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# The composition of sacarine substrates for ethanol production and the fermentative capacity *Saccharomyces cerevisiae* Pedra-2

# A composição de substratos sacarinos para produção de etanol e a capacidade fermentativa da *Saccharomyces cerevisiae* edra-2

# La composición de sustratos de sacarina para la producción de etanol y la capacidad fermentativa da *Saccharomyces cerevisiae* Pedra-2

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**Rebeca Fasioli Silva**
ORCID: https://orcid.org/0000-0002-9649-0619
State University of Mato Grosso do Sul, Brazil
E-mail: beca_fasioli@hotmail.com
**Maria do Socorro Mascarenhas Santos**
ORCID: https://orcid.org/0000-0002-5343-4502
State University of Mato Grosso do Sul, Brazil
E-mail: maria_mascarenhas@outlook.com
**Larissa Pires Mueller**
ORCID: https://orcid.org/0000-0003-0134-7033
State University of Mato Grosso do Sul, Brazil
E-mail: laripiresmueller@gmail.com
**Claudia Andrea Lima Cardoso**
ORCID: https://orcid.org/0000-0002-4907-0056
State University of Mato Grosso do Sul, Brazil
E-mail: claudiacardosouems1@gmail.com
**Margareth Batistote**
ORCID: https://orcid.org/0000-0001-9865-2362
State University of Mato Grosso do Sul, Brazil
E-mail: margareth@uems.br

**Abstract**

The production of ethanol in Brazil is based on sugarcane juice, however other biomasses can be used for this process, such as sweet sorghum. However, some nutrients can interfere with fermentation, such as the presence of metals, carbon and nitrogen sources, which can affect

the fermentation capacity of yeasts. Thus, this study aims to analyze the presence of fundamental nutrients present in saccharine substrates, as well as their assimilation and conversion of ethanol by the yeast Pedra-2. Samples of sugarcane and sorghum juice were obtained, in which analysis of the presence of metals was carried out using acid digestion and the levels determined by atomic flame absorption spectroscopy. The amino acid analysis was performed on the saccharine substrates at a concentration of 22 ºBrix, before and after fermentation, and analyzed by high-performance liquid chromatography and the concentration of ethanol by gas chromatography. The sorghum broth showed higher amounts of available amino acid metals. The yeast Pedra-2 showed better fermentative performance in the sorghum broth. We can conclude that the sorghum broth represents an important substrate to be used to increase the sustainability and production of ethanol in Brazil.

**Keywords:** Nutrients; Sugarcane juice; Sweet sorghum; Fermentation.

**Resumo**

A produção de etanol no Brasil tem como matéria-prima o caldo de cana-de-açúcar, contudo outras biomassas podem ser utilizadas para este processo, como o sorgo sacarino. Todavia, alguns nutrientes podem interferir na fermentação como a presença de metais, as fontes carbono e de nitrogênio que podem afetar a capacidade fermentativa das leveduras. Assim, este estudo visa analisar a presença nutrientes fundamentais presentes em substratos sacarinos, bem como sua assimilação e conversão de etanol pela levedura Pedra-2. Foram obtidas amostras de caldo de cana e sorgo, nas quais foram realizadas análises de presença de metais utilizando uma digestão ácida e os teores determinados por espectroscopia de absorção atômica de chama. A análise de aminoácidos foi realizada nos substratos sacarinos na concentração de 22 ºBrix, antes e após a fermentação, e analisadas por cromatografia líquida de alta eficiência e a concentração de etanol por cromatografia gasosa. O caldo de sorgo apresentou maiores quantidades de metais de aminoácidos disponíveis. A levedura Pedra-2 apresentou melhor desempenho fermentativo no caldo de sorgo. Podemos concluir que o caldo de sorgo representa um importante substrato a ser utilizado para aumentar a sustentabilidade e produção /do etanol no Brasil.

