STARCH EXTRACTION FROM THE PEACH PALM (*Bactris gasepaes* Kunth.) FRUIT: A MODEL APPROACH FOR YIELD INCREASE

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ABSTRACT: The objective was to study the starch extraction process of the peach palm (*Bactris gasipaes* Kunth.) fruit focused on increasing yield and in preserving the quality characteristics the extracted product. Two experimental designs are carried out in order to investigate the effects of NaOH and NaHSO$_3$ concentrations, as well as the decantation time, on the yield of starch and on product color. Firstly, a 2^3 full factorial design provided a linear model with 95% of confidence, indicating major tendencies for optimization. Secondly, a 2^2 central composite design rendered a quadratic model which allowed the maximization of yield (it 9.0% higher) without compromising the color of the product. The maximum yield of 23.90%, validated with triplicate experiments, is considerably higher than the yield of starch from other fruits reported in literature.

KEYWORDS: design of experiments, response surface methodology, native starch.

INTRODUCTION

Starch is extracted from the edible part of cereals, tubers, roots, rhizomes and fruits. It is the main source of energy stored in higher plants and provides 70 to 80% of the calories consumed by humans (LEONEL et al., 2003; AGUILERA et al., 2009) as thickener, colloidal stabilizer, gelling agent, glue, adhesive, among others (LEONEL & CEREDA, 2002). Food industries, then, are the greatest consumers of this raw material. This multipurpose polymer is also used in a large number of processes, especially in the cosmetics, pulp and paper, chemical, textile and pharmaceutical industries. A more recent application has been in the development of biomaterials (APLEVICZ & DEMIATE, 2007; ARAUJO-FARRO et al., 2010). Research institutions and industries, therefore, are becoming more interested in identifying native starches with specific characteristics, as well as improving the extraction process towards greater purity and yield (LEONEL, 2007).

The peach palm (*Bactris gasipaes* Kunth.), native from Costa Rica, has cylindrical trunks which can reach up to 20 meters tall until adulthood. It is globally recognized by its stem manufacture from the palm heart, and Brazil is considered to be the largest producer (SOUSA et al., 2011). The tree produces fleshy fruits rich in carotene (pro-vitamin A), carbohydrates, oils and starch, hence with great nutritional value and energy. Although these fruits have potential for application in food, they are discarded during the stem production. They are entirely used for obtaining seeds (FARIAS NETO et al., 2013), which are considered a highly polluting waste in major producing regions of the country. Therefore, the extraction of starch from these fruits would increase their added-value also contributing to a more sustainable production of stem from palm heart.

Previous study MELO NETO et al. (2015) investigated the chemical characteristics and functional properties of the starch from the peach palm fruit, concluding that it is similar to traditional starches sources, such as the starch from cassava and corn, so that it might be used for commercial application. The starch extraction from the peach palm fruit, therefore, is a potential alternative to add value to a current waste from the stem manufacture. This post-processing would diminish the environmental impact while contributing to the economy and sustainability of the

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process. In this context, this paper attempts to maximize the yield of starch extracted from the peach palm fruit.

The are several studies on the extraction and characterization of starch in literature, as well as on the relationship between the extraction processes and molecular structure of the final product (LEONEL, 2007; DENARDIN & SILVA, 2009). Studies on the optimization of the extraction process, are still an open issue in literature and is the focus of the present contribution. LIMA et al. (2012) studied three industries of cassava starch with the aim of get viable alternatives for improvement in the production process. They found that optimization of extraction would be of fundamental importance for the development of the sector, however, the authors found no literature on work related to the optimization of starch extraction.

