyphenols) found in fruits, which are important for health.

To overcome this problem, other non-thermal promising conservation methods are in the process of development for liquid food such as membrane filtration, pulsed electric field, UV radiation … But these techniques are impractical for small and medium industries in Senegal.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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