**Palavras-chave:** Nutrientes; Caldo de cana; Sorgo sacarino; Fermentação.

**Resumen**

La producción de etanol en Brasil se basa en jugo de caña de azúcar, sin embargo se pueden utilizar otras biomasas para este proceso, como el sorgo dulce. Sin embargo, algunos

nutrientes pueden interferir con la fermentación, como la presencia de fuentes de metales, carbono y nitrógeno que pueden afectar la capacidad de fermentación de las levaduras. Así, este estudio tiene como objetivo analizar la presencia de nutrientes fundamentales presentes en los sustratos sacarinos, así como su asimilación y conversión de etanol por la levadura Pedra-2. Se obtuvieron muestras de jugo de caña de azúcar y sorgo, en las cuales se realizaron análisis de presencia de metales mediante digestión ácida y los niveles determinados por espectroscopia de absorción de llama atómica. El análisis de aminoácidos se realizó sobre los sustratos dulces a una concentración de 22 ºBrix, antes y después de la fermentación, y se analizó por cromatografía líquida de alta resolución y la concentración de etanol por cromatografía de gases. El caldo de sorgo mostró mayores cantidades de metales aminoácidos disponibles. La levadura Pedra-2 mostró un mejor desempeño fermentativo en el caldo de sorgo. Podemos concluir que el jugo de sorgo representa un sustrato importante para ser utilizado para incrementar la sostenibilidad y producción de etanol en Brasil.

**Palabras clave:** Nutrientes; Jugo de caña de azúcar; Sorgo dulce; Fermentación.

## 1. Introduction

Renewable biomasses emerge on the world stage as an alternative to non-renewable natural resources, especially concerning their use as a raw material for obtaining energy, with a view also to the demand for this resource, which is related to society's consumption modes modern (Azevedo & Azevedo Lima, 2016). However, new standards are being outlined and propose obtaining alternative sources of renewable energy that pay attention to the precepts of sustainable development such as the mitigation of environmental impacts and the greenhouse effect that influence climate change (Barbieri, 2017).

Thus, countries that can produce large quantities of natural resources with energy potential are in promising conditions (Ferreira, Silva & Ferreira, 2013). Furthermore, the growing interest in biomass transformation processes and the possibilities of application in different industrial processes have been demanding the development of technologies that provide a more efficient energy conversion from biomass.

In this context, Brazil has numerous advantages compared to other countries, as it has a large territorial extension, with fertile soils, water availability and tropical climate, favourable conditions for the production of a variety of plant varieties with bioenergetic potential. Sugarcane (Saccharum officinarum), is a successful example of biomass with

energy potential, as it has important characteristics such as the high content of fermentable sugars, high suitability for the tropical climate with the possibility of regrowth and with that being used for periods successive (Alves & Paixão, 2018), in addition to having a high yield with lower production cost when compared to other crops, being used both for the manufacture of sugar for food and the production of biofuels and, more recently, for the generation of electricity according to Golderberg (2016). However, the demand for fuel ethanol has been driving research on other biomasses that can be used in this process.

Thus, sweet sorghum (*Sorghum bicolour* L. Moench), presents itself as promising biomass, given its characteristics, as it is a culture of the same family and genus of sugarcane, storing sugars in its culms fermentable, this plant also has the advantage of tolerating abiotic stresses and a faster phenological cycle (Da Silva, 2017). Another factor for the use of sorghum in ethanol production according to Lucena et al. (2013), would be the possibility of using sorghum as a complement to the off-season of sugarcane, since it can be processed with the same equipment, presenting a good relationship between costs and benefits in production.

The production of Brazilian ethanol has as its raw material, saccharine substrates, which undergoes a clarification and pH correction treatment, in which the yeasts *Saccharomyces cerevisiae* are added, responsible for fermentation (Gonçalves et al., 2015). In this process, selected yeasts highly adapted to the industrial fermentation environment are used, which guarantees production with quality and good yields (Della-Bianca et al., 2013), among the selected yeasts, Catanduva-1 and Pedra-2 stand out, which are responsible for the highest productivity of ethanol in Brazil. However, in this substrate metals can be found that in excess can damage the yeast's physiological functions and interfere with the conversion of sugars (Basso, Basso & Rocha, 2011).

Throughout the fermentation process, nutrients such as carbon and nitrogen sources must be available in the substrates for efficient assimilation by the yeast and effective ethanol production, such nutrients as amino acids and sucrose, have their availability influenced by plant cultivation conditions, maturation stage and variety (Vázques-Lima et al., 2014). The concentration and composition of these sources can affect yeast metabolism and influence the production of ethanol and aromatic compounds such as higher alcohols (Dzialo et al., 2017). Thus, this study aims to analyze the presence of minerals in saccharine substrates, evaluate the presence of amino acids and the consumption of these nutrients by the yeast Pedra-2, as well as evaluate the concentration of ethanol.