The response surface methodology (RSM) is a collection of mathematical and statistical techniques used for developing, improving and optimizing processes, and can be used to evaluate the relative significance of many factors that are affected by complex interactions. The main objective of RSM is to determine the optimal operating conditions for a system or to determine a region that satisfies the operating specifications (SANTANA et al.; 2007). By put forward better results for optimization than other statistical methods, the RSM was utilized in this work. The independent variables investigated are the concentrations of sodium hydroxide (NaOH) and sodium bisulfite (NaHSO$_3$), in addition to decantation time, according to previous analysis and contributions of literature (ROCHA et al., 2008; DEVEREUX et al., 2011; MADRIGAL-ALDANA et al., 2011).

So the objective was to study the starch extraction process of the peach palm (*Bactris gasipaes* Kunth.) fruit focused on increasing yield and in preserving the quality characteristics the extracted product. Since the color is the main quality parameter of the starch, this response is also investigated through luminosity and chromaticity indices, which are constraints of the optimization problem. In order to accomplish the optimization, two sequential designs of experiments are carried out attempting to validate an empirical model which can satisfactorily describe the extraction process.

MATERIAL AND METHODS

Materials

Plantations owned by the Federal Institute of Baiano Uruçuca Campus and by INACERES S.A. supplied the mature peach palm (*Bactris gasipaes* Kunth.) fruits Additional materials are: sodium hydroxide (NaOH) P.A., sodium bisulfite (NaHSO$_3$) P.A. and absolute ethyl alcohol. All analytical grade reactants are made by the Synt Company.

Methods

Starch extraction

The starch was obtained from the peach palm fruit according to the methodology described by LEONEL et al. (2003) and applied by MELO NETO et al. (2015). The fruits were peeled, shredded and put into plastic containers. A "Croton" type knife mill with a 10 screen mesh grinded the samples in order to separate the mulch from the starch milk which was purified in 100 and 200 mesh sieves. The mulch was then successively washed with running water in order to separate the starch by decanting into plastic containers. The decanted starch was purified with absolute alcohol, filtered and dehydrated at 35°C for 12 hours in a dryer containing silica gel. The obtained starch was then reduced to powder and passed through a 48 mesh sieve in order to obtain the sample for analysis. Attempting to increase the process yield, the mulch from the first extraction was further purified in a 10 mesh sieve.

Extraction yield

The yield of starch extraction was calculated based on the amount of starch extracted and on the total quantity of fruit used, according to:

Biano A. de Melo Neto, Renata C. F. Bonomo, Marcelo Franco, et al.

150

$$\text{Yield (\%)} = \left(\frac{\text{mass of dried starch(g)}}{\text{total mass of peach palm fruit (g)}}\right) \times 100\% \tag{1}$$

Colorimetric analysis

The color was measured by means of direct analysis in a Color Quest XE colorimeter (Hunter Lab). The operating parameters during analysis were: 10° angle, D65 illuminant and a RSIN calibration mode. The color was determined by the international color scale (CIE-International Commission on Illumination) which uses the following coordination: L* represents luminosity (light reflecting ability), ranging from 0 (totally black) to 100 (totally white) - the closer to 100, the more white and clear is the starch; a* represents the color transition from green (-60) to red (+60) - the closer to zero, the less green and/or red pigments mixed with the starch granules; b* represents the color transition from blue (-60) to yellow (+60) - the closer to zero, the less blue and/or yellow pigments exists (LEONEL et al., 2009; YOSHIDA & ANTUNES, 2009). Calorimetric analysis also allows the determination of saturation index and other variables.

Experimental design

First design of experiment: full 2^3

In order to investigate the effects and significance of the independent variables on both responses, extraction yield and starch color, a 2^3 full experimental design is carried out. The levels of the independent variables, summarized in Table 1, were determined based on preliminary tests as discussed above. In order to assess the experimental reproducibility, as well as to provide an extra degree of freedom for the model, three runs at the central point are carried out, as the design matrix in Table 3 illustrates.

TABLE 1. Levels of the independent variables - 2^3 experimental design.

