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Total Soluble Solids from Banana: Evaluation and Optimization of Extraction Parameters

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Abstract Banana, an important component in the diet of the global population, is one of the most consumed fruits in the world. This fruit is also very favorable to industry processes (e.g., fermented beverages) due to its rich content on soluble solids and minerals, with low acidity. The main objective of this work was to evaluate the influence of factors such as banana weight and extraction time during a hot aqueous extraction process on the total soluble solids content of banana. The extract is to be used by the food and beverage industries. The experiments were performed with 105 mL of water, considering the moisture of the ripe banana (65%). Total sugar concentrations were obtained in a beer analyzer and the result expressed in degrees Plato (°P, which is the weight of the extract or the sugar equivalent in 100 g solution at 20 °C), aiming at facilitating the use of these results by the beverage industries. After previous studies of characterization of the fruit and of ripening performance, a 2^2 full-factorial star design was carried out, and a model was developed to describe the behavior of the dependent variable (total soluble solids) as a function of the factors (banana weight and extraction time), indicating as optimum conditions for extraction 38.5 g of banana at 39.7 min.

Keywords Banana · Soluble sugars · Extraction process · Experimental design · Response surface

Introduction

Nowadays, biotechnology includes a wide range of diverse technologies that may be applied in each of the different food and agriculture sectors. In this context, several studies

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to obtain foods or beverages from fruits, such as orange, grape, or apple, have been carried out. With the development of the processing industries of different fruits and the increase in production of processed fruit products (e.g., as chips, flours, dried pulps and jam, and beverages), potential perspectives of control and/or treatment and extraction processes of its compounds are in constant increase [1, 2].

Banana, one of the most significant foodstuffs in the world, is one of the oldest fruits known to mankind as an important energy-producing food and is also a good source of mineral salts and vitamins [3]. Furthermore, sensorial attributes of the banana, as flavor, taste, texture, and color, are significantly influenced by its chemical composition, especially by acids, sugars, and phenolic compounds. Studies have revealed physicochemical, nutritional, and sensorial differences according to the region of origin of the bananas [4, 5].

Banana production in tropical and subtropical regions is an important financial resource and is the main economic activity for many countries, as well as an essential component in the diet of the global population. According to Food and Agriculture Organization of the United Nations—FAO [6], among the major producers of banana worldwide, in 2006, India was the first with 11.7×10^6 tonnes, which corresponded to 16.5% of the world production, and Brazil was the second with 7.1×10^6 tonnes (approximately 10% of the world production).

In the last decades, experimental design and other related statistical methods have been used to systematically examine different types of problems that arise within the fields of, e.g., research, development, and production [7, 8]. These designs include blocking and factorial experiments for determining the path of steepest ascent in order to identify the effect of individual factors and to approach the neighborhood of the optimum response [9].

In the present work, the response surface methodology was used to verify the influence of factors as wet weight of banana and time on the total soluble solids extraction from banana during an aqueous extraction process under heating. This work was preceded by preliminary studies of characterization of the fruit and of its ripening performance.

Materials and Methods

Raw Material

Good quality green banana fruits of the variety *Prata* (*Musa* spp.) were provided by EMATER (Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais) in Cristina, state of Minas Gerais, Brazil. This variety was chosen by considering the great production and availability in Brazil, which results in accessible price at markets. They were sanitized with running potable water, manually peeled, cut in the form of discs (thickness, 0.5 cm), and triturated in a blender before use.

Initially, the fruits were characterized and evaluated at three different times throughout their ripening. This characterization was performed using a scale representing ripening stages for *Prata* banana classified by the color of banana peel, ranging from 1 to 8, which was developed by Loeseck [10], as follows: green (1), traces of yellow (2), more green than yellow (3), more yellow than green (4), yellow with green endings (5), completely yellow (6), yellow with slight brown specks (7), and yellow with more brown specks (8). Thus, for each stage evaluated in this work (stages: 3—more green than yellow, 6—completely yellow, and 8—yellow with more brown specks), samples were chemically analyzed for their contents of moisture, starch, and soluble sugars (reducing, non-reducing, and total sugars), expressed as gram of carbohydrates in 100 g of wet banana pulp, as well as, concentrations of Ca, Mg, K, Na, Al, and Fe. Stages of maturation of the fruit were controlled in laboratory at room temperature (20–25 °C).

