Assimilation of amino acids present in must based on sugarcane juice by 
Saccharomyces cerevisiae under fermentative stress

Assimilação de aminoácidos presentes em mosto à base de suco de cana-
de-açúcar por Saccharomyces cerevisiae sob estresse fermentativo

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ABSTRACT
In Brazil, sugar cane juice and molasses are used as a substrate for the production of ethanol. This substrate is called wort and is rich in carbon source and low in nitrogen source. Thus, this study aims to evaluate the assimilation profile of amino acids present in the must based on sugarcane juice by the yeasts Saccharomyces cerevisiae under different conditions of

Nislene Pires dos Santos
Mestre em Recursos Naturais pelo Programa de Pós-Graduação em Recursos Naturais/PGRN
Universidade Estadual de Mato Grosso do Sul/UEMS
Cidade Universitária, Rodovia Itahum, Km 12 s/n - Jardim Aeroporto,
Dourados - MS, 79804-970, Brasil
E-mail: nislene5@hotmail.com

Maria do Socorro Mascarenhas Santos
Doutoranda do Programa de Pós-Graduação Graduação em Recursos Naturais/PGRN
Universidade Estadual de Mato Grosso do Sul/UEMS
Cidade Universitária, Rodovia Itahum, Km 12 s/n - Jardim Aeroporto,
Dourados - MS, 79804-970, Brasil
E-mail: maria_mascarenhas@outlook.com

Claudia Andrea Lima Cardoso
Doutora em Química Instituto de Química de Araraquara -UNESP
Docente do Programa de Pós-Graduação Graduação em Recursos Naturais/PGRN
Universidade Estadual de Mato Grosso do Sul/UEMS
Cidade Universitária, Rodovia Itahum, Km 12 s/n - Jardim Aeroporto,
Dourados - MS, 79804-970, Brasil
E-mail: claudiacardosouems1@gmail.com

Margareth Batistotete
Doutora em Biotecnologia pela Universidade Estadual Paulista Júlio de Mesquita Filho - Instituto de Instituto de Química
Docente do Programa de Pós-Graduação Graduação em Recursos Naturais/PGRN
Universidade Estadual de Mato Grosso do Sul/UEMS
Cidade Universitária, Rodovia Itahum, Km 12 s/n - Jardim Aeroporto,
Dourados - MS, 79804-970, Brasil
E-mail: margareth@uems.br

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fermentative stress. Catanduva-1 and Red Star strains were used, grown in the medium (2% YPD), sterilized at 120 °C for 20 minutes and incubated at 30 °C for 10 hours to produce biomass that was inoculated in the base fermentation medium. of wort in concentrations of (18, 22 and 25) °Brix at temperatures of 30 °C and 40 °C, and aliquots were collected at different times for the analysis of amino acid assimilation. Amino acid quantification was performed by high performance liquid chromatography (HPLC). It is possible to observe that there was a difference in the assimilation profile of the amino acids present in the must by Saccharomyces cerevisiae, mainly in relation to the concentrations of the amino acids valine, methionine, alanine, threonine and tryptophan. According to the discriminant analysis, fermentation time and temperature were determining factors for the consumption of yeast amino acids.

Keywords: Carbon and nitrogen sources, Yeast, Metabolism, Ethanol production.

RESUMO
No Brasil, o suco e o melaço de cana-de-açúcar são utilizados como substrato para a produção de etanol. Este substrato recebe o nome de mosto e é rico em fonte de carbono e pobre em fonte de nitrogênio. Assim, este estudo visa avaliar o perfil de assimilação de aminoácidos presentes no mosto à base de suco de cana-de-açúcar pelas leveduras indústrias Saccharomyces cerevisiae em diferentes condições de estresse fermentativo. Foram utilizadas as linhagens Catanduva-1 e Red Star, cultivadas no meio (YPD 2%), esterilizadas a 120 °C por 20 minutos e incubadas a 30 °C por 10 horas para produção de biomassa que foi inoculada no meio de fermentação à base de mosto em concentrações de (18, 22 e 25) °Brix a temperaturas de 30 °C e 40 °C, e aliquotas foram coletadas em diferentes momentos para análise da assimilação de aminoácidos. A quantificação de aminoácidos foi realizada por cromatografia líquida de alta eficiência (HPLC). É possível observar que houve diferença no perfil de assimilação dos aminoácidos presentes no mosto por Saccharomyces cerevisiae, principalmente em relação às concentrações dos aminoácidos valina, metionina, alanina, treonina e triptofano. De acordo com a análise discriminante, o tempo e a temperatura de fermentação foram fatores determinantes para o consumo de aminoácidos pelas leveduras.