Variables		Level		
		-1	0	**+1**
Sodium hydroxide (mol)	X_1	0,02	0,06	0,10
Sodium bisulfite (%)	X_2	0,10	0,25	0,40
Time decantation (min)	X_3	60,0	90,0	120,0

Second design of experiment: 2^2 central composite design

The first design indicated major tendencies towards optimal yield, as discussed next in Results section. Based on these findings, the levels of independent variables were redefined as Table 2 illustrates, considering the axial level in an attempt to obtain a quadratic model. As in the first design, here three runs at the central point are carried out.

TABLE 2. Levels of the independent variables - 2^2 central composite design.

Variables		Level				
		$-\alpha$	-1	0	+1	$+\alpha$
Sodium hydroxide (mol)	X_1	0,03	0,05	0,10	0,15	0,17
Time decantation (min)	X_2	77,7	90,0	120,0	150,0	162,3

Data analysis

When carrying out an experimental design, one may obtain a model, according to:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i<j}\sum_j \beta_{1j} X_1 X_j + \varepsilon \tag{2}$$

where,

Y is the output variable;

β_0 is the mean;

β_i is the linear coefficient corresponding to the independent variable I;

β_{ii} is the quadratic coefficient corresponding to the independent variable I;

β_{ij} is the interaction coefficient between independent variables i and j, and

X represents the encoded independent variables.

In order to assess the statistical significance of the proposed model within a confidence level, an analysis of variance (ANOVA) has to be carried out. The data analysis here considered a confidence level of 95%. Only and only if the mathematical model is statistically significant, the response surface methodology (RSM) may be carried out in order to optimize the response. All results were evaluated with the software Statistics for Windows, version 8.0.

RESULTS AND DISCUSSION

Table 3 presents the design matrix of the first full 2^3 design as well as both responses, yield and color. Pareto charts of standardized effects are illustrated in Figure 1 for each investigated response.

TABLE 3. Design matrix - full 2^3.

Runs	Independent variables			Responses			
	X_1	X_2	X_3	Yield (%)	L*	a*	b*
1	-1	-1	-1	18,00	95,81	0,086	3,670
2	+1	-1	-1	16,20	97,03	-0,216	2,313
3	-1	+1	-1	9,00	96,01	-0,040	2,390
4	+1	+1	-1	15,30	97,67	-0,266	2,160
5	-1	-1	+1	19,80	97,62	-0,063	1,990
6	+1	-1	+1	20,70	97,75	-0,233	2,093
7	-1	+1	+1	12,60	96,53	0,043	3,053
8	+1	+1	+1	15,30	97,91	-0,133	1,783
9	0	0	0	15,29	97,81	-0,113	2,006
10	0	0	0	15,30	98,05	-0,140	1,716
11	0	0	0	15,50	97,77	-0,050	2,243

When analyzing the yield from Figure 1a, $NaHSO_3$ has the greatest effect. As mentioned before, it was added to the extraction process in order to avoid darkening of the sample. As $NaHSO_3$ is a salt derived from an acid, a saline hydrolysis reaction may have occurred with the NaOH, producing water and then reducing the concentration of NaOH. This unexpected reaction might have consumed part of NaOH, diminishing its desired role, i.e., to release the starch granules and then to increase the yield. This finding is corroborated by the significant interaction effect between NaOH x $NaHSO_3$, even higher than the main effect of NaOH. The main effects of NaOH and DT are positive and significant as expected. The interaction effect $NaHSO_3$ x DT indicates that a longer DT affords the saline hydrolysis reaction, therefore decreasing the effect of NaOH during the decantation period.

The main role of the $NaHSO_3$ is to whiten the sample so that a significant positive effect on color was expected. However, as Figure 1b and 1c indicates, it has no significant influence on the color parameters L* and a*. As discussed before regarding Figure 1, $NaHSO_3$ might have been consumed by a saline hydrolysis reaction, what diminished its main role. The significant effect of NaOH corroborates this hypothesis. Some authors report that NaOH contributes to whitening the product, not only by preventing darkening reactions, but also by degrading starch's natural pigmentations (LEONEL et al., 2003). NaOH has a positive effect on luminosity L*, which measures the black and with scale, hence in accordance with literature. On the other hand, its effect

Biano A. de Melo Neto, Renata C. F. Bonomo, Marcelo Franco, et al.