Initial Characterization: Starch, Soluble Carbohydrates, and Mineral Salts

The starch quantification in the banana pulps was determined according to the Instituto Adolfo Lutz—IAL [11] after some modifications. In many cases, starch quantification is not made directly; it is calculated after its total hydrolysis to glucose. Thus, samples were initially defatted with diethyl ether (5 g banana pulp treated with three volumes of 20 mL solvent), and the soluble sugars were removed with 70% ethanol at 83–87 °C/1 h (100 mL ethanol with 0.5 g calcium carbonate). After natural cooling and rest for 15 h, the volume was completed to 250 mL with ethanol 95%. In this stage, sediment (residue) and supernatant (liquid) were separated for determination of the starch concentration and soluble carbohydrates, respectively.

After washing the residue with 50 mL ethanol 70%, the residue, together with the filter paper, was transferred to an Erlenmeyer with 150 mL of deionized water. Four drops of sodium hydroxide solution 10% was added to mixture, and it was put at autoclave for 1.0 atm/121 °C/1.0 h. The starch was hydrolyzed adding 5 mL of hydrochloric acid in the resultant solution and treating again at autoclave for 1.0 atm/121 °C/0.5 h. The resultant solution was neutralized with sodium hydroxide 10%, and the volume was completed to 250 mL with deionized water. Starch levels (grams per 100 g fresh pulp) were determined by the correlation with the values of reduced sugars in glucose obtained by Somogy–Nelson method [12, 13]. Using the supernatant obtained early in the extraction process and after evaporation of the liquid phase, the resultant residue was mixed with solution of neutral acetate of lead until complete dissolution (3.8 mL). The final volume was completed to 500 mL (deionized water), and sodium sulfate was added (dry solids) until total precipitation of the lead in excess. After filtration, samples of this solution were used to quantification of soluble reduction sugars in the pulp (Somogy–Nelson method) and total sugars by hydrolysis procedure. Non-reduction sugars were obtained by the difference between the total sugars and the reduction sugars.

Quantification of Ca, Mg, K, Na, Al, and Fe were obtained in the three ripening stages of the banana pulps evaluated in this work. These elements were determined by atomic absorption spectrometry (model Analyst 800, PerkinElmer) after partial digestion in acid media (HCl/HNO_3) and total digestion of organic matter by peroxide (decomposition of the sugars and organic materials), followed by analytical determination.

Extraction Experiments of Total Soluble Solids from Banana

Extractions were made by immersing different amounts of banana paste in deionized water and heating in a thermo-regulated device at 84 °C for selected periods of time (Table 1). In all cases,

Table 1 Experimental range and levels of the independent variables according to the 2^2 full-factorial central composite design.

Independent variable	Symbol	Range and levels				
		$-2^{1/2}$	−1	0	+1	$+2^{1/2}$
Wet weight (g)	X_1	13.3	17.0	26.0	35.0	38.7
Extraction time (min)	X_2	23.8	30.0	45.0	60.0	66.2

the moisture of the banana was taken into account. After the extraction procedure, the juice was centrifuged at 1,400×*g* for 15 min, and the supernatant was used to determine the total soluble solids at 20 °C (Beer Analyzer 2, Anton-Paar, Austria), with the results expressed in degrees Plato (°P) [14], which is the weight of the extract or the sugar equivalent present in 100 g of the solution (reflecting the soluble sugars concentration after extraction).