Palavras-chave: Fontes de carbono e nitrogênio, Leveduras, Metabolismo, Produção de etanol.

1 INTRODUCTION
Environmental problems related to global warming and fossil fuel reserves scarcity have promoted the search for new raw materials to compose the diversification in the energy matrix. Thus, the research on biofuels are being carried out in order to make it a viable alternative to fossil fuels (ISMAIL et al., 2014; ZABED et al., 2016). As an example, there is the ethanol that has arousing world interest by being a renewable energy source that can contribute positively to reducing environmental problems related to the emission of greenhouse gases (BELLIDO et al., 2013; ZABED et al., 2016).

In Brazil, the ethanol production process uses sugarcane as raw material resulting in a successful large-scale bioprocess because besides being economically competitive and
causing less impact to the environment (DELLA-BIANCA; GOMBERT, 2013), being called first generation ethanol (AZHAR et al., 2017). This country stands out as an important developer of technologies and is the second largest producer of this fuel in the world (TÁVORA, 2011; NOGUEIRA and CAPAZ, 2013). Among the several microorganisms producers of ethanol the yeast *Saccharomyces cerevisiae* stands out being widely used by industries of this sector (MA and LIU, 2010). According to Souza and Monteiro (2010), in the process of ethanolic fermentation other compounds are produced in addition to ethanol, such as glycerol, organic acids, acetaldehyde, acetoin, butylene glycol that can be used in different industrial processes. The advantages of using yeasts range from cultivation to employment in different industrial sectors, composing a source of research for biotechnological processes (HERNÁNDEZ-CARBAJAL et al., 2013).

In these processes, in which the yeasts *Saccharomyces cerevisiae* are present, the growth substrates must contain compounds such as carbon source, nitrogen source and also mineral salts and vitamins. However, the main compounds used by these microorganisms as carbon source are the monosaccharides (fructose, glucose and galactose) and disaccharides (maltose and sucrose) and as sources of nitrogen, peptides, amino acids and ammonium salts (SANTOS et al., 2013). These microorganisms are used by man since ancient times, being notorious the numerous benefits they provide and play a key role in the industrial processes of foods, pharmaceuticals, ethanol production, among other (KURTZMAN, FELL and BOEKHOUT, 2011).

In Brazil, ethanol production consists of a fermentation process in which yeasts are added to a substrate constituted by a mixture of sugarcane juice and molasses, the must$^{13}$ which is rich in carbon source and low in nitrogen source, composed of high percentages of sugars with contents between 15.5-24% of sucrose, 0.2-1 , 0% glucose and 0.0-0.5% fructose plus 75-82% water and presence of acids, waxes, fats, dyes and inorganic salts, SiO, K2O, CaO, MgO, among others (HARI, JEBITTA AND SIVARAMAN, 2013; MARQUES et al., 2008). For this process yeasts are required that have a high viability rate, since it is carried out in batches with cell recycling (SILVA et al., 2016).

Nitrogenous sources have a high molecular weight and are composed by proteins (albumin, nucleins and albuminose), amino acids, aspartic acid and glutamic acid, alanine, leucine, glycine, lysine, thyroxine, valine, isoleucine, can be metabolized by yeasts (BUTZKE and PARK, 2011). Therefore, the availability of carbon and nitrogen sources can affect the metabolism of yeasts during fermentation process, and can contribute to limit the formation of
secondary products (GUTIÉRREZ-RIVERA et al., 2015; CARRILLO et al., 2012). On the other hand, the assimilation of amino acids can accelerate the speed of substrate degradation leading to an increase in cell viability rate resulting in higher ethanol production (WANG et al., 2012). Thus, this study aims to evaluate the assimilation profile of amino acids present in the must based on sugarcane juice by the yeasts Saccharomyces cerevisiae under different conditions of fermentative stress.

2 MATERIAL AND METHODS

2.1 MICROORGANISM

The strain Catanduva-1 and the Red Star.

