Characterization and optimization of production process of alcoholic fermentation of pineapple

Caracterização e otimização do processo de produção do fermentado alcoolico de abacaxi

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Abstract

Fruits are natural sources of vitamins, minerals, carbohydrates and other nutrients. Tropical fruits are consumed and appreciated all over the world due to the taste and flavour. This work aims to evaluate the physicochemical composition of fermented pineapple beverages, study associated fermentation kinetics and optimize fermentation conditions through the use of multiple linear regression and surface response analysis. Pineapple juice was characterized physiochemically using measures of soluble solids (brix), acidity, density and pH. It was determined that a fermentation temperature of 25 °C, pH between 3.0 and 4.0, and inoculum quantity of 4–5 g·L-1 resulted in a high-quality fermented beverage with 8.1% (v/v) ethanol content. A kinetic study was performed on the alcoholic fermentation process, monitoring the following variables: Brix, acidity, density, and alcohol content. The results showed that higher concentrations of yeast results in higher productivity and was possible to optimize the processes to maximize the results. This process is technically viable and can yield a value-added product that can generate revenue as well as other significant economic benefits for the beverage market.

Keywords: Fermentation; Optimization; Kinetics; Pineapple; Physicochemical; Beverages

Resumo

Frutas são fontes naturais de vitaminas, minerais, carboidratos e outros nutrientes. Frutas tropicais são consumidas e apreciadas em todo o mundo devido ao gosto e sabor. Este trabalho tem como objetivo avaliar a composição físico-química de bebidas fermentadas de abacaxi, estudar a cinética de fermentação e otimizar as condições de fermentação através do uso de regressão linear múltipla e análise de resposta superficial. O suco de abacaxi foi caracterizado fisico-quimicamente utilizando medidas de sólidos solúveis (°brix), acidez, densidade e pH. Foi determinado que uma temperatura de fermentação de 25 ° C, pH entre 3,0 e 4,0 e quantidade de inóculo de 4-5 g · L-1 resultou em uma bebida fermentada de alta qualidade com 8,1% (v / v) de etanol. Um estudo cinético foi realizado no processo de fermentação alcoólica, monitorando as seguintes variáveis: °Brix, acidez, densidade e teor alcoólico. Os resultados mostraram que concentrações mais altas de levedura resultam em maior produtividade e foi possível otimizar os processos para maximizar os resultados. Este processo é tecnicamente viável e pode gerar um produto de valor agregado que pode gerar receita, além de outros benefícios econômicos significativos para o mercado de bebidas.

Palavras-chave: Fermentação; Otimização; Cinética; Físico-química; Bebidas fermentadas

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1 Introduction

Brazil is one of the largest fruit producers in the world, an achievement that has garnered many economic benefits including increased employment and development in several cities. Unfortunately, there are also many problems related to waste. Raw materials easily degrade, and in the commercialization process, all fruits must have quality for the internal and external markets, consistent with a standard profile, without defects including shells, sizes, colouring, consistency, among other factors (TESSARO et al., 2010). This post-harvest waste is a serious problem and generates many losses (GOMES, 2007). An alternative for minimizing these problems is the investment in biotechnological processes and the agroindustry in order to transform waste from this raw material into a viable product. This solution would enable a reduction in postharvest losses and further develop rural areas (DIAS, SCHAWN and LIMA, 2003; GOMES, 2007; FONTAN, 2011). Utilization of fruit juice not only reduces waste, but also creates additional value. As an example, there are some fruits such as apple, peach and pineapple.

Pineapple (*Ananas comosus*) is a monocotyledonous plant of the bromeliaceous family in the subfamily Bromelioideae. The pineapple is among the most well-known tropical fruits in the whole world, not only due to its flavour and aroma characteristics, but also because of its nutritional composition such as water, mineral salts, carbohydrates, proteins, organic acids, pigments, and vitamins. The quantity of sugar in tropical fruits is a favourable characteristic for application in biotechnological processes, such as, alcoholic fermentation. Fermentation is a natural process for obtaining energy, which is still performed by microorganisms like fungi and bacteria, and widely accepted today as a technique for developing new products (MUNDARAGI; THANGADURAI, 2017). Alcoholic fermentation is widely used for the production of alcoholic beverages. The chemical equation of alcoholic fermentation is as follows (1):

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2 + Energy \qquad (1)$$

Wine is considered to be one of the oldest alcoholic fermented beverages, which is generally obtained by maturing natural fruit juice, mainly that of grape (*Vitis vinifera*). The other fruits used for beverage fermentation are referred to by the name of the fruit, as in this work on alcoholic fermented pineapple (CORAZZA; RODRIGUES, 2001).