## 2. Methodology

### 2.1 Sugar cane juice and sweet sorghum

The sugarcane juice was obtained directly from the Bunge mill process and the sorghum juice from Embrapa Agropecuária Oeste-Dourados, with its extraction by grinding in a conventional mill. Both were stored in sterile bottles and transported at 4 ºC to the Biotechnology, Biochemistry and Biotransformation laboratory (CERNA/UEMS).

The sugarcane juice was obtained from the industrial process and transported to the laboratory in sterile bottles at a temperature of 4 ºC. This material was filtered through filter paper to remove impurities. The Brix was concentrated by evaporation and accompanied by a portable refractometer and calibrated at 22 °Brix. The pH was checked by pH meter and adjusted to 5.0, with the addition of hydrochloric acid (1 mol $L^{-1}$).

### 2.2 Mineral analysis

The organic matter was oxidized using 25 ml of sugarcane and sorghum juice in digestion tubes and 20 ml of nitric acid (65-70%) were added and kept at rest for 30 minutes, then they were placed in a digestion block and heated to 100 °C ± 10 °C for 1 hour, after the temperature was raised to 180 °C ± 10 °C and 10 mL of a nitroperchloric solution (3: 1) was added, after volatilization of the solution plus 10 mL of the solution the sample was added.

The end of digestion was considered when the mixture exhaled white smoke. Soon afterwards, the samples were diluted in 50 ml of deionized water and the levels of metals were determined utilizing atomic flame absorption spectroscopy, brand Perkin Helmer. The elements were determined according to the methodology of Welz and Sperling, (1999).

### 2.3 Fermentative condition

#### 2.3.1 Pre-inoculum

The pre-inoculum used as the liquid medium YPD 2%, composed of 1.0% (p $v^{-1}$) of yeast extract: 1.0% (p $v^{-1}$) peptone; 2.0% (p $v^{-1}$) glucose, sterilized in an autoclave at 120 ºC for 20 minutes, in which 0.10 grams of lyophilized yeast Pedra-2 were inoculated and incubated at 30 °C for 10 hours at 250 rpm. After this period, the cells were collected by

centrifugation (800g, 20min), washed for three consecutive times in sterile saline solution (0.85%) resulting in a final concentration of 10 mg $mL^{-1}$ of wet mass, being used in the experiments fermentative.

### 2.3.2 Fermentation

Fermentation was carried out on sweet substrates based on sugarcane juice and sweet sorghum, at a concentration of 22 °Brix in 125 ml Erlenmeyer flasks containing 50 ml of sterile broths, in which the biomass was inoculated and incubated for 10 hours at room temperature. from 30 ºC to 250 rpm. Aliquots were collected for the analysis of amino acid consumption. The experiment was carried out in triplicate.

### 2.4 Amino acid analysis

The samples for amino acid analysis were prepared and processed according to the method described by Torres et al. (2018). The identification of amino acids was performed using the Sigma standards, $\geq$ 97% (alanine, arginine, cysteine, isoleucine, methionine, proline, serine, threonine, tryptophan and valine) and analyzed by comparing the retention times and spectra of the amino acid patterns, in the region of 200 to 800 nm. The analysis was performed in triplicate.

### 2.5 Evaluation of fermentative efficiency

The ethanol concentration was determined with a gas chromatograph (CG) 3900 with flame ionization detector (Varian), according to Batistote et al., (2010).

### 2.6 Data Processing

The data were analyzed using Excel 2016 software and GraphPadPrism 7.0.

## 3. Results and Discussion

According to the analysis of the content of minerals present in the saccharine substrates, the sorghum juice presented a greater quantity of these nutrients, such as

phosphorus 223.04, potassium 465.14 and magnesium 285.14, with the sugarcane juice standing out potassium and iron 128.54. Such elements are essential to activate enzymatic actions and countless metabolic reactions during the fermentation process (Table 1).

In the fermentation environment, the yeasts *Saccharomyces cerevisiae* need some elements, aiming to activate innumerable physiological mechanisms, which act directly on the activation of enzymes such as magnesium (Mg), which acts as a cofactor in the activation of enzymes of the glycolytic pathway in fermentation (Trofimova Y; Walker & Rapoport, 2010). The calcium that acts in cell protection in the presence of high levels of ethanol, as it acts as an intracellular signal against homeostasis (Couchesne et al., 2011).