2.2 PRE-INOCULUM

For the pre-inoculum we used the classic cultivation midst YPD 2%, composed of 1.0% of yeast extract, 1.0% peptone, 2.0% glucose, with pH adjusted to 5.0 with hydrochloric acid 1N and autoclaved at 120 ºC for 20 minutes, in which were inoculated 0.10g of lyophilized yeast and incubated at 30 ºC for 24h at 200 rpm. After the growth period the cells were centrifuged (800 g, 20 min), then resuspended and rinsed three times in sterile saline solution (0.85%), resulting in a concentration of 10 mg mL⁻¹ of wet mass.

2.3 FERMENTATIVE MIDST

The fermentative assays were performed on Erlenmeyer flasks 125 mL containing 50 mL of must based on sugarcane juice at concentrations of (18, 22 and 25) °Brix, which were promptly adjusted with a saccharimeter, without correction of pH, and sterilized at 120 ºC for 20 minutes. After cooling, the biomass was inoculated and incubated at temperatures of 30 and 40 ºC at 250 rpm. Aliquots were collected at different fermentation times and used for the analyses of amino acid assimilation.

2.4 ANALYTIC METHODS

2.4.1 Amino acid determination

Furthermore, during the fermentation process, the yeasts are exposed to numerous stressing factors such as abundance or scarcity of nutrients, temperature, substrate concentration, ethanol, glycerol, acidic compounds and contaminants. In this sense, the interaction of the carbon and nitrogen sources on metabolism of the yeast is an important factor...
for fermentative efficiency and consequently, it is necessary to know the metabolism of yeasts with biotechnological potential for ethanol production. Thus, the study aims to assess the profile of assimilation of amino acids present in must based on sugarcane juice by Saccharomyces cerevisiae industries under fermentative stress.

For identification and quantification of the amino acids, the standards (serine, threonine, alanine, valine, methionine, isoleucine and tryptophan) were analyzed to obtain the analytical curves, by external standard method, under the same conditions employed for the real samples. The data obtained in the analyses of the standards, at different concentrations, were used for the construction of analytical curves and to obtain the correlation coefficient, angular coefficient and linear coefficient values.

2.5 STATISTICAL ANALYSIS

To assess whether there are significant differences between the amino acid composition of the different groups of samples, discriminant analyses were applied using each amino acid concentrations identified in the samples. The value of Wilks lambda was used to indicate the separation of the groups, in which values close to 0 indicate that the groups are different from each other, while values close to 1 indicate high overlap of groups and, consequently no significant difference between them. In addition, we used the value of p < 0.05 for indication of significant differences.

3 RESULTS AND DISCUSSION

The analysis of consumption of amino acids profile by strains of *Saccharomyces cerevisiae* under fermentative stress, showed that the yeasts studied presented differences in relation to assimilation and to the concentration of these compounds at longer times of fermentation. Table 1 shows the amino acids present in must based on sugarcane juice.
Table 1 – Concentration of amino acids, present in must based on sugarcane juice under different fermentative conditions of strains Catanduva-1 and Red Star.