152

on a*, which measures the scale from green to red, is negative. Therefore, it is important to monitor the color of the starch in the optimization procedure. DT exerted positive influence on luminosity, unlike expected. This positive effect is associated to the constant temperature of the washing water, 5°C, which might have avoided most darkening reactions (OLIVEIRA, 2008; DAUDT, 2012). The Pareto analysis for the b* color parameter is not shown here since it does not indicate any statistically significant variable.
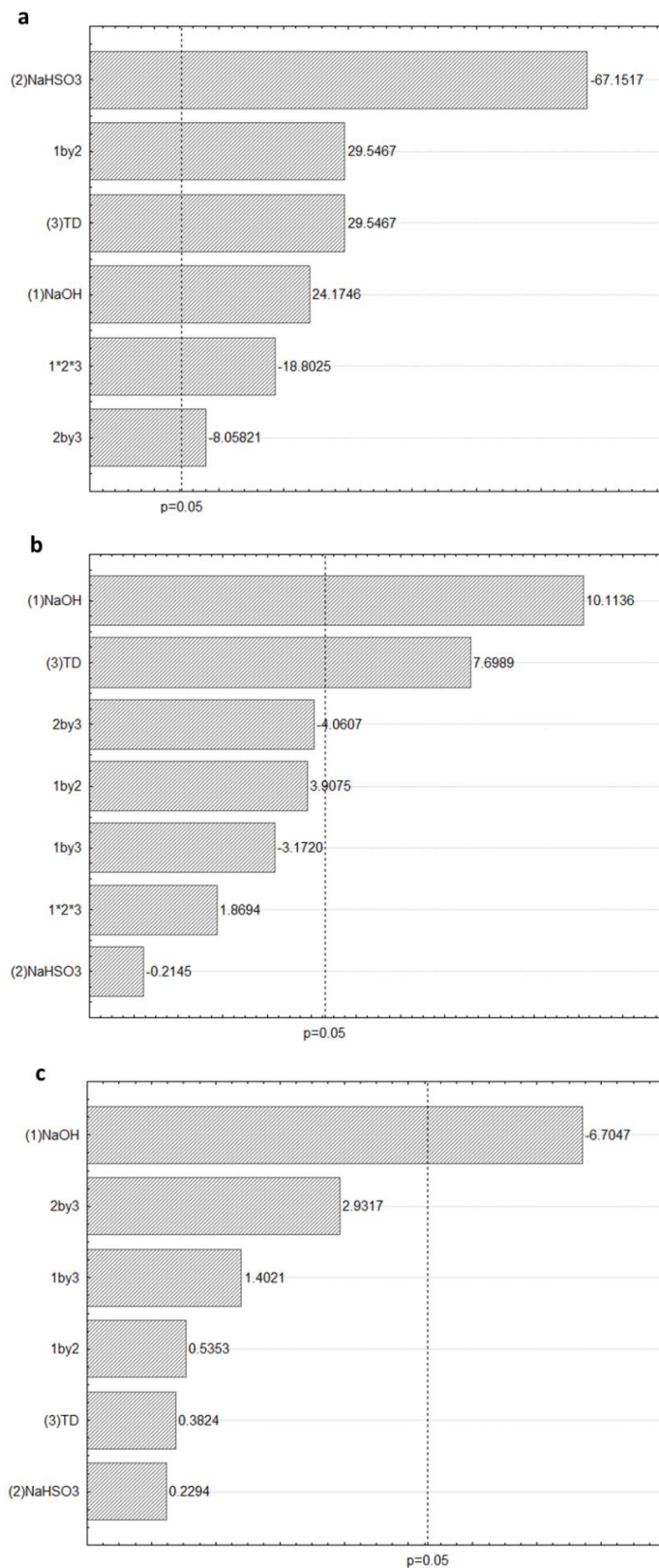

FIGURE 1. Pareto chart for full experimental design: a) yield; b) factor L*; c) factor a*.