Experimental Design and Statistical Analysis

A 2^2 full-factorial central composite design with five coded levels leading to 12 sets of experiments was performed. For statistical calculation, the variables were coded according to Eq. 1, where xi is the independent variable coded value, X_i is the independent variable real value, X_0 is the independent variable real value on the center point, and ΔX_i is the step change value. The range and the levels of the variables investigated in this study are given in Table 1.

$$xi = (X_i - X_0)/\Delta X_i \qquad (1)$$

Total soluble solids concentration obtained from banana was taken as the dependent variables or responses of the design experiments. The quadratic model for predicting the optimal point was expressed according to Eq. 2, where Y represents the response variable, b_0 is the interception coefficient, b_1 and b_2 are the linear terms, b_{11} and b_{22} are the quadratic terms, and X_1 and X_2 represent the variables studied.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad (2)$$

The Design expert (version 5.0) and Statistica (version 5.0) software were used for regression and graphical analyses of the data obtained. The statistical significance of the regression coefficients was determined by Student's *t* test, the second-order model equation was determined by Fischer's test, and the proportion of variance explained by the model obtained was given by the multiple coefficient of determination, R^2.

Results and Discussion

Initial Raw Material Characterization and Ripening Performance Studies

The results obtained in the initial raw material characterization and ripening performance are shown in Fig. 1 and Table 2. During natural ripening, evaluated between stages 3 (more

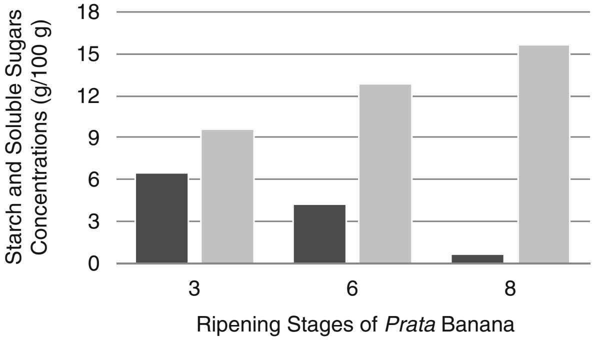

Fig. 1 Levels of starch (*gray bars*) and total soluble sugars (*black bars*) in *Prata* banana during ripening stages, scale according to Loeseck [10]: *1* green, *2* traces of yellow, *3* more green than yellow, *4* more yellow than green, *5* yellow with green endings, *6* completely yellow, *7* yellow with slight brow specks, *8* yellow with more brown specks

Table 2 Levels of moisture (percent *w*/*w*) and reduction, non-reduction, and total sugars (percent *w*/*w*) obtained in different ripening stages.

Ripening stages	Moisture[a] (% *w*/*w*)	Sugars[a] (% *w*/*w*)		
		Reduction	Non-reduction	Total
(3) More green than yellow	62.13±0.12	7.39±0.34	2.20±0.22	9.59±0.56
(6) Completely yellow (ripe fruit)	63.00±0.08	8.36±0.37	4.52±0.54	12.88±0.87
(8) Yellow with more brown specks	65.12±0.16	12.45±0.44	3.20±0.23	15.65±0.66

[a] Experiments and measurements made in triplicates

green than yellow) and 8 (yellow with more brown specks) at approximately 25 days of storage, the starch concentration decreased significantly from 6.46% *w*/*w* to 0.48% *w*/*w*, while the total soluble sugars increased from 9.59 to 15.65% *w*/*w*, respectively.

These results are similar to those reported by Adão and Glória [15], Fernandes et al. [16], and Loeseck [10]. Fernandes et al. [16], also working with banana of the variety *Prata*, observed a decrease in the starch concentration from 7.80% *w*/*w* to 0.63% *w*/*w*, and Adão and Glória [15] observed an increased in the total soluble sugars concentration from 1.26 to 14.8% *w*/*w*.

According to Zhang et al. [17], the average starch content drops from 70% to 80% in the pre-climacteric (prior to starch breakdown) period to less than 1% at the end of the climacteric period, while sugars accumulate to more than 10% of the fresh weight of the fruit. In this work, we found 4.28% *w*/*w* of starch concentration in stage 6 (completely yellow). These results are close to starch levels found by Mota et al. [18] for ripe bananas, between 2.5% and 5.2% *w*/*w*.