| Code | Serine | Threonine | Alanine | Methionine | Isoleucine | Tryptophan |
|------|--------|-----------|---------|------------|------------|------------|
| Temperature 30°C | | | | | | |
| 0 h | 11.00±0.04 | 5.87±0.09 | 10.95±0.01 | 9.15±0.02 | 8.30±0.01 | 3.40±0.00 | 4.06±0.01 |
| 20 h | 10.76±0.01 | 5.35±0.04 | 10.92±0.02 | 7.74±0.03 | 6.36±0.02 | 1.41±0.02 | 2.47±0.02 |
| 40 h | 10.56±0.01 | 5.25±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 60 h | 10.52±0.01 | 5.21±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 25°C Box | | | | | | |
| 0 h | 11.00±0.04 | 5.87±0.09 | 10.95±0.01 | 9.15±0.02 | 8.30±0.01 | 3.40±0.00 | 4.06±0.01 |
| 20 h | 10.76±0.01 | 5.35±0.04 | 10.92±0.02 | 7.74±0.03 | 6.36±0.02 | 1.41±0.02 | 2.47±0.02 |
| 40 h | 10.56±0.01 | 5.25±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 60 h | 10.52±0.01 | 5.21±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| Temperature 40°C | | | | | | |
| 18°C Box | | | | | | |
| 0 h | 11.00±0.04 | 5.87±0.09 | 10.95±0.01 | 9.15±0.02 | 8.30±0.01 | 3.40±0.00 | 4.06±0.01 |
| 20 h | 10.76±0.01 | 5.35±0.04 | 10.92±0.02 | 7.74±0.03 | 6.36±0.02 | 1.41±0.02 | 2.47±0.02 |
| 40 h | 10.56±0.01 | 5.25±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 60 h | 10.52±0.01 | 5.21±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 25°C Box | | | | | | |
| 0 h | 11.00±0.04 | 5.87±0.09 | 10.95±0.01 | 9.15±0.02 | 8.30±0.01 | 3.40±0.00 | 4.06±0.01 |
| 20 h | 10.76±0.01 | 5.35±0.04 | 10.92±0.02 | 7.74±0.03 | 6.36±0.02 | 1.41±0.02 | 2.47±0.02 |
| 40 h | 10.56±0.01 | 5.25±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
| 60 h | 10.52±0.01 | 5.21±0.01 | 10.90±0.02 | 7.36±0.03 | 6.06±0.02 | 1.28±0.02 | 2.05±0.02 |
In the fermentative processes the main compounds assimilated by the yeasts are the sources of carbon and nitrogen, which alter the metabolic flow of these microorganisms. In this study we were able to quantify the amino acids serine, threonine, alanine, valine, methionine, isoleucine and tryptophan present in must, among them the tryptophan stands out acting directly on the stress responses to high concentrations of substrates and tolerance to ethanol, constituting an important amino acid to be consumed by the yeasts. Proline has protective function to thermal and oxidative stress (BUTZKE and PARK, 2011). The free amino acids and ammonia provide most of the nitrogen source necessary for fermentation, however, the amount of free amino acids in must can vary depending on the sugarcane, time of maturation, harvest and age of the sugarcane.

The presence of free amino acids in the fermentative midst provides the yeast with less synthesis of these compounds, effectively contributing in cell viability resulting in greater fermentative efficiency (GUTIÉRREZ-RIVERA et al., 2015). The same yeast may have different fermentative performances, in relation to the composition of the substrate during the fermentation process (HERNÁNDEZ-CARBAJAL et al., 2013). Studies with must regarding the assimilation of carbon and nitrogen sources by yeasts, in alternating temperature ranges and substrate concentration, are necessary not only to understand the physiology and biochemistry during the fermentation process but also to differentiate these microorganisms (CARRILLO et al., 2012; WANG et al., 2012).

Among the biotechnological processes, fermentation has been reported as an important industrial process in which the yeast *Saccharomyces cerevisiae* has been used, an agent responsible for the production of breads, beverages and ethanol (PITT and HOCKING 2009). In industrial processes, several methods of monitoring are applied aimed at productivity. Similarly, different yeast strains can be applied in different industrial processes, due to their physiological characteristics in relation to biotechnological processes.

The data showed that there were similarities in the values of concentrations of amino acids in 3 independent experiments evaluated by mean and standard deviation. Therefore, to assess whether the concentrations of amino acids were affected by temperature and fermentation time for each of the yeast strains analyzed a discriminant analysis was applied (Figure 1). For the strain Catanduva-1 the discriminant analysis showed significant differences in the concentration of amino acids over time of fermentation (Wilks' lambda = 0.000; F = 53.028; p <= 0.0000), with significant separation into groups. Notably the variable temperature was not a determining factor, except for the time of 20 hours of fermentation, because there
was a separation of the groups. The first canonical root explained 90.7% of the groups separation and the second 0.9% and the significant amino acids for the separation were valine, methionine and serine (Figure 1). Similar behavior has occurred for the Red Star strain (Wilks’ lambda = 0.001; F = 41.936; p<= 0.0000), and the results showed significant difference once the ellipses of the dispersion diagram were separated according to the time of fermentation; in no time the temperature affected the performance of the amino acids in this strain of yeast. The first canonical root explained 97% of separation and the second 0.2%; the concentration of the following amino acids were responsible for this separation: alanine, threonine and tryptophan.

Figure 1 – Dispersion diagram of discriminant analyses showing the two canonicals roots of differentiation between fermentation times and temperatures in relation to the assimilation of the nitrogen sources of yeasts Red Star (A) and Catanduva-1(B), employing the concentrations of amino acids.