**Table 1.** Evaluation of the presence of minerals in (mg $L^{-1}$) in saccharine substrates that can be fermented.

| | Saccharine substrates | |
|---|---|---|
| Elements | **Sorghum juice (mg $L^{-1}$)** | **Sugarcane juice (mg $L^{-1}$)** |
| **P** | 223.04 | 13.45 |
| **K** | 465.58 | 265.59 |
| **Ca** | 219.14 | 29.29 |
| **Mg** | 285.18 | 32.18 |
| **S** | 78.49 | 24.95 |
| **Zn** | 2.01 | 0.51 |
| **Mn** | 4.54 | 3.65 |
| **Fe** | 28.59 | 128.54 |
| **Cu** | 0.46 | 0.19 |
| **B** | 0.78 | 0.95 |
| **Na** | 7.24 | 1.13 |

(P-Phosphorus, K-Potassium, Ca-Calcium, Mg-Magnesium, S-Sulfur, Zn-Zinc, Mn-Manganese, Fe-Iron, Cu-Copper, B-Boron, Na-Sodium). Source: Authors.

Zinc acts on the physiology of cells, as a cofactor for many proteins, mainly from the "zinc-finger" group according to Zhao & Bai (2012). Thus, it is observed that the composition of the substrate directly influences the efficiency and yield in ethanol production. Besides, as Souza (2016) points out, the minerals present in the fermentative medium are an important factor for yeast metabolism.

Table 2 shows the consumption of amino acids by the yeast Pedra-2 in 10 hours of fermentation, it can be observed a difference concerning the concentration of amino acids (µg $L^{-1}$) found in the saccharine substrates before and after fermentation, however, the sorghum broth showed higher amounts of amino acids. Regarding the consumption of amino acids by

the yeast Pedra-2, it presented a different profile, since for the sorghum juice the amino acids consumed with the best efficiency were tryptophan (90.1%), cysteine (84.0%), proline (80.6%), arginine (74.7%), alanine (64.4%), threonine (61.6%) and isoleucine (52.7%). In the fermentation using sugarcane juice, the assimilated by yeast were cysteine (90.5%), alanine (84.0%), proline (80.0%), arginine (78.0%), tryptophan (68.6%), threonine (65.2%) and serine (53.0%). for other amino acids, consumption was below 50%.

According to Auesukaree (2017), the amino acids proline, tryptophan and arginine, promote a protective action for yeasts about ethanolic stress. In this study, these amino acids were also the most assimilated by the yeast Pedra-2, which were present in the saccharine substrates, possibly the assimilation of such amino acids is essential to maintain the fermentative capacity and the physiological integrity of the yeast during anaerobic conditions.

**Table 2.** Availability of amino acids present in saccharine substrates and efficiency of consumption by yeast.

| | **Sorghum juice** | | | **Sugarcane juice** | | |
|---|---|---|---|---|---|---|
| Amino acids | Initial (μg $L^{-1}$) | Last (μg $L^{-1}$) | Consumption (%) | Initial (μg $L^{-1}$) | Last (μg $L^{-1}$) | Consumption (%) |
| Serine | 60.77 ± 0.14 | 25.99 ± 0.03 | 18.0 | 36.12 ± 0.05 | 16.99 ± 0.19 | 53.0 |
| Threonine | 13.51 ± 0.05 | 5.18 ± 0.04 | 61.6 | 10.33 ± 0.04 | 3.59 ± 0.05 | 65.2 |
| Alanine | 43.14 ± 0.06 | 15.34 ± 0.05 | 64.4 | 27.44 ± 0.06 | 4.41 ± 0.02 | 84.0 |
| Valina | 3.19 ± 0.05 | 1.89 ± 0.01 | 40.7 | 2.09 ± 0.01 | 1.58 ± 0.01 | 24.4 |
| Methionine | 2.39 ± 0.03 | 1.61± 0.02 | 32.6 | 2.06 ± 0.02 | 1.41 ± 0.04 | 31.5 |
| Isoleucine | 6.83 ± 0.05 | 3.23 ± 0.01 | 52.7 | 3.96 ± 0.04 | 2.02 ± 0.01 | 49.0 |
| Tryptophan | 11.36 ± 0.08 | 1.12 ± 0.05 | 90.1 | 7.43 ± 0.02 | 2.33 ± 0.02 | 68.6 |
| Arginine | 43.06 ± 0.09 | 10.89 ± 0.11 | 74.7 | 23.98 ± 0.08 | 5.29 ± 0.02 | 78.0 |
| Proline | 2.12 ± 0.03 | 0.41 ± 0.02 | 80.6 | 2.06 ± 0.02 | 0.41 ± 0.03 | 80.0 |
| Cysteine | 2.07 ± 0.02 | 0.33 ± 0.01 | 84.0 | 2.01 ± 0.01 | 0.19 ± 0.02 | 90.5 |