According to Adão et al. [15], *Prata* is one of the most consumed banana varieties in Brazil. Working with carbohydrate changes during ripening, these authors detected three types of soluble sugars in *Prata* banana: fructose, glucose, and sucrose. Table 2 shows that reducing sugars (as glucose and fructose) levels were predominant during all the three stages evaluated in our work. These results are in agreement with those previously obtained by Fernandes [16]. However, according to these authors, sucrose (representing non-reduction sugars) levels are higher than glucose and fructose levels in early stages (e.g., green and traces of yellow stages, stages 1 and 2), followed by a decrease during the subsequent stages of *Prata* banana ripening.

There was an increase in water content of the banana pulp from 62.13% *w*/*w* to 65.12% *w*/*w* during the ripening stages evaluated (Table 2). According to Loeseck [10] and Fernandes

Table 3 Performance obtained of mineral salts concentration during ripening stages in *Prata* banana values represented in oxide element.

Chemical element (in its oxide compound)	Mineral salts concentration (%)		
	Stage 3 (little ripe fruit)	Stage 6 (ripe fruit)	Stage 8 (more ripe fruit)
Iron (Fe_2O_3)	1.590	1.230	0.737
Calcium (CaO)	0.120	0.092	0.081
Sodium (Na_2O)	0.338	0.213	0.121
Potassium (K_2O)	42.952	42.613	42.327
Magnesium (MgO)	0.104	0.095	0.079
Aluminum (Al_2O_3)	0.382	0.217	0.100

Table 4 Experimental design and results according to the 2^2 full-factorial central composite design.

Runs	Independent variables				Dependent variable (response factor)
	Real levels		Coded levels		
	Weight (g)	Time (min)	Weight	Time	EB [a] (°P)
1	17.0	30.0	−1	−1	4.12
2	35.0	30.0	+1	−1	6.68
3	17.0	60.0	−1	+1	4.82
4	35.0	60.0	+1	+1	6.63
5	26.0	45.0	0	0	5.54
6	26.0	45.0	0	0	5.57
7	26.0	45.0	0	0	5.07
8	26.0	45.0	0	0	5.55
9	13.3	45.0	$-2^{1/2}$	0	3.53
10	38.7	45.0	$+2^{1/2}$	0	7.51
11	26.0	23.8	0	$-2^{1/2}$	5.45
12	26.0	66.2	0	$+2^{1/2}$	3.53

[a] Extraction of total soluble solids from banana (EB)

et al. [16], the increase in moisture during ripening is due to carbohydrates utilized in respiration and osmotic transfer from the peel to the pulp. Adão and Glória [15] explained that this happens as a marked difference in osmotic pressure between peel and pulp develops during ripening because sugar content increases more rapidly in the pulp than in the peel.

This increase in moisture during ripening stages can explain the changes obtained in mineral salts content (such as calcium, potassium, sodium, aluminum, iron, and magnesium) of the banana used in this work. During the ripening stages, there was a decrease of the values of their concentrations (in terms of their oxide compounds), presented in Table 3.

Experimental Design: Total Soluble Solids Extraction from *Prata* Banana

The effects of variables such as extraction time [minute] and wet weight of banana [gram] on the extraction performance of total soluble solids [°P] from *Prata* banana were simultaneously investigated employing a full-factorial design consisting of 2^2 trials plus a star configuration ($\alpha=\pm 2^{1/2}$) and four replicates at the center point. In earlier published work [8], different conditions of extraction time [minute] and temperature [degree Celsius]

Table 5 Estimated effects, standard errors, Student's *t* test, and significance levels for extraction of total soluble solids from *Prata* banana (EB) using the 2^2 full-factorial star design.

