

Alcohol production from sweet potato varying the fermentation and hydrolysis conditions

Produção de álcool de batata-doce variando as condições de fermentação e hidrólise

Producción de alcohol de batata al variar las condiciones de fermentación e hidrólisis

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Eveline Gheller dos Santos

ORCID: <https://orcid.org/0000-0002-4981-8538>

Universidade Estadual do Centro-Oeste, Brasil

E-mail: evgheller@hotmail.com

Edson Perez Guerra

ORCID: <https://orcid.org/0000-0002-0082-4267>

Universidade Estadual do Centro-Oeste, Brasil

E-mail: epguerra@unicentro.br

Juliano Tadeu Vilela de Resende

ORCID: <https://orcid.org/0000-0001-5226-7813>

Universidade Estadual de Londrina, Brasil

E-mail: jvresende@uol.com.br

Fabielli Priscila de Oliveira

ORCID: <https://orcid.org/0000-0001-9570-0592>

Universidade Estadual do Centro-Oeste, Brasil

E-mail: fabellioliveira@hotmail.com

Nathalia Campos Vilela Resende

ORCID: <https://orcid.org/0000-0002-7825-3070>

Universidade Federal de Lavras, Brasil

E-mail: nathaliacvr@yahoo.com.br

Abstract

Bioethanol is a renewable fuel as an alternative source of energy of worldwide importance. The present study aimed to evaluate alcohol production from sweet potato, under varying reaction conditions for fermentation and hydrolysis. A genotype was selected to obtain dried flour and processed by acid hydrolysis and enzymatic hydrolysis with α -amilase and amilogucosidase. The experiment was carried out in a completely randomized block design

with three central point replicates combined with response surface methodology. The variables were pH, temperature and fermentation time. The alcohol content was determined by distillation with subsequent spectrophotometric analysis. Raw materials were characterized according to moisture, reducing sugars and starch, and 4.13%, 4.04% and 71.80% were obtained, respectively. Acid hydrolysis showed higher alcohol percentages of distilled samples. The highest values for acid hydrolysis and enzymatic hydrolysis in fermentations were 26.42% and 25.04%, respectively, carried out at pH 5.0 and 36°C for 5 days. The fermentation time was the most important variable, with higher and significant values for both types of hydrolysis. Acid hydrolysis presented higher potential for alcoholic production than enzymatic hydrolysis in sweet potato fermentation.

Keywords: *Ipomoea batatas* L.; *Saccharomyces cerevisiae*; Hydrolysis; Bioethanol.

Resumo

O bioetanol é um combustível renovável como fonte alternativa de energia de importância mundial. O presente estudo teve como objetivo avaliar a produção de álcool a partir de batata-doce, sob diferentes condições de reação para fermentação e hidrólise. Um genótipo foi selecionado para obtenção de farinha seca e processado por hidrólise ácida e hidrólise enzimática com α -amilase e amiloglucosidase. O experimento foi realizado em delineamento de blocos completamente casualizados com três repetições de pontos centrais, combinadas com a metodologia da superfície de resposta. As variáveis foram pH, temperatura e tempo de fermentação. O teor de álcool foi determinado por destilação com subsequente análise espectrofotométrica. As matérias-primas foram caracterizadas quanto à umidade, açúcares redutores e amido, obtendo-se 4,13%, 4,04% e 71,80%, respectivamente. A hidrólise ácida mostrou maiores porcentagens de álcool nas amostras destiladas. Os maiores valores de hidrólise ácida e hidrólise enzimática nas fermentações foram 26,42% e 25,04%, respectivamente, realizados a pH 5,0 e 36 ° C por 5 dias. O tempo de fermentação foi a variável mais importante, com valores mais altos e significativos para os dois tipos de hidrólise. A hidrólise ácida apresentou maior potencial de produção alcoólica do que a hidrólise enzimática na fermentação da batata-doce.

Palavras-chave: *Ipomoea batatas* L.; *Saccharomyces cerevisiae*; Hidrólise; Bioetanol.