Saccharomyces cerevisiae is the main yeast used for fermentation. Yeast consumes the sugar in the fruit, specifically sucrose, glucose, and fructose, effectively turning them into alcohol. Temperature control is an important consideration during alcoholic fermentation that can, in this case, facilitate the growth of microorganisms (ARROYO-LÓPEZ et al., 2009; MUNDARAGI; THANGADURAI, 2017).

The speed of the reaction can be analyzed through a kinetic study. It is important to understand the transformation of the substrate into the product, and measure the velocities of cell growth, substrate consumption, and product formation (MELO, 2011; BORZANI, 2001). There are many works on fermentation kinetics surrounding the behaviour of the grape, but similar work is less common for other fruits. An investigation into the production, yield, and efficiency are essential components in the overall analysis of the process (PAVLAK et al., 2011).

The optimization of the process, used to maximize the yield of the product, can be calculated through several statistical approaches (Matlab, Origin e Excel). These statistical tools are used and applied in bioprocess technology to analyse the influence of soluble solids and yeast concentration in the alcoholic fermentation process (percent conversion and productivity). The most important consideration is that the tool provides accurate results and have good efficacy.

Thus, the main aim of this study is to investigate alcoholic fermented pineapple production through physicochemical characterization, evaluation of alcoholic fermentation kinetics, and linear regression analysis to propose a statistically significant probabilistic model for alcoholic fermentation.

2 Materials and methods

2.1 Materials

The materials used for the fruit fermentation process were pineapple purchased in supermarket, 4 litres fermenter without temperature control, crystal Sugar (sucrose) purchased in supermarket, distilled water, commercial instant dry yeast (Dr. Oetker brand), °Brix, pH, acidity, density, and alcohol content using a DMA 4500 M digital densimeter manufactured by Anton Paar, pH papers, an industrial blender in stainless steel blender to beat the pineapple, a filter paper and Gelatine (Royal brand) to clarify the fermented.

2.2 Manufacture of fermented beverages

An industrial blender was used to prepare and homogenize the fruit juice, comprising a total volume of 1.0 L. The juice was kept at rest for 1 h, with a measured pH of 3.0. While the juice was resting, a syrup was prepared by dissolving the sugar in hot water and letting the mixture cool. Subsequently, the syrup was put inside of fermenter at room temperature, followed by the yeast (*Saccharomyces cerevisiae)*, and finally the fruit juice. The whole mixture was then homogenized using a glass stick. Before closing the fermenter, the pH was verified to be between 3.0 and 4.0. A small reactor was placed next to the larger fermenter in order to, with the aid of a hose, collect the carbon dioxide produced during fermentation. This design effectively prevented external air contamination and facilitated observation of the fermentation process from beginning to end, specifically the intensity of bubble release in the attached tube. The reactors were maintained at room temperature for 15 days. The product was clarified using colourless gelatine at a concentration

of 1 g·L⁻¹ (TORRES NETO et al., 2006). At the end of the fermentation process the fermented beverage was removed from the fermenter and was heated to 65 ° C for 20 min to thereby be conserved into a bottle for longer at room temperature.

2.3 Kinetic, product formation and substrate consumption

Kinetic studies were conducted on the fermented beverage using must aliquots obtained periodically throughout the fermentation. The fermenter was opened for a few minutes and the aliquots were measured with the apparatus. Measurements were taken at 1 h intervals for the first 24 h of the process, and at increasingly longer intervals until the end of fermentation. For each of the must aliquots, four experimental measurements were taken: Brix, density, alcohol, and acidity.