Effects	Estimated	Standard errors	*t* value	Probability
Average	5.5790	0.0937	59.5273	0.0000[a]
WW[b]	2.4996	0.1711	14.6083	0.0007[a]
ET[c]	−0.5163	0.1711	−3.0175	0.0569
ET^2	−0.7370	0.1874	−3.9318	0.0293[a]

[a] Significant level of the variables and their interactions at 95% (5% probability level)

[b] WW wet weight (X_1)

[c] ET extraction time (X_2)

Table 6 Regression coefficients, standard errors, Student's *t* test, and significance levels for the model representing the extraction of total soluble solids from *Prata* banana (EB).

Independent variables	Parameters	Coefficients	Standard errors	*t* value	Probability
Intercept	Constant	5.5790	0.0937	59.5273	0.0000[a]
WW[b]	X_1	1.2498	0.0856	14.6083	0.0007[a]
ET[c]	X_2	−0.2582	0.0856	−3.0175	0.0569
ET^2	X_2^2	−0.3685	0.0937	−3.9318	0.0293[a]

R^2 =0.83 and adjusted R^2 =0.77

[a] Significant level of the variables and their interactions at 95% (5% probability level)

[b] WW wet weight (X_1)

[c] ET extraction time (X_2)

for hot aqueous extraction of banana juice showed significant effects on the extraction of total soluble solids [degree Brix], as well as on organoleptic characteristics (odor and taste) of the final product. Based in these results, the extraction temperature used in the present work was fixed at 84 °C.

The design matrix with the real and coded levels of wet weight and extraction time and the amounts of extracted sugars is shown in Table 4. The highest extraction of total soluble solids from banana (EB) occurred when the real levels of the weight and time variables were 38.7 g and 45.0 min, respectively. On the other hand, the lowest sugars extraction occurred when the real levels of the weight and time variables were 13.3 g and 45.0 min and also 26.0 g and 23.8 min, respectively.

The estimated effects, standard errors, and Student's *t* test for the 2^2 full-factorial star design are shown in Table 5. According to the Student's *t* test shown, the main effect (or linear) of the factor wet weight of banana (WW) and the quadratic effect of the factor extraction time (ET^2) significantly influenced the response function (sugars extraction from banana, EB) at a 5% probability level. For instance, increasing the wet weight of banana in the extraction process from 26.0 to 38.7 g (at the time of 45 min) increased from 5.55 to 7.51 °P the extracted total soluble solids.

Regression analysis was performed to fit the response function with the experimental data (Table 6). Although only the main effect of wet weight (X_1) and the quadratic effect of time (X_2^2) were significant at a 5% probability level, in order to minimize the error determination and due to the situation of existent hierarchy, the main effect of the time factor (X_2) was also kept in the model. Thus, the model expressed by Eq. 3, where the

Table 7 Analysis of variance (ANOVA) for the model regression representing extraction of total soluble solids from *Prata* banana (EB).

Sources of variations	Sum of squares	Degrees of freedom	Mean square	*F* value	Probability
Model	13.9349	3	4.6450	5.3900	0.0127[a]
Lack of fit	2.5905	5	0.5181	8.8477	0.0513
Pure error	0.1757	3	0.0586	–	–
Residual	2.7662	8	0.3458	–	–
Total	16.7011	11	–	–	–

R^2 =0.83 and adjusted R^2 =0.77; medium absolute error=0.3253; standard error=0.5881

[a] Significant level of the variables and their interactions at 95% (5% probability level)