Based on the significant effects within 95% of confidence level, the mathematical model proposed for the yield (Y_1) is given by:

$$Y_1 = 15{,}73 + 1{,}01X_1 - 2{,}81X_2 + 1{,}24X_3 + 1{,}24X_1X_2 - 0{,}34X_2X_3 - 0{,}79X_1X_2X_3$$

(3)

which,

ANOVA has to be carried out in order to verify its statistical significance.

As Table 4 shows, the calculated F value for the regression is much higher than the tabulated, indicating that the model is significant within 95% of confidence. The coefficient of determination, $R^2 = 99.34\%$, corroborates the quality of fit, since it explains 99.97% of the variations in the observed data. Despite the lack of fit also being significant (Fcalculated = 22.98 > Ftabulated = 19.00), the predicted *versus* observed is random and there is no systematic deviation.

TABLE 4. ANOVA ($p \leq 0{,}05$) for the yield - 2^3 experimental design.

Source of variation	Quadratic sum	Degrees of freedom	Mean square (MS)	$F_{calculated}$	$F_{tabulated}$
Regression	101,86	6	16,98	100,90[1]	6,16[3]
Residue	0,67	4	0,17	-	-
Lack of fit	0,64	2	0,32	22,98[2]	19,00[4]
Pure error	0,03	2	0,01	-	-
Total	102,53	10	10,25	-	-

[1]MS$_{Regression}$/MS$_{Residue}$; [2]MS$_{Lack of fit}$/MS$_{Pure error}$; [3]$F_{95\%,6,4}$; [4]$F_{95\%,2,2}$.

Table 5 presents the analysis of variance (ANOVA) regarding the color factor L*. The model obtained is not statistically significant since $F_{calculated}$ is slightly higher than $F_{tabulated}$. The coefficient of determination, $R^2 = 60.0\%$, corroborates the low quality of fit. The ANOVA regarding the color factor a* was not performed since the Pareto chart indicated only one significant effect.

Once validating the model that predict yield, equation (3), the response surfaces might be analyzed. Figure 2a shows yield *versus* NaHSO$_3$ and NaOH concentrations while the DT is fixed at the central point. The surface makes more evident the interaction effect between the NaOH and NaHSO$_3$ and its non-linear behavior.

TABLE 5. ANOVA ($p \leq 0{,}05$) for the factor L*- 2^3 experimental design.

Source of variation	Quadratic sum	Degrees of freedom	Mean square	$F_{calculated}$	$F_{tabulated}$
Regression	3,77	2	1,89	5,71[1]	4,46[3]
Residue	2,64	8	0,33	-	-
Lack of fit	2,59	6	0,43	18,28[2]	19,33[4]
Pure error	0,05	2	0,02	-	-
Total	6,42	10	0,64	-	-

[1]MS$_{Regression}$/MS$_{Residue}$; [2]MS$_{Lack of fit}$/MS$_{Pure error}$; [3]$F_{95\%,2,8}$; [4]$F_{95\%,6,2}$.

At a fixed NaOH concentration, an increase in NaHSO$_3$ concentration reduces yield since it affords the hydrolysis reaction which reduces the NaOH concentration, as discussed before. At lower concentrations of NaHSO$_3$, the interaction effect becomes a negligible.

Figure 2b and 2c illustrates the interaction effects of DT with NaOH and NaHSO$_3$ on yield. The response surfaces indicate that yield can be optimized towards lower concentrations of NaHSO$_3$, higher concentrations of NaOH and higher decantation times. These tendencies are corroborated by the analysis of Figure 2. Therefore, the levels might be re-defined (Table 2) in order to carry out a second design of experiment towards yield maximization. Since the NaHSO$_3$ does not present the desired effect, its level is maintained at the lower level while NaOH concentration and DT levels are increased. Axial points are considered in order to further verify the statistical significance of a quadratic model.