Resumen

El bioetanol es un combustible como fuente alternativa de energía de importancia mundial. El presente estudio tuvo como objetivo evaluar la producción de alcohol a partir de batata, en

diferentes condiciones de reacción para la fermentación e hidrólisis. Se seleccionó un genotipo para obtener harina seca y se procesó por hidrólisis ácida e hidrólisis enzimática con α -amilasa y amiloglicosidasa. El experimento se llevó a cabo en un diseño de bloques completamente al azar con tres repeticiones de puntos central, combinadas con la metodología de la superficie de respuesta. Las variables fueron pH, temperatura y tiempo de fermentación. El contenido de alcohol se determinó por destilación con análisis espectrofotométrico posterior. Las materias primas se caracterizaron por la humedad, los azúcares reductores y el almidón, obteniendo 4,13%, 4,04% y 71,80%, respectivamente. La hidrólisis ácida mostró mayores porcentajes de alcohol en las muestras destiladas. El 26,42% y el 25,04% valores fueron los más altos de hidrólisis ácida e hidrólisis enzimática en las fermentaciones, respectivamente, realizadas a pH 5,0 y 36°C durante 5 días. El tiempo de fermentación fue la variable más importante, con valores más altos y significativos para ambos tipos de hidrólisis. La hidrólisis ácida mostró un mayor potencial para la producción de alcohol que la hidrólisis enzimática en la fermentación de batata.

Palabras clave: *Ipomoea batatas* L.; *Saccharomyces cerevisiae*; Hidrólisis; Bioetanol.

1. Introduction

Alcohol can be produced from different types of vegetable raw material sources. These renewable energy sources may have considerable amounts of fermentable carbohydrates. They are classified into three specific groups: sugary, starchy and cellulosic materials (Canilha et al., 2012).

Sweet potato (*Ipomoea batatas* L.) is the sixth most cultivated food crop in the world after rice, wheat, potatoes, maize and cassava. The world production was 112.84 million ton of sweet potato. The China ranked in first with 72.03 million ton and 58.6% of the world production and Brazil with 0.77 million ton and approximately 0.6% of world production (FAO, 2017).

The tuberous roots have high levels of sugar and starch, and are a significant source for alcohol production. Other positive factors are the rusticity of the culture, the use of byproducts and the adaptation to different types of environment. Sweet potato has a great capacity to accumulate energy per unit of area. The branches and tuberous roots of sweet potato have been largely used in human and animal feed, as well as raw material in the food industry, fabrics, papers, cosmetics and alcohol fuel. Silva et al. (2018) obtained a high yield of bioethanol in a small industrial plant from sweet potato of 161.4 L t⁻¹ corresponding to

10,598 L ha⁻¹, based on the productivity of 65.5 t ha⁻¹ of the evaluated cultivar. The result were 46% superior to the ethanol yield of sugarcane, 34% superior to that of sugar beet and 149% superior to that of corn ethanol.

The amount of water from a substrate or food is one of the most valued indexes. It has great economic importance as it reflects the solids content of a product and its perishability. Moisture transfer results in loss of chemical stability, physiological changes and microbiological spoilage and therefore affects food quality (Amit et al., 2017).

The present study aimed at determining the flour quality of a sweet potato genotype and its alcoholic yield, using both acid and enzymatic hydrolysis under, varying conditions of pH, temperature and time of fermentation.

2. Materials and methods

The experiment was carried out at the Plant Physiology and Horticulture Laboratory of Midwestern State University-UNICENTRO, located in the municipality of Guarapuava-Paraná, Brazil. Sweet potato clone number 119 of the genotypes collection was used. This clone has a white outer film and a pale cream pulp with elongated roots. It is an average cycle material that can be harvested at 120 days with great commercial profit and productivity around 45 t ha⁻¹, with 38.56% dry mass, of which 91.12% is represented by starch and sugars (Camargo et al., 2013).

The tuberous roots were washed in order to remove the dirt and them submersed in chlorinated water with 50 ppm of chlorine for 10 minutes. These samples were placed in runoff and dried to remove the excess of external moisture and then weighted.