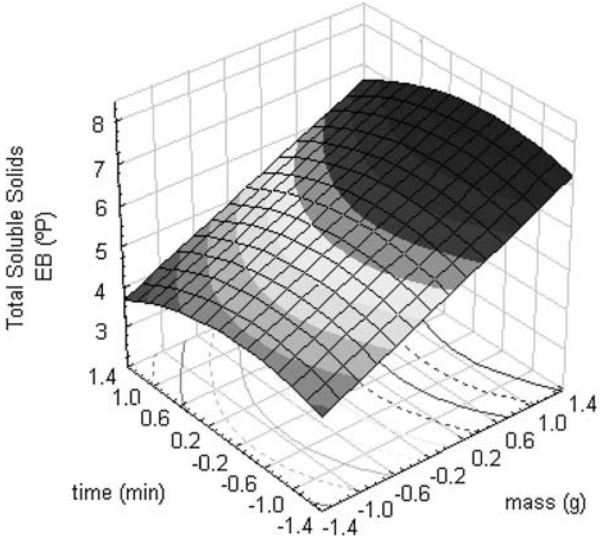

Fig. 2 Response surface described by the model *Y*, which represents the extraction of total soluble solids from *Prata* banana (*EB*)

variables take their coded values, represents the extraction of total soluble solids (Y) as a function of the variables: wet weight (X_1) and extraction time (X_2).

$$Y = 5.5790 + 1.2498X_1 - 0.2582X_2 - 0.3685X_2^2 \quad (3)$$

The statistical significance of the second-order model equation (Table 7) was evaluated by the F-test analysis of variance (ANOVA), which revealed that this regression is statistically significant ($P<0.05$) at a 95% of confidence level. The model did not show significant lack-of-fit, and the determination coefficient (R^2) value indicated that the model allows explaining 83.4% of the variability in the responses. The response surface described by the model equation (Y) is represented in Fig. 2.

According to these results, the optimum point for extraction of total soluble solids of *Prata* banana was found in the time established in the level −0.350 (equivalent to 39.75 min) and in the higher level of wet weight evaluated +1.4142 (equivalent to 38.7 g). In these conditions, the model presents a predicted response of total soluble solids of 7.40 °P. In previous studies, it was observed that for higher wet weight values than that shown at the level 1.4142, the

Table 8 Extraction processes conducted under optimized conditions (laboratory scale 105 mL) and on experimental conditions similar to the first assay of the factorial design (pilot scale 105 L).

Experimental conditions for extraction of total soluble solids from *Prata* banana (EB)			
Laboratory scale, 105 mL—100 rpm/84 °C; optimized condition, 38.70 g/39.75 min		Pilot scale, 105 L—100 rpm/84 °C; experimental condition, 17 kg/30 min	
Predicted response[a]	Observed value[b]	Predicted response[a]	Observed value
7.39±0.37 (°P)	7.69±0.65 (°P)	4.22±0.21 (°P)	4.22 (°P)

[a] Predicted response of total soluble solids showed by empirical model

[b] Experiments and measurements made in triplicates

resultant solution (banana juice) presents unviable characteristics from the practical point of view for further application as, e.g., occurrence of fermentation.

In order to validate the empirical mathematical model representative of the response function (Eq. 3), as well as the response surface itself (Fig. 2), extraction processes were conducted under optimized conditions. In addition, a scale-up procedure was also conducted following the same conditions of experiment 1 shown in the initial experimental design. For this, the runs of the scaled-up process were carried out in a stainless steel tank (105 L volume) with controlled conditions of temperature and agitation.

All these results are shown in Table 8, indicating that the model fitted the experimental data and thus described the region studied and that the results obtained at a pilot scale are in agreement with those previously obtained at a laboratory scale. The next step should be to evaluate and explore potential applications in different biotechnological processes, particularly in the beverage or other food processing industries.

Conclusions

This work started with the evaluation of the maturation stage of *Prata* bananas; maturation involved an increase in moisture and soluble sugar content and, at the same time, a decrease in starch content. The results obtained with the response surface methodology were adjusted by a quadratic model ($Y=5.58+1.25X_1-0.26X_2-0.37X_2^2$) in order to obtain the optimal extraction of the total soluble solids. The values that optimized the extraction of total soluble solids (Y) were 38.5 g banana weight (X_1) and 39.7 min (X_2) of extraction time. These values were compared with the model predicted values indicating that the model fitted the experimental data and thus described the region studied. Furthermore, the assays performed on a scaled-up equipment demonstrated the efficiency and the possibility of industrial implementation of the process studied in this work.

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