Biano A. de Melo Neto, Renata C. F. Bonomo, Marcelo Franco, et al.

154

3.2 Second experimental design - 2^2 central composite design

Based on the findings of the first design, a 2^2 central composite design was carried out, having NaOH concentration and DT as independent variables. Their levels are given in Table 2 and Table 6 summarizes the design matrix and the responses yield, (L*) and (a*).

TABLE 6. Design matrix - 2^2 central composite design.

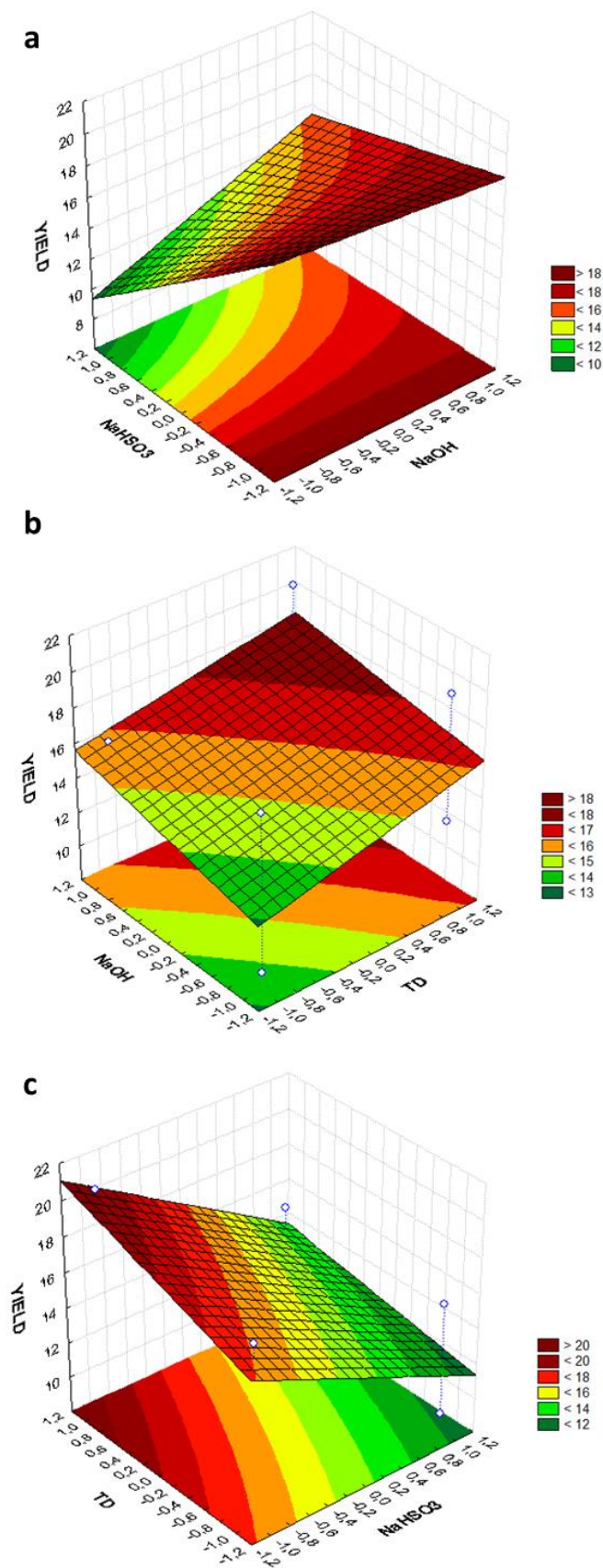| Experiments	Leves of the independent variables		Dependent variables (responses)		
	X_1	X_2	L*	a*	Yield (%)
1	-1	-1	97,85	0,183	17,0
2	-1	1	97,75	-0,231	17,5
3	1	-1	96,90	-0,085	21,8
4	1	1	97,46	-0,249	23,0
5	$-\alpha$	0	97,33	-0,133	16,0
6	$+\alpha$	0	97,68	-0,245	22,0
7	0	$-\alpha$	96,89	0,073	20,0
8	0	$+\alpha$	97,95	-0,154	20,6
9	0	0	97,22	-0,143	23,4
10	0	0	97,06	-0,187	22,8
11	0	0	97,56	0,153	23,1