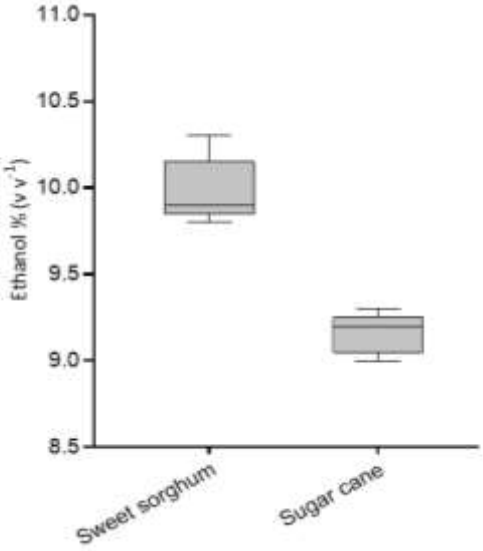
Source: Authors.

Amino acids are responsible for the integrity of the yeast's metabolic functions during fermentation, acting as structural components that collaborate in the formation of proteins and the maintenance of metabolic pathways (Ljungdahl And Daignan-Fornier, 2012). Also, for there to be a good fermentative performance for yeast, the substrate must contain the necessary quantities and nutrients, so that the yield in conversion from sugar to ethanol (Tognete, 2017) occurs effectively.

In the evaluation of the ethanol concentration from the saccharine substrates, differences were observed in the production of this compound by the analyzed yeast concerning the evaluated saccharine substrates. It was found that the sorghum juice provided an ethanol conversion efficiency for the yeast with 10% (v $v^{-1}$) and the sugarcane juice 9.0%

(v v $^{-1}$), as shown in Figure 1.

**Figure 1.** Evaluation of the ethanol concentration by the yeast Pedra-2 in saccharine substrates for 10 hours of fermentation at a temperature of 30 °C.




Source: Authors.

The yeast Pedra-2 has a high fermentative capacity with ethanol production ranging from 10 to 12% (v v $^{-1}$) and great adaptability to the environment considered inhospitable, which are the vats (Amorim & Lopes, 2013). In the studies developed by Mueller et al. (2019), using sugarcane juice and sorghum in concentrations of 22 °Brix and yeast FT858, observed that there was ethanol production in both substrates and that yeast was more efficient in sorghum juice with an ethanol concentration of 9.6% (v v $^{-1}$). The results of Mueller et al. (2019) corroborate the data of this study, considering that, although the yeast used is of different strains, the best ethanol production occurred in sweet sorghum broth.

The yeast Pedra-2 has characteristics such as advantages, a great capacity for ethanol bioconversion, low glycerol accumulation and high viability during cell recycling (SANTOS et al., 2017). Possibly the greater availability of metals and amino acids in the sorghum broth has provided better assimilation and fermentative efficiency of the yeast Pedra-2 in this substrate that resulted in a greater production of ethanol. Sorghum broth proved to be an important fermentative medium to be used since this biomass; is rich in nutrients that can be directly fermented and can add greater productivity to the sugar-energy sector due to its availability between harvest. As well as maintaining Brazil at the forefront of sustainability and ethanol production; this important product associated with the best technologies for biotechnological development.

## 4. Conclusion

The sorghum juice showed higher amounts of metals in relation to the sugarcane juice, as well as a greater amount of available amino acids and the most assimilated by yeast, were: were tryptophan, cysteine, proline, arginine, alanine, threonine and isoleucine, as a result of this, this substrate has a great potential to be used in the production of ethanol.

The yeast Pedra-2 showed fermentative performance in both substrates, however, the best accumulation of ethanol occurred in the fermentation with sweet sorghum. Which leads us to suggest that the sorghum juice represents an important substrate to be used to leverage ethanol production in Brazil.

The results demonstrate that sweet sorghum has potential for the production of bioethanol. Thus, future studies could be carried out using other yeasts S*accharomyces cerevisiae* to evaluate the fermentative profile, yield and efficiency of this biomass, which can also be used for other biotechnological processes as a source of bioenergy.

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**Percentage of contribution of each author in the manuscript**

Rebeca Fasioli Silva – 30%

Maria do Socorro Mascarenhas Santos – 20%

Larissa Pires Mueller – 15%

Claudia Andrea Lima Cardoso – 15%

Margareth Batistote – 20%