2.4 Yield, Productivity and Kinetic parameters of alcoholic fermentation

The yield (R) of the alcoholic fermentation, expressed as a percentage, quantitatively evaluates the efficacy of the yeast in converting sucrose into ethanol. The yield, R (%), and the productivity, Pr (g·L⁻¹·h⁻¹), of the production process were calculated using Equations 2 and 3 (LOPES and SILVA, 2006):

$$R = \frac{P_{exp}}{P_{teo}} \, x100 \qquad (2)$$

$$\mathbf{P} = \frac{P_{exp}}{t} \qquad (3)$$

P_{exp} = concentration (mol/L) of experimental ethanol,
P_{teo} = concentration (mol/L) of theoretical maximum ethanol (stoichiometric), and
t = fermentation time (h).

The product yield ($Y_{p/s}$) was calculated using Equation 4 (ALMEIDA et al., 2006);

$$Y_{p/s} = \frac{P_f - P_0}{S_0 - S_f} \qquad (4)$$

P_0 and P_f = initial and final ethanol (mol. L⁻¹) concentrations;
S_0, S_f = the initial and final sucrose concentrations (mol·L⁻¹), respectively.

2.5 Statistic analysis

To statistically analyse the influence of the input variable (concentration of soluble solids) on the response variables (percent conversion and productivity). The excel program was used to apply linear regression and variance analysis and the Origin program was used to plot graphics.

3 Results and discussion

3.1 Process for obtaining the fermented fruit

The three stages of the fermentation process are outlined in Tables 1 and 2. Table 1 provides values for the fermentation process parameters used to produce the fermented beverage, and Table 2 provides values of soluble solid content in °Brix, acidity (g·L⁻¹), density (kg·m⁻³), and pH before fermentation process.

Table 1— values of process of fermentation

Formulation of must	added sugar (g)	water added (mL)	juice volume (L)
	300	150	0.9

Formulation of inoculum	amount of yeast (g.L⁻¹)	°Brix	pH
	6	27.6	3.0

Table 2 — Pineapple juice before fermentation process

	Pineapple
Brix	5.0
Density (kg/m³)	1.017
Acidity (g.L⁻¹)	1.018
pH	3.5

3.2 Physical and chemical characterization

Table 3 provides the values and characteristics of fermented beverages in the final 7 days of fermentation. The main parameters analyzed were soluble solid content in °Brix, acidity (g·L⁻¹), density (kg·m⁻³), alcohol, turbidity, and pH.

Table 3 — Physical and chemical parameters

	°Brix	Acidity (g/cm³)	Density (kg/m³)	Alcohol (%v/v)	Turbidity (NTU)	pH
Pineapple	6	0.9982	987.3	8.1	1461	3.7

The physicochemical analysis of the pineapple fermentation was compared with works in the literature. As shown in Table 4, the pH of pineapple falls within the optimum pH range (3.5 to 4.5) for fermentation conduction (LOPES and SILVA, 2006). Table 4 lists the values for pH, °Brix, and alcohol content measurements for pineapple and others tropical fruits to verify if there are also many differences in relation to the fermentation process. The comparison verifies that the parameters evaluated for the fermented pineapple do not differ considerably from

those of the other fermented fruits. The pH measurement is prevalent in industrial alcoholic fermentations because of its importance in bacterial contamination control, fermentation rates, by-product formation, and yeast growth (JONES et al., 1981).

According to Aquarone et al. (1983), the pH range between 3.8 and 4.0 allows for rapid alcoholic fermentation in addition to the inhibition of undesirable bacteria. Based on this information, the pineapple juice used in this study falls within the appropriate pH range to perform a successful fermentation. Our product yielded an ethanol content of 8.1% v/v at 20 °C and presented characteristics similar to those fermented in accordance with current legal standards. However, in order to verify the beverage's acceptance in the market, more studies are needed to determine its sensory and conservation characteristics, among others.