The tuberous roots were peeled with a stainless steel knife, cut into fragments of approximately 3.0 mm deep each, and dried in a warm house equipment, for 24 h at 60°C. These samples were stored in sealed glasses bottles to avoid the moisture accumulation, and after grinded in an electric mill using knives with a 30 mesh sieve to obtain the flour.

Flour samples were used to determine moisture level using the 413/IV method (IAL, 2005). Samples were subjected to a pre-drying stage at 65 °C for 24 hours in an oven with air circulation, obtaining a DAS (dry by air sample). Five porcelain capsules were warmed in an oven at 130°C and them chilled until room temperature and immediately weighed. Flour samples (2.0 g) were collected, weighed and subjected to drying in an oven at 130°C, for 1h. Samples were chilled until achieving room temperature and the mass was determined.

The levels of reducing sugar and glucose were determined using the Fehling reagents,

according to the 038/IV method by IAL (2005) based on the reduction of copper ion method by Lane-Eynon. The amount of reducing sugar was determined by titration.

Two types of hydrolysis were performed: acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis was carried out with an adaptation of the method published by Woiciechowski et al. (2002), using 1:10 (m/v) transferring 9.0 g of sweet potato flour to an Erlenmeyer, adding 90 mL of hydrochloric acid (HCl) 1%. Samples were autoclaved at 120 °C, with 1.0 atm for 10 minutes. Samples were chilled and centrifuged at 3,000 rpm for 8 minutes in centrifuge.

An adaptation of the method published by Pavlak et al. (2011) was used to perform the enzymatic hydrolysis of the starch. A sample of 9.0 g of the flour and 150.0 mL of Mc Ilvaine solution (formed by citric acid solution 0.1 mol L⁻¹ and Na₂HPO₄ 0,2 mol L⁻¹) with pH 5.5 were mixed in an Erlenmeyer, and placed in a thermostatic bath for 15 minutes at 90°C. After that, 13 µL g⁻¹ of α-amylase were added and kept in a thermostatic bath at 90°C for 1 hour, while stirring and homogenizing the system in every 15 minutes. Samples were then cooled and the pH adjusted for 4.5 with HCl 0.1 mol L⁻¹. Then 54 µL g⁻¹ of amyloglucosidase were added, keeping in a thermostatic bath for 1 hour at 60°C, while stirring and homogenizing the system. To obtain the supernatant, samples were centrifuged at 3,000 rpm for 8 minutes.

The fermentative conditions was carried out in a completely randomized design with three central point replicates with varying pH, temperature and the fermentation time. To perform the fermentative process, sweet potato was autoclaved for 10 minutes at 120°C and 1.0 atm. The addition of *Saccharomyces cerevisiae* in concentrations of 8.0 g L⁻¹ started the fermentative process. Three variables were used in the fermentation in triplicate: pH (4, 4.5 and 5), temperature (20°C, 28°C and 36°C), and time (1, 3 and 5 days). The yeast consumed the sugars obtained by the hydrolysis. As a result, there was a conversion and alcohol production with the release of CO₂.

The alcohol acquired was separated by distillation. The distillation was performed in simple reflux system (75°C), using a *Vigreux* column. The alcohol was collected in an ice bath in order to keep its stability, stored in a corked glass bottle, and kept in a regular cooler of 10°C. Determination of the level of alcohol in the sample was performed according to a standard absorbance curve in function of previously known alcohol concentrations.

Pearson's linear correlation coefficient was used to determine the linear regression analysis, varying from -1 to 1, to quantify the correlation between the hydrolysis methods used. In this interval, the values were distributed considering 0 (zero) for nonexistent correlations, 0.3 to 0.7 for moderate correlation, and from 0.7 to 1.0 for strong correlation.

The data was submitted to analysis of variance (ANOVA), and reported in the average and standard deviation. The averages were compared by Tukey test ($p < 0.05$). The answers and Pareto's graphics were generated by the software Statistica version 8.0 (StatSoft, USA).