At the beginning of fermentation of grape must, wine yeasts use the nitrogen of ammonia salts for growth, and subsequently nitrogen of free amino acids. Among these, particularly, arginine, glutamic acid, glutamine, aspartic acid, asparagine, threonine and serine, are assimilated (KURTZMAN et al., 2015; PEREIRA et al., 2015). Thus, the biosynthesis of amino acids have important function such as tolerance to ethanol and cell viability rate according to Zhao and Bai (2009).
Studies on beer must, aiming to assess the interference of the free amino acid composition when supplemented with three commercial proteases (Neutrase, Flavorzyme and Protamex), and monitor the assimilation of free amino acids during the fermentation used the yeast Saccharomyces pastorianus. The data showed that the must supplemented with proteases affected the fermentation performance of the yeast, and the proteases Neutrase and Protamex propitiated the largest ethanol production and formation of volatile compounds. However the different processes of fermentation provided different profiles of assimilation of free amino acids, yeasts of high fermentation used leucine, arginine, phenylalanine, asparagine and valine, in normal fermentation were used lysine, leucine, arginine and histidine (MORENO-ARRIBAS and POLO, 2009).

In must of winery supplemented with 20 amino acids and testing wild yeast strains (W16, W34 and W35) and commercial (C5 and C11), obtained as a result, employing High Performance Liquid Chromatography (HPLC), that the commercial yeasts presented higher consumption of amino acids in relation to the wild (MORENO-ARRIBAS and POLO, 2009). In natural environments, the yeasts find a great diversity of sources of ammonia nitrogen, proline, arginine and nitrates, among others, which can be used for their metabolism and maintenance of cell integrity (LEI et al., 2013; SIMANCAS et al., 2015).

4 CONCLUSIONS

Analyzing the profile of assimilation of amino acids present in must based on sugarcane juice by Saccharomyces cerevisiae under fermentative stress, there were differences in relation to the concentrations of the amino acids. According to the discriminant analysis significant differences occurred in function of the fermentation time, except for the time of 20 hours of fermentation.

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REFERENCES

AZHAR, S. H. M.; ABDULLA, R.; JAMBO, S. A.; MARBAWI, H.; GANSAU, J. A.; FAIK, A. A. M.; RODRIGUES, K. F. Yeasts in sustainable bioethanol production: A review. Biochemistry and Biophysics Reports, (10):52-61, 2017.

BELLIDO, C.; BENITO, G. G.; COCA, M.; LUCAS, S.; CUBERO, M. T. G. Influence of aeration on bioethanol production from ozonized wheat straw hydrolysates using Pichia stipites. Bioresource Technology, (133):51-58, 2013.

BUTZKE, C. E.; PARK, S. K. Impact of Fermentation Rate Changes on Potential Hydrogen Sulfide Concentrations in Wine. Journal Microbiol Biotechnol, 21:519-524, 2011.

CARRILLO, E. P.; SALDIVAR, S. O. S.; HERNANDEZ, C. C.; CALLEJAS, M. L. C. Addition of protease during starch liquefaction affects free amino nitrogen, fusel alcohols and ethanol production of fermented maize and whole and decorticated sorghum mashes. Biochemical Engineering Journal, (67):1-9, 2012.

DELLA-DIANCA, B. E.; GOMBERT, A. K. Stress tolerance and growth physiology of yeast strains from the Brazilian fuel ethanol industry. Antonie van Leeuwenhoek, 104:1083-1095, 2013.

GUTIÉRREZ-RIVERA, B.; ORTIZ-MUÑIZ, B.; GÓMEZ-RODRÍGUEZ, J.; CÁRDENASCÁGAL, A.; GONZÁLEZ, J. M. D.; AGUILAR-USCANGA, M. G. Bioethanol production from hydrolyzed sugarcane bagasse supplemented with molasses “B” in a mixed yeast culture. Renewable Energy, (74):399-405, 2015.

HARI, S.; JEBITTA, R.; SIVARAMAN, K. Production and characterization of sugar cane juice powder. Journal of Sugarcane Research, 3(1):20-34, 2013.

HERNÁNDEZ-CARBAJAL, G.; RUTIAGA-QUIÑONES, O. M.; PÉREZ-SILVA, A.; SAUCEDO-CASTAÑEDA, G.; MEDEIROS, A.; SOCCOL, C. R.; SOTO-CRUZ, N. Ó.
Screening of native yeast from Agave duranguensis fermentation for isoamyl acetate production. Brazilian Archives of Biology and Technology, 56(3), 357-363, 2013.