The quantity of sugar stabilized as a consequence of the non-fermentable compounds present in the medium (AQUARONE et al., 2001). The use of yeast (*Saccharomyces cerevisiae*) was adequate to obtain an alcoholic content within the standards required for alcoholic fermentation. Silva (2008) found that ideal yeast concentrations for ethanol production is approximately 20 g·L^{-1}.

Table 4 — Comparison of the composition of the fermentation of pineapple

Fruits	pH	°Brix	Alcohol %v/v	References
Pineapple	3.7	6.0	8.1	This work
Pineapple	3.8	7.0	-	Belchior et al., 2013
Pineapple	3.1	14	12.3	Oliveira et al., 2012
Watermelon	4.1	6.0	10.0	Fontan et al., 2011
Orange	3.3	8.0	10.6	Corazza, Rodrigues e Nozaki, 2001
Caja	2.6	0.0	12.0	Dias, Schawn e Lima, 2003

3.3 Kinetics of alcoholic fermentation

Figure 1 illustrates the fermentative kinetics of the fermented pineapple beverage. This graphic shows the substrate consumption (S) and ethanol production (P) profiles, each expressed as g·L^{-1}, as a function of the fermentation time. In the first 24 h, sucrose decreased, and ethanol production increased dramatically. According to Silva (2009), the control variables in the fermentation process are very important for a production that is high in quality and safe for consumption. Substrate and product concentrations stabilized around 45 h of fermentation, with values of 60 g·L^{-1} and 65 g·L^{-1} respectively. According to Aquarone et al. (2001), the stabilization of the sugar content is explained by the presence of fermentation inhibitory compounds, like the alcohol in the medium.

Figure 1 — Sugar consumption and ethanol production as a function of time of cultivation of Saccharomyces cerevisiae
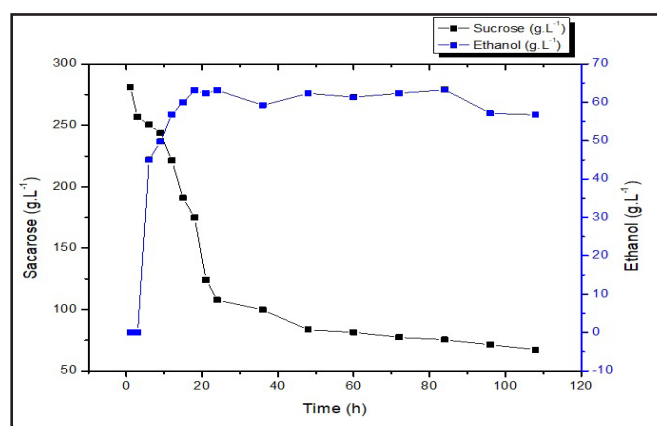
The conversion of the substrate into ethanol began to occur 15 h after fermentation process was initiated. While studying the preparation of fermented cashews, Torres Neto et al. (2006) observed that at around 10 h of fermentation, the microorganism reaches the middle of the adaptation phase.

Table 5 shows the kinetic profiles of the must throughout fermentation. The physicochemical parameters analyzed during the first 5 days of the fermentation process were °Brix, acidity, density, and alcohol. The results verify the continuous decay of sugar content in the pineapple throughout fermentation. The acidity parameter experienced little variation throughout the process. Similarly, there was also little variation in the density of the fermented pineapple, falling almost always within the range of 0.900–0.999 g·cm^{-3}. Finally, alcohol production became evident after 6 h of fermentation.

Table 5 — Fermentative kinetics

Time (h)	Brix °	Acidity	Density	Alcohol (%v\v)
1	27,6	1,116	1,114	0
3	25,2	1,004	0,999	0
6	24,6	0,973	0,951	5,7
9	23,9	0,917	0,940	6,3
12	21,7	0,871	0,909	7,2
15	18,7	0,811	0,934	7,6
18	17,1	1,073	0,974	8
21	12,1	1,045	0,970	7,9
24	10,5	1,041	0,974	8
36	9,7	0,948	0,917	7,5
48	8,1	1,024	0,900	7,9
60	7,9	1,025	0,907	6,5
72	7,5	1,013	0,930	7,8
84	7,3	1,006	0,907	8,0
96	6,9	1,005	0,949	7,9
108	6,5	1,006	0,955	8,02

Table 6 presents the results of yield ethanol, productivity, yield (Yp/s) of pineapple fermented.

