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PRODUCTION OF NATURAL AROMA BY YEAST IN WASTEWATER OF CASSAVA STARCH INDUSTRY

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SIMONE M. M. OLIVEIRA[1], SIMONE D. GOMES[2], LUCIANE SENE[3], DIVAIR CHRIST[4], JULIA PIECHONTCOSKI[5]

ABSTRACT: 2-Phenylethanol (PE) is an aromatic alcohol with a characteristic odor of roses, widely used in food industry to modify certain aroma compositions in formulations with fruit, jam, pudding, and chewing gums, and also in cosmetic and fragrance industry. This compound occurs naturally in low concentrations in some essential oils from flowers and plants. An alternative to plants extraction are biotechnological processes. This study evaluated 2-phenylethanol's production in cultivation of *Saccharomyces cerevisiae* in cassava wastewater originated from starch industry. The substrate was supplemented with glucose and L-phenylalanine in order to obtain higher 2-phenylethanol concentrations and better efficiency in glucose/2-phenylethanol conversion. It was performed using Rotatable Center Composite Design and response surface analysis. Cultures were performed under aerobic conditions in a batch system in Erlenmeyer flasks containing 50 mL of medium in shaker at 150 rpm and 24 ± 1 ºC. The highest PE values were obtained with supplementation of 20.0 $g.L^{-1}$ of glucose and 5.5 $g.L^{-1}$ of L-phenylalanine, which has been experimentally validated, obtaining a PE production of 1.33 $g.L^{-1}$ and PE/glucose yield factor of 0.070 $g.g^{-1}$, equivalent to 74.3 and 89.7% of desirability values according to the validated model.

KEYWORDS: 2-phenylethanol, flavor, aerobic cultivation.

PRODUÇÃO DE AROMA NATURAL POR LEVEDURA EM ÁGUA RESIDUÁRIA DE INDÚSTRIA DE FÉCULA DE MANDIOCA

RESUMO: O 2-feniletanol (FE) é um álcool aromático com odor característico de rosas, amplamente utilizado na indústria alimentícia para modificar composições de aroma em formulações com frutas, geleias, pudins, gomas de mascar e também na indústria de cosméticos e perfumes. Este composto é encontrado naturalmente, em baixas concentrações, em óleos essenciais de algumas flores e plantas. Uma alternativa à extração de vegetais são os processos biotecnológicos. Neste trabalho, foi avaliada a produção de 2-feniletanol no cultivo de *Saccharomyces cerevisiae* em água residuária de indústria de fécula de mandioca. O substrato foi suplementado com glicose e L-fenilalanina, a fim de obter as condições de maior geração de 2--feniletanol e maior eficiência de conversão glicose/2-feniletanol. Foi realizada otimização através de planejamento composto central rotacional e de análise de superfície de resposta. Os cultivos foram mantidos em condições aeróbias, em processo em batelada, em frascos Erlenmeyer com capacidade para 250 mL, contendo 50 mL de meio, em agitador regulado a 150 rpm e 24±1ºC. Os maiores valores de FE foram alcançados com a suplementação de 20,0 $g.L^{-1}$ de glicose e de 5,5 $g.L^{-1}$ de L-fenilalanina, os quais foram validados experimentalmente, obtendo-se produção de FE de 1,33 $g.L^{-1}$ e fator de rendimento FE/glicose de 0,070 $g.g^{-1}$, que equivaleram a 74,3 e 89,7% dos valores de desejabilidade, segundo o modelo validado.

PALAVRAS-CHAVE: 2-Feniletanol, bioaroma, cultivo aerado.

[1] Farmacêutica, Aluna, UNIOESTE/CASCAVEL/CCET/PGEAGRI, Cascavel - PR Fone (45)9907-3500 - simone_meneoli@yahoo.com.br
[2] Engª Agrônoma, Prof. Doutor. UNIOESTE/CASCAVEL/CCET/PGEAGRI, Cascavel - PR - simone.gomes@unioeste.br
[3] Farmacêutica, Prof. Doutor, UNIOESTE/CASCAVEL/CCET/PGEAGRI, Cascavel - PR - luciane.sene@unioeste.br
[4] Engº Agrícola, Prof. Doutor, UNIOESTE/CASCAVEL/CCET/PGEAGRI, Cascavel - PR - divair.christ@unioeste.br
[5] Aluna, UNIOESTE/CASCAVEL/CCET/PGEAGRI, Cascavel - PR - piechontcoskiju@hotmail.com
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INTRODUCTION