3. Results and Discussions

The sweet potato flour presented $4.13 \pm 0.70\%$ of moisture. The raw material should never exceed a moisture value of 15.0% (ANVISA, 2005), since it may influence the enzymatic activity or the acid hydrolysis. Alves et al. (2012) observed similar moisture level of 5.80% dry bases used for alcohol production. Variations in moisture may occur due to the processing, drying time and how the sample is stored. Flour can be stored in dry and fresh locations for 6 months or beyond. The values obtained were within the satisfactory interval for analysis. Tian et al. (2018) evaluating 12 genotypes of sweet potato tubers with different storage times, observed that the starch content was weakly correlated with other ingredients, while the percent dry content and fermentable sugars contents had a close correlation with starch content of the flour and there were significant differences in the bioethanol production with the same storage time and different sweet potato genotype.

The reducing sugars obtained from the flour were $4.04 \pm 0.74\%$, which is considered a low value. These levels vary from 4.80% to 7.80% according to the storage when starch is converted to sugar. The higher the conversion is, the higher the alcohol yield with lower cost will be, considering there is no need of large amounts of hydrolytic enzymes such as amylases and glucosidases. Schweinberger et al (2019) observed that drier sweet potatoes result in higher ethanol potentials and they made an equation to estimate total reducing sugars based on their moisture content. Results showed that ethanol potential increases non-linearly with increasing concentrations of sweet potato mash in the fermenting medium.

The starch value of $71.80 \pm 1.32\%$ observed is important for alcohol production since the amount of sugars obtained by hydrolysis is directly proportional to the expected yield.

The fermentation efficiency influences energy consumption during the process of alcohol generation, indicating the need of industrial yeast with good performance during fermentation (Ferrari et al., 2013). A pilot test was performed until the best results were obtained. A calibration curve produced the line equation (1) used to determine the level of alcohol.

$$Y = 0.0670 X + 0.025 \quad (R^2 = 0.99) \quad (1)$$

Table 1. Alcohol level obtained by acid hydrolysis (AH) and enzymatic hydrolysis (EH) from sweet potato flour and the coded levels. Midwestern State University, Guarapuava, Brazil.

Test	pH	(CL)	T	(CL)	Time	(CL)	Alcohol level*	
							(°C)	(days)
1	4.0	(-1)	20	(-1)	1	(-1)	16.72 ± 0.45 fg A	17.25 ± 1.11 cde A
2	5.0	(+1)	20	(-1)	1	(-1)	15.41 ± 0.29 g A	15.47 ± 0.02 ef A
3	4.0	(-1)	36	(+1)	1	(-1)	16.86 ± 0.33 fg A	16.20 ± 0.07 de A
4	5.0	(+1)	36	(+1)	1	(-1)	16.43 ± 1.47 g A	13.96 ± 0.82 f B
5	4.0	(-1)	20	(-1)	5	(+1)	21.94 ± 0.40 cd A	20.71 ± 0.18 b B
6	5.0	(+1)	20	(-1)	5	(+1)	23.43 ± 0.38 bc A	21.94 ± 1.11 b B
7	4.0	(-1)	36	(+1)	5	(+1)	25.30 ± 0.45 ab A	24.22 ± 0.51 a A
8	5.0	(+1)	36	(+1)	5	(+1)	26.42 ± 0.16 a A	25.04 ± 0.02 a B
9	4.5	(0)	28	(0)	3	(0)	20.32 ± 0.07 de A	15.69 ± 0.30 ef B
10	4.5	(0)	28	(0)	3	(0)	19.78 ± 0.43 e A	18.10 ± 0.07 cd B
11	4.5	(0)	28	(0)	3	(0)	18.53 ± 0.07 ef A	18.43 ± 0.03 c A

*average ± standard deviation (n=3); same small letters in the same column do not have significant difference (p<0.05); same capital letters in the same line do not have significant difference (p<0.05); studied intervals: pH: 4.0 to 5.0; temperature: 20.0 a 36.0 °C; time: 1 to 5 days; alcohol level is expressed in % of ethanol alcohol; CL- Coded levels; T- Temperature (°C).