Table 6 – Fermentation parameters at the end of the process

Fruits	Yield (%)	Productivity (g / L·h)	YP/S
Pineapple	45	1,30	0,44

The results in Table 6 were compared to those reported in the literature of other fermented fruit studies. Some parameters for pineapple juice indicated a low efficacy comparatively, such as the conversion percentage contrasted with the mandacaru fruit (91.82%) (ALMEIDA et al., 2007). Fontan et al. (2011) similarly achieved a high conversion percentage (94.0%) in the production of fermented watermelon, in addition to a productivity of 1.65 g·L^{-1}·h^{-1}. In this study, the product yield obtained in relation to the substrate (°Brix) was 6.5. On the other hand, lower values were obtained for the conversion percentage (55.166) and the product yield substrate (0.282). Through such comparisons, it is possible to affirm that the parameters determined in this research, namely conversion percentage, productivity, and product yield in relation to the substrate, are reasonably within the ranges of the related literature. Therefore, the production of fermented pineapple on a commercial scale is viable, provided that modifications are made to some experiments and technical feasibility is achieved.

3.5 Statistic analyse

In order to examine the influence of input variables, including soluble solid and yeast concentrations, on the response variables, such as conversion and productivity, experimental data was submitted to a multiple linear regression analysis using the Excel program. This step was performed in an effort to optimize the processes and define the optimal ranges of process operations to maximize the results.

Equations 5 and 6 show the empirical regression models of the experimental data, adjusted for the conversion (%) and productivity (g·L^{-1}·h^{-1}) of the fermented pineapple, respectively.

$$\text{Conversion}(\%) = 218 - 60.8Y + 0.306B \qquad (5)$$

$$\text{Productivity}(g·L^{-1}·h^{-1}) = -287.8 + 104.28Y - 0.57B \qquad (6)$$

Where Y and B represent yeast concentration (g·L^{-1}) and °Brix, respectively. Table 7 shows the results that were obtained throughout the process using the equation 3 and 4.

The coefficients are statistically significant at the 95% confidence interval for the pineapple fermentation. Tables 8 presents the parameters of the variance analysis (coefficient of determination R^2 and test F) for model adjustment.

Table 7 — Conversion and productivity of the fermented pineapple

Yeast (g.L^{-1})	Brix (g.L^{-1})	Productivity	Conversion (%)
5	281,03	112,25	0,00
4,8	256,72	33,04	0,00
4,5	250,64	15,97	31,91
4,2	243,55	10,22	35,27
4	221,27	6,66	40,30
3,9	190,88	4,23	42,54
3,7	174,67	3,04	44,78
3,5	124,02	2,30	44,22
3,3	107,81	2,04	44,78

Table 8 — Parameters of the variance analysis for the percent conversion and productivity of the fermented pineapple

Parameters	% Conversion	Productivity (g L^{-1}h^{-1})
R^2	~0,80	0,75
test F	36,9	6,34
F$_{calculated}$/F$_{tabulated}$	6,38	1,23

The conversion and productivity models presented in Equations 5 and 6 are proven statistically significant by satisfactory coefficients of determination (R^2) and F$_{calculated}$ / F$_{tabulated}$ ratios above 1 at the 95% confidence interval (BARROS NETO et al., 1995). Thus, the operating ranges of the input variables can be maximized through the analysis of a response surface graph. Figures 2 and 3 show the coefficient determination graphs.

Figures 4 and 5 show the effects of the input variables, °Brix and yeast concentration, on the response variables, conversion and productivity. Figure 4 illustrates the conversion (%) increase and indicates that the influence of Brix is greater than the initial concentration of yeast on the conversion (%) response. The highest conversions were obtained when the amount of yeast was lower.