2-Phenylethanol (PE) is an aromatic alcohol with odor similar to roses, widely used in cosmetics, perfume, and food (HUA et al., 2010). According to ZHU et al. (2011) it occurs naturally in essential oils of flowers as hyacinth, jasmine, lilies and daffodils, however, concentrations are too low to justify extraction (SENDOVSKI et al., 2010), with the exception of rose oil that contains up to 60% PE. High percentages of FE can be obtained by steam drag distillation and solvent extraction. PE can also be obtained chemically, but compound purification is expensive and generates impurities such as metallic chlorine (ETSCHMANN et al., 2002).

An alternative to PE extraction in vegetables are the biotechnological processes. The more efficient approach for 2-phenylethanol obtaining is through the conversion of L-phenylalanine into 2-phenylethanol by yeast, through Ehrlich pathway. In this way, occurs amino acid transformation into phenylpyruvate by enzymes action, then in phenyl acetaldehyde and finally in PE. Moreover, the aromatic compound can also be formed by the shikimate pathway. Glycolysis and Pentose phosphate pathway origin shikimate, wich changes to phenylpyruvate, then phenyl acetaldehyde and finally PE (ETSCHMANN et al., 2002). SAKAI et al. (2007) demonstrated that L-phenylalanine is more effective in 2-phenylethanol obtaining than its dextrorotatory isomer.

The cost of 2-phenylethanol obtained from flowers and plants essential oils approaches US$ 1.000/kg, while the chemically synthesized cost is around US$ 5/kg (KIM et al., 2014). One of the biggest impact factors in economic viability of aroma production compounds by microorganisms through fermentation is the cost of substrate preparation. Thus, an alternative would be agro-industrial waste use as raw material for this process, which could contribute to economic viability (BICAS *et al.*, 2010). The use of this waste can also assist in minimizing environmental problems arising from the high organic load (ALEXANDRINO et al., 2007; DAMASCENO et al., 2003). WANG et al. (2013) and LI et al. (2014) obtained PE with organic residues of tobacco and molasses, respectively.

Saccharomyces cerevisiae is an yeast easily found and used in ethanol production (FLORÊNCIO et al. 2013) and food industry (BELDA et al., 2014). BOONNOP et al. (2009) used *S. cerevisiae* to increase by-products nutritional value of cassava by fermentation processes. The yeast was used in PE production studies for present phenylpyruvate pathway which allows obtaining this type of alcohol (OLIVEIRA et al., 2013; MEI et al., 2009; SENDOVSKI et al., 2010).

An agro industrial waste of abundant production in the western region of Paraná comes from cassava starch industries. Most of starch industry that exists in Brazil are located in the States of Parana, Mato Grosso do Sul, São Paulo and Santa Catarina (ABAM, 2012).

Residual liquid of cassava processing (*Manihot esculenta* Crantz) is generated in large volumes and researchers have studied it in biotechnological processes, such as volatile compounds production (DAMASCENO et al., 2003), in treatment of tilapia slaughterhouse effluent (KUMMER et al., 2011) and 2-Phenylethanol production (OLIVEIRA et al., 2013).

Cassava wastewater of starch industry is a problem for environment due to high levels of organic compounds that participate in many fermentation processes and also contain high levels of cyanogenic glycosides (DAMASCENO et al., 2003). Hydrocyanic acid released by these glycosides presents a serious health hazard if ingested or inhaled, and can cause extreme cases of poisoning (CAGNON et al, 2002). Through this context this study aimed optimize 2-phenylethanol production by *Saccharomyces cerevisiae* using cassava wastewater obtained from a starch industry and check the interference of glucose and L-phenylalanine aminoacid in PE yield.

MATERIAL AND METHODS

Microorganism

The yeast used was *Saccharomyces cerevisiae*, obtained from Food Microbiology Laboratory of UNIOESTE which was submitted to replication in YMA medium (Yeast Malt Agar) containing increasing waste concentrations, up to 100%.

Substrate

The substrate used in the tests was cassava wastewater , collected in a starch industry located in the city of Toledo (PR). Samples were collected at the exit of the process, before the incoming at wastewater treatment system. The liquid waste was filtered on cotton fabric and stored at -15 ° C.