FIGURE 2. Response surface; a) yield versus NaOH and NaHSO$_3$; b) yield versus DT and NaOH; c) yield versus DT and NaHSO$_3$.

Figure 3 shows a Pareto chart for the yield, where (L) and (Q) indicate the linear and quadratic term, respectively. It is interesting to note that only the quadratic effect of DT is significant within the re-defined space of independent variables. The linear effect of NaOH is in

Biano A. de Melo Neto, Renata C. F. Bonomo, Marcelo Franco, et al.

156

accordance with the first design and the quadratic term is also significant. Analysis for the luminosity (L*) and chromacity (a*) does not indicate any statistically significant variable, therefore are omitted here.
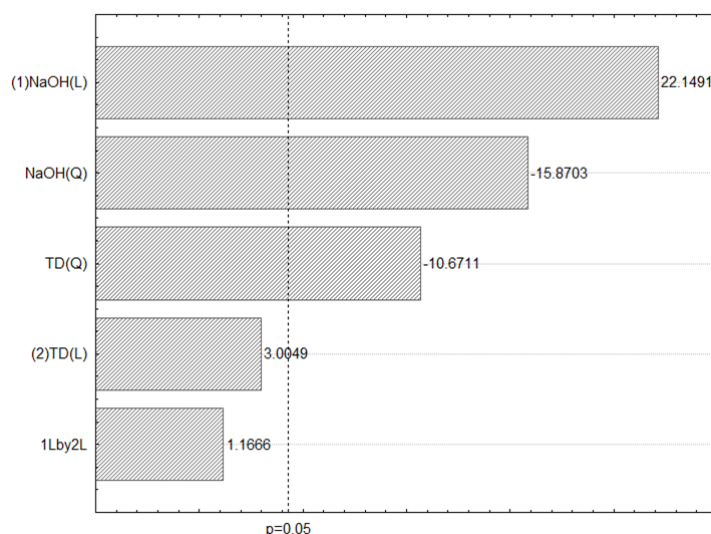

FIGURE 3. Pareto chart of for the yield - 2^2 central composite design.

Based on the Pareto chart analysis, a model for the yield (Y_2) might be proposed, according to:

$$Y_2 = 23,10 - 1,92X_1^2 + 2,47X_1 - 1,40X_2^2$$

(4)

The ANOVA, shown in Table 7, indicates that this model is statistically significant within 95% of confidence since the $F_{calculated}$ for the regression is much higher than the $F_{tabulated}$. The lack of fit is not significant, corroborating the quality of fit. Furthermore, the coefficient of determination, $R^2 = 98.20\%$, indicates that it explains 99.80% of the variation from the observed data.

TABLE 7. ANOVA ($p \leq 0,05$) for the yield - 2^2 central composite design.

Source of variation	Quadratic sum	Degrees of freedom	Mean square	$F_{calculated}$	$F_{tabulated}$
Regression	81,20	3	27,10	140,45[1]	4,35[3]
Residue	1,35	7	0,20	-	-
Lack of fit	1,20	5	0,23	2,60[2]	19,30[4]
Pure error	0,20	2	0,10	-	-
Total	82,50	10	8,25	-	-

[1]$MS_{Regression}/MS_{Residue}$; [2]$MS_{Lack\ of\ fit}/MS_{Pure\ error}$; [3]$F_{95\%,3,7}$; [4]$F_{95\%,5,2}$.