As illustrated in Figure 5, the two input variables (Brix and yeast concentration) influence the response (productivity). Evidently, with the increase of yeast concentrations, the productivity of the process increases. Large amounts of brix and small amounts of yeast decrease the productivity parameter. According to Parente (2014) the increase in yeast concentration provides a higher productivity increase than the increase in soluble solids concentration, showing that the yeast concentration exerts a greater influence on the productivity than the soluble solids concentration. The fermentation process demonstrated that higher concentrations of yeast result in higher productivity; however, the conversion is independent of yeast. Alternatively, based on the analysis of the Brix parameters, higher concentrations of Brix correlated with high conversion percentage results.

Figure 2 — Graphs coefficients of determination (R2) of productivity in relation to °Brix
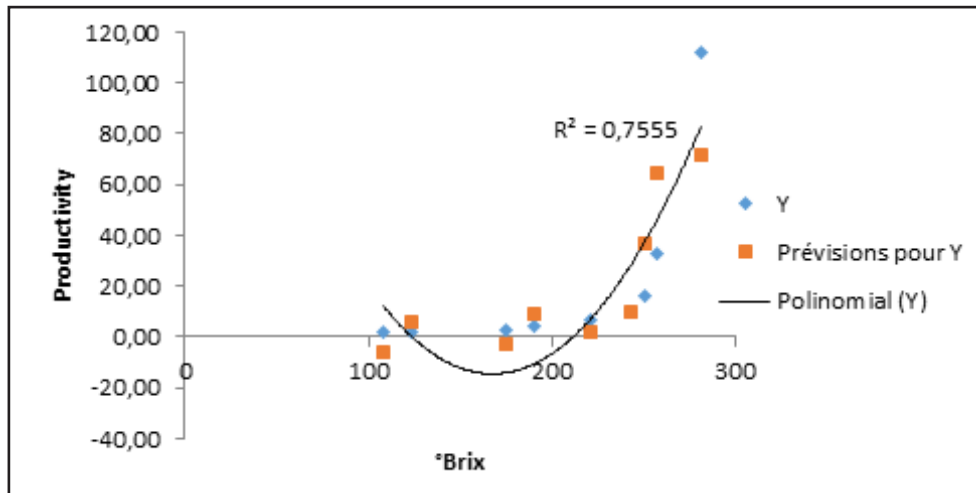

Figure 3 — Graphs coefficients of determination (R2) of percent conversion as a function of °Brix
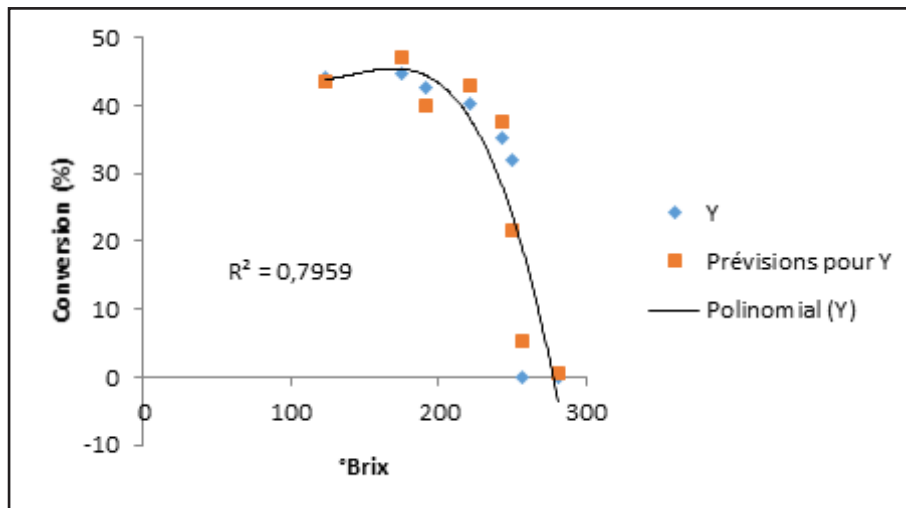