The values of alcohol level using two types of hydrolysis and the real and coded levels of the variables used are shown in Table 1. The acid hydrolysis of samples 1 and 3, with pH 4.0 and time of 1.0 day, and the samples number 2 and 4, with pH 5.0 and time of 1.0 day, processed with 20°C and 36°C, respectively, did not result in significant differences, varying from 15.41% to 16.86% of alcohol. Despite the different conditions of pH and temperature, in the first day all samples presented an amount of alcohol produced without differences.

When fermented for 5.0 days, samples 5, 6, 7 and 8 resulted in an increase in alcohol production proving that the longer the period is, the higher the alcohol volume will be.

The highest alcohol contents were 24.2% and 25.0% obtained from the enzymatic hydrolysis of samples 7 and 8, with pH 4.0 and 5.0, respectively, at 36°C for 5 day. It was

followed by samples 5 and 6, with 20.7% and 21.9% of alcohol contents, but at a temperature of 20°C, with no significant differences between them (Table 1).

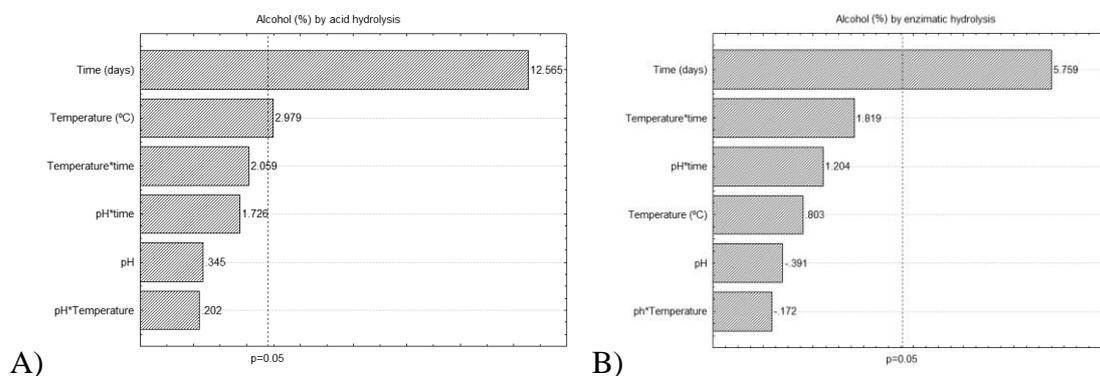
A Pearson's correlation of 92.90% was found. There is a positive and strong correlation between alcohol values obtained by acid hydrolysis and enzymatic hydrolysis (Table 1). The final models was obtained using the production of alcohol by acid hydrolysis (AH) (Equation 2) and enzymatic hydrolysis (EH) (Equation 3), due to pH, temperature (T) and time (t).

$$\text{Alcohol (AH)} = 20.10 + 0.11 \text{ pH} + 0.93 \text{ T} + 0.96 \text{ t} + 0.06 \text{ pH}*\text{T} + 0.54 \text{ pH}*t + 0.64 \text{ T}*t \quad (2)$$

$$\text{Alcohol (EH)} = 18.82 + 0.25 \text{ pH} + 0.51 \text{ T} + 3.63 \text{ t} - 0.11 \text{ pH}*\text{T} + 0.76 \text{ pH}*t + 1.15 \text{ T}*t \quad (3)$$

The Pareto Diagram shows that the linear coefficients of time (days) and temperature (°C) were significant for alcohol production by acid hydrolysis (Figure 1A). Only the linear coefficients of time (days) was significant for enzymatic hydrolysis (Figure 1B).

Figure 1 – Pareto Diagram for standard purposes – Alcohol (%) obtained by acid hydrolysis (A) and enzymatic hydrolysis (B) of sweet potato flour.