The wastewater was characterized regarding the parameters: pH, total and volatile suspended solids, COD (chemical oxygen demand), Total Kjeldahl nitrogen and Ammoniacal Nitrogen (APHA, 2012), and reducing sugar content (SOMOGY, 1945). Results are shown in Table 1.

TABLE 1. Wastewater composition obtained from a cassava starch industry.

Variable	Concentration
pH	5.1
	mg L^{-1}
Reducing sugars	570.0
Total Suspended Solids	3540.0
Volatile Suspended Solids	3140.0
COD	16088.0
Total Kjeldhal nitrogen	158.2
Ammoniacal nitrogen	14.4

Inoculum preparation

Inoculum preparation was performed using 50 ml of cassava wastewater, sterilized, inoculated with a pure culture handle, and incubated in 250 ml Erlenmeyer flask, shaked at 150 rpm and 28 °C for 24 hours. Subcultures were done, keeping volume proportion and inoculum transfer of 10% (v/v), in 500 mL and 1000 mL Erlenmeyer flask (DAMASCENO et al., 2003).

Determination of the inoculum concentration was performed by spectrophotometry correlating optical density at 600 nm with cell concentration (cells/mL) (DAMASCENO et al., 2003), standardizing the amount of inoculums cells between 5.7 to 8.9 x 10^8 cells.mL^{-1}.

Evaluation of 2-phenylethanol production

Stage 1 - Avaliation of carbon source and L-phenylalanine concentrations

The cultivation was performed aerobically, in a batch system in 250 mL Erlenmeyer flasks, containing 50 ml of medium in shaker set at 150 rpm and 24 ± 1 °C (DAMASCENO et al., 2003). Samples corresponding to the total volume of flask were collected at the end of 72 hours.

The following cultivations sequence was performed:

a) Initial modeling with experimental design, varying glucose and L-phenylalanine (PHE) amino acid levels. The following points were tested: 0; 25 and 50 $g.L^{-1}$ of glucose and 0; 1.5 and 3.0 $g.L^{-1}$ of PHE, with 3 repetitions at the centre point.

b) Based on PE production data it was made maximum inclination path using the points 30, 40, 50 and 60 $g.L^{-1}$ of glucose and 3.0; 4.5; 5.1 and 6.6 $g.L^{-1}$ of PHE in duplicate.

c) The treatment that produced more PE was placed as the centerpiece of a Rotatable Central Composite Design (RCCD), which evaluated the points: 36, 40, 50, 60 and 64 $g.L^{-1}$ of glucose and 3.0; 3.6; 5.1; 6.6 and 7.2 $g\ L^{-1}$ of PHE, with 3 repetitions at the centre point.

d) Again, the treatment of higher PE production was placed as the centerpiece of a new RCCD, which evaluated the points: 20.0; 31.6; 60.0; 88.4 and 100.0 g L^{-1} of glucose and 1.0; 1.9; 4.0; 6.1 and 7.0 g.L^{-1} of PHE, with 3 repetitions at the centre point.

These steps were performed evaluating graphically PE production, and glucose/2-phenylethanol conversion, in other words, specific 2-phenylethanol production according to glucose consumption. When the samples results showed that bioconversion efficiency began to decrease and there was no increase of PE concentration, it was applied desirability function, in which were obtained response surface and desirability graphics, indicating the best glucose and PHE concentrations for greater PE production and bioconversion.

Stage 2 – Validation

To validate the experimental results, the best condition for glucose and PHE obtained on stage 1 for PE production and factor glucose / 2-phenylethanol conversion (g.g^{-1}) was repeated to obtain kinetics of PE formation over time, under the same growing conditions, in triplicate, with sampling every 12 h, keeping the cultivation for 120 h.

Analytical Methods

Samples were centrifuged at 2200 rpm for 20 min. It was analyzed in supernatant the reducing sugar contents (SOMOGY, 1945), L-phenylalanine and 2-phenylethanol.

The volatile compounds contained in 5 ml of supernatant were extracted with 15 ml of dichloromethane, following the method by JANSSENS et al. (1988).

Identification and determination of 2-Phenylethanol

The PE determination was carried out on a Shimadzu GC 2010 gas chromatograph (GC) , equipped with flame ionization detector (FID) using fused silica capillary column Nukol 30m x 0.25 mm x 0.25 µm thick film, using hydrogen as carrier gas at a flow 5mL.min^{-1}, injector temperature of 250 ° C and detector temperature