The response surface is then illustrated in Figure 4, where the point of maximum yield can be clearly seen around $X_1 = 0.65$ and $X_2 = 0$. It corresponds to NaOH concentration at 0.1325 M and decanting time of 120 minutes, therefore to an yield of 23.90% of the starch from the peach palm fruit. In order to validate this optimal point, it was experimentally reproduced in triplicate, obtaining 23.86±0.07 (%). Comparing the optimum yield with the ones reported in Tables 3 and 6, the effectiveness of the response surface methodology (RSM) for the optimization of the extraction of starch from the peach palm fruit is notable.
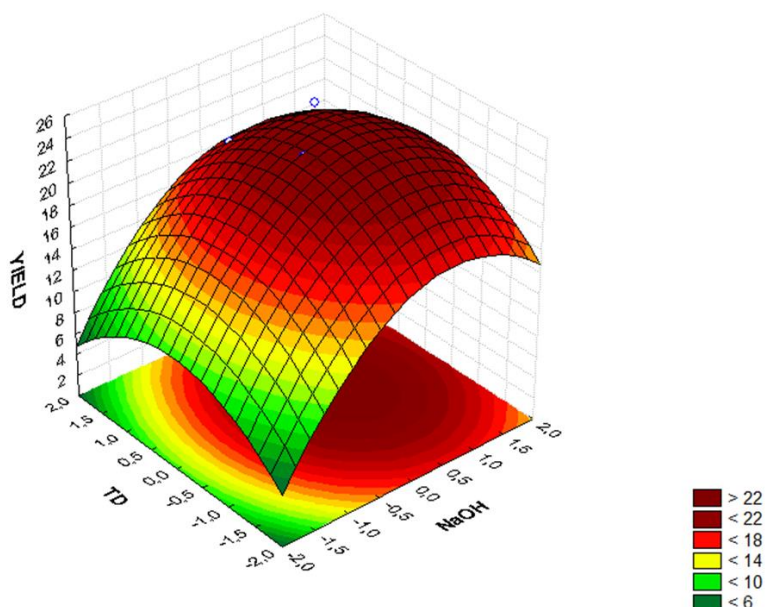
FIGURE 4. Surface the response of yield as function of NaOH and DT.

The yield of starch obtained from the peach palm fruit is much higher than the yield from other starch sources reported in literature as Table 8. It indicates the potential of the peach palm fruit as a competitive source of starch so that this current waste of the stem manufacture can contribute to a more sustainable process.

TABLE 8. Extraction yield from several sources reported in literature.

Source of starch	Yield	Authors
banana Nanicão (*Musa sp.* AAA)	5%	FREITAS & TAVARES (2005)
banana Terra (*Musa sapientum*)	8%	FREITAS & TAVARES (2005)
arrowroot (*Maranta arundinaceae* L.)	24.43%	LEONEL et al. (2002)
cassava (*Manihot esculenta* Crantz)	33.25%	NUNES et al. (2009)
ahipa (*Pachyrhizus ahipa*)	43%	LEONEL et al. (2003)
cowpea-bean (*Vigna unguiculata* L.)	48%	SALGADO et al. (2005)

CONCLUSIONS

The design of experiments and the response surface methodology indicated main and interacting effects allowing the optimization of the yield of extraction from the peach palm fruit. The design identified that the $NaHSO_3$, a clarifying agent, indeed reduces yield in the presence of NaOH, which is enough to ensure a pure and white starch. It further indicated the importance of NaOH and decantation time in increasing yield. Two sequential design of experiments allowed the maximization of the yield to 23.90% at 0.1325 M of NaOH and at 120 min, which was validated with triplicate experiments. The optimum yield is much higher than some reported in literature for other sources of starch. Therefore, the starch from the peach palm fruit presents itself as an alternative for higher scale production. Since the peach palm fruit is currently discarded during the steam manufacture, its usage as a source of starch might contribute significantly to a more sustainable process.

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Biano A. de Melo Neto, Renata C. F. Bonomo, Marcelo Franco, et al.

158

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