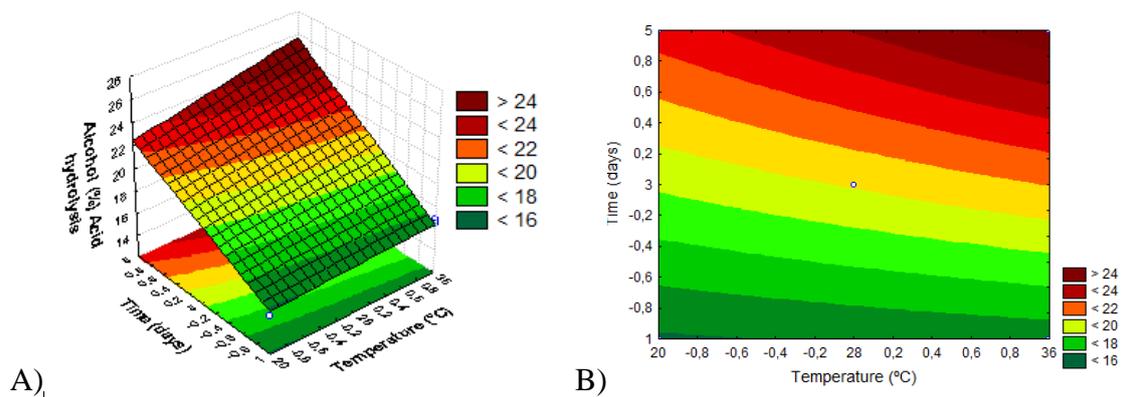


Font: Santos (2019).

The model presented significant regression (95.0% of trust) with R^2 equals to 0.97 for acid hydrolysis and 0.91 for enzymatic hydrolysis (Figure 1). The analysis of variance of the model (pH, time and temperature) for alcohol production presented an average square of 70.73 and 138.28 ($p < 0.01$) to the model (2 degrees of freedom) and 0.40 and 0.35 of residue average square (8 degree of freedom) to the acid hydrolysis and enzymatic hydrolysis, respectively.

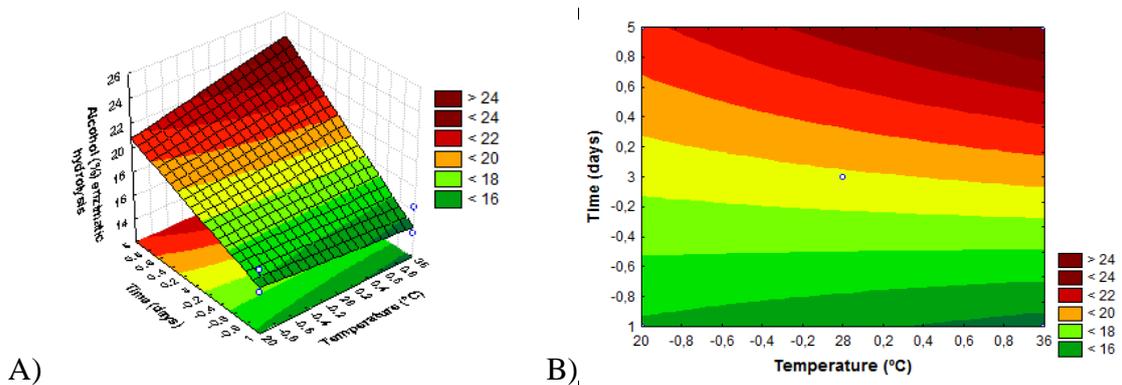
The linear response surface (Figure 2) shows the interaction of the variables effects on the alcohol production. The area that presents the highest values of alcoholic content was the increase in temperature and fermentation period. The contour levels (Figure 2A) were generated by the model (Equation 1). Figure 2B shows in the darkest area the highest values of alcohol levels, which are related to an increase in temperature and time of fermentation.

Figure 2. Linear response surface (A) and contour level (B) of alcohol production from sweet potato by acid hydrolysis in function of temperature and time.



Font: Santos (2019).

Figure 3. Surface of linear response (A) and contour levels (B) of alcohol production from sweet potato through enzymatic hydrolysis in function of time and temperature.