ISMAIL, K. S. K.; SAKAMOTO, T.; HASUNUMA, T.; ZHAO, X. Q.; KONDO, A. Zinc, magnesium, and calcium ion supplementation confers tolerance to acetic acid stress in industrial Saccharomyces cerevisiae utilizing xylose. Biotechnology Journal, (9):1519-1525, 2014.

KURTZMAN, C. P.; FELL, J. W.; BOEKHOUT, T. (Eds). The yeasts: a taxonomic study. Amsterdam: Elsevier, ed, 5, 2011.

KURTZMAN, C. P.; MATEO, R. Q.; KOLECKA, A.; THEELEN, B.; ROBERT, V.; BOEKHOUT, T. Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways. FEMS Yeast Research, (15):1-17, 2015.

LEI, H.; ZHENG, L.; WANG, C.; ZHAO, H. Effects of worts treated with proteases on the assimilation of free amino acids and fermentation performance of lager yeast. International Journal of Food Microbiology, (161):76-83, 2013.

MA, M.; LIU, Z. L. Mechanisms of ethanol tolerance in Saccharomyces cerevisiae. Applied Microbiol Biotechnol, 87:829-845, 2010.

MARQUES, M. O.; MUTTON, M. A.; NOGUEIRA, T. A. R.; TASSO JÚNIOR, L. C.; NOGUEIRA, G. A.; BERNARDI, J. H. Tecnologias na agroindústria canavieira. Jaboticabal: FCAV, 9-16 2008.

MORENO-ARRIBAS, M. V.; POLO, M. C. Special wines production. Wine Chemistry and Biochemistry. Madrid: Springered. Springer-Verlag, Nova Iorque 2009, 59p.

NOGUEIRA, L. A. H.; CAPAZ, R. S. Biofuels in Brazil: Evolution, achievements and perspectives on food security. Global Food Security, (2):117-125, 2013.
PEREIRA, A. F.; SILVA, P. H. A.; PINHEIRO, P. F.; BRAGA, L. M.; BRAGA PINHEIRO, C. A. Adição de fontes de nitrogênio e de duas linhagens de levedura na fermentação alcoólica para produção de cachaça. *Revista de Engenharia Química e Química*, (1):45-59, 2015.

PITT, J. I.; HOCKING, A. D. Fungi and Food Spoilage. 3 ed. Springer Dordrecht, Heidelberg, London, New York, 2009.

SANTOS, E. F. S.; SCHAUTZ, L. C. A.; CARDOSO, C. A. L.; ERNANDES, J. R.; BATISTOTE, M. O efeito da complexidade estrutural da fonte de carbono e nitrogênio no desempenho fermentativo de leveduras indústrias. *Ciência e Natura*, 2:09-014, 2013.

SILVA, R. O. D.; CEREDA, M. P.; GOMES, E.; MARTINS, G. M.; PAGNOCCA, F. C.; SILVA, R. D. Selection of xilose-fermenting yeast strains. *Brazilian Archives of Biology and Technology*, 59, 2016.

SIMANCAS, N. B.; GIESE, E.; ARÉVALO-VILLENA, M.; ÚBEDA, J.; BRIONES, A. Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. *Food Chemistry*, 127: 441-446, 2015.

SOUSA, J. L. U.; MONTEIRO, R. A. B. Fatores Interferentes na Fermentação Alcoólica para a Produção de Etanol. *FAZU em Revista*, 2:100-107, 2012.

TÁVORA, F. L. História e Economia dos Biocombustíveis no Brasil. Centro de Estudos da Consultoria do Senado. 2011; p. 89.

WANG, K.; MAO, Z.; ZHANG, C.; ZHANG, J.; ZHANG, H.; TANG, L. Influence of nitrogen sources on ethanol fermentation in an integrated ethanol–methane fermentation system. *Bioresource Technology*, (120):206-211, 2012.

ZABED, H.; SAHU, J. N.; BOYCE, A. N.; FARUQ, G. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. *Renewable and Sustainable Energy Reviews*, (66):751-774, 2016.
ZHAO, X. Q.; BAI, F. W. Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. *Journal of Biotechnology*, (144):23-30, 2009.