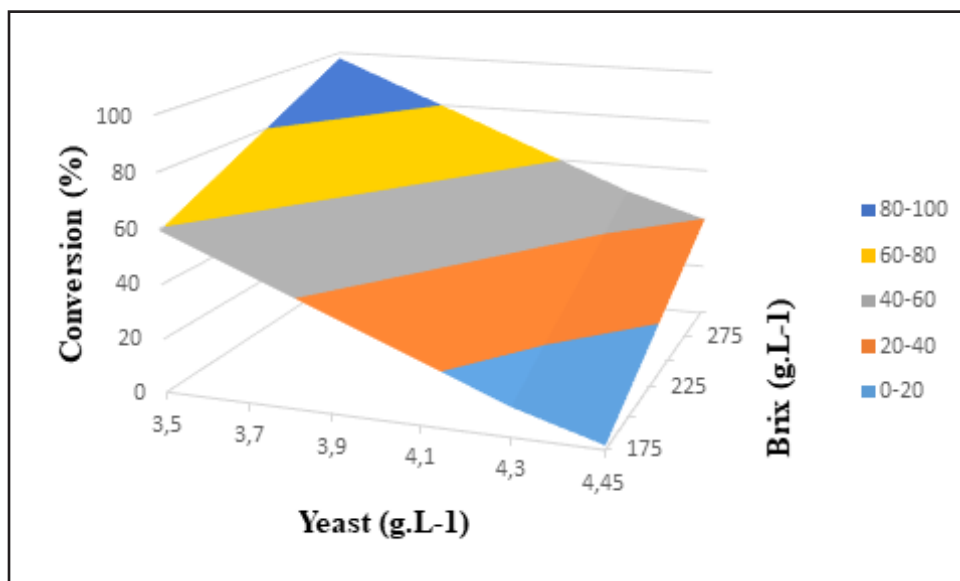Figure 4 — Response surface of the percent conversion as a function of Brix and yeast concentrations
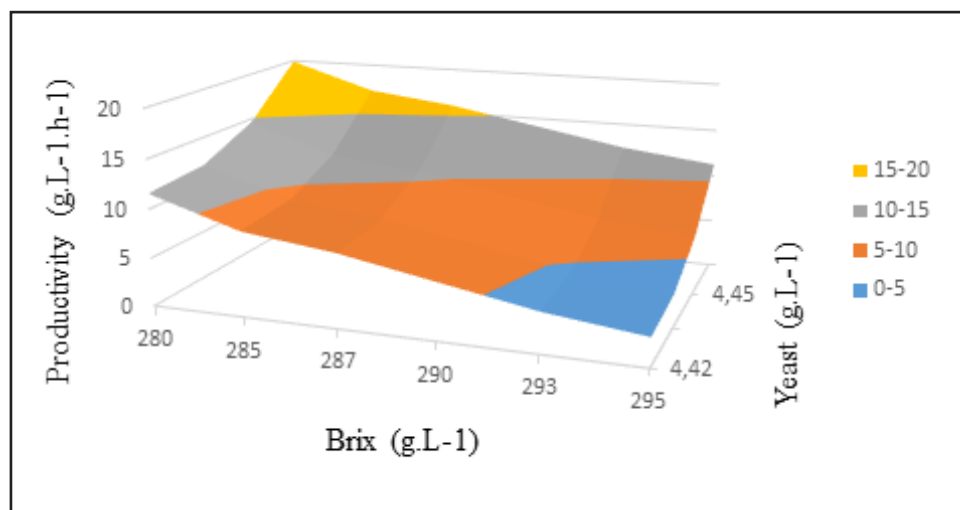
Figure 5 — Response surface of the productivity as a function of °Brix and yeast concentrations

4 Conclusions

We developed a process for alcoholic pineapple fermentation that is technically feasible. The results of the fermentation indicate that higher concentrations of yeast results in higher productivity; however, the conversion percentage is independent of the yeast concentration. The resulting product had characteristics similar to those of other fruit ferments reported in the literature. The must remained in fermentation for 20 days; however, 48 h into the process, no further change was observed in either the substrate or product concentrations. The fermentation parameters obtained demonstrate that the commercial yeast *Saccharomyces cerevisiae* performed satisfactorily in the alcoholic fermentation process of pineapple juice. For large-scale production, final adjustments to the production process are necessary.

Acknowledgments

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