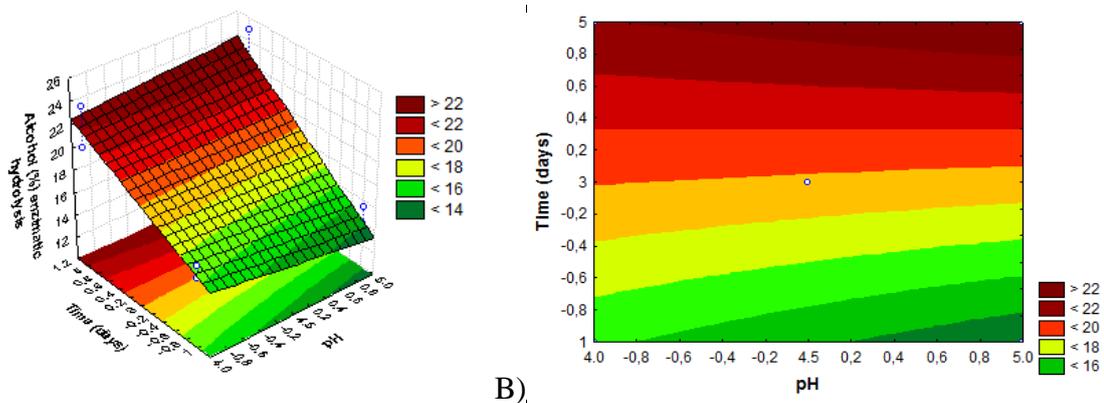
Font: Santos (2019).

The linear response surface (Figure 3A) and the contour levels (Figure 3B) shows that there is more alcohol production by enzymatic hydrolysis in the darkest area with the higher values of time, confirming the effect analysis. Srichuwong et al. (2012) studied biofuel production from sweet potatoes obtaining 15.1 and 15.4% (v/v), that is, 88.8% and 90.6% of the provided theoretical incomes, during the period of 48 and 72 hours. Cantos-Lopes et al.

(2018) studied fermentation of flour from two sweet potato genotypes, suggested that alcohol production is more efficient within 72 hours of fermentation.

The linear response surface (Figure 4A) and the contour levels (Figure 4B) confirmed the effect analysis performance. The area with higher values for alcohol level produced is the same test performed with longer duration. This result reinforces the hypothesis that longer times of fermentation generate higher alcohol contents. Such process would be more expensive though. Pereira et al. (2017) obtained high glucose yield with less energy demand and relatively lower enzymatic cost, converting sweet potato granular starch into fermentable sugars using enzymatic hydrolysis by *Aspergillus niger* to optimize amylase production.

Figure 4. Linear response surface (A) and contour levels (B) for alcohol production from sweet potato by enzymatic hydrolysis in function of time and pH.



Font: Santos (2019).

The response surface presented in Figure 2, 3 and 4 suggests that the longer is the fermentation time, the higher the levels of alcohol will be. The results indicate that acid hydrolysis is more efficient for obtaining alcohol from sweet potato.

4. Conclusion

Both acid hydrolysis and enzymatic hydrolysis were efficient in fermentation to produce alcohol from reducing sugars and starch present in sweet potato flour.

Temperature and fermentation time were statistically significant for acid hydrolysis. However, only fermentation time was significant for the enzymatic hydrolysis. Despite the different conditions of pH and temperature, in the first day all samples presented an amount of

alcohol produced without differences. Thus, for both types of hydrolysis the longer the time is, the higher the alcoholic level will be. But such process would be more expensive though.

The greatest values of alcoholic content were obtained from distillate samples fermented at temperatures of 36°C for 5 days. In these conditions, acid hydrolysis was more efficient than enzymatic hydrolysis for alcohol production.

Acid hydrolysis was more efficient than enzymatic hydrolysis for sweet potato fermentation, presenting greater potential for high alcohol yield.

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Contribution percentage of each author to the manuscript

Eveline Gheller dos Santos – 30%

Edson Perez Guerra – 30%

Juliano Tadeu Vilela de Resende – 30%

Fabielli Priscila de Oliveira – 5%

Nathalia Campos Vilela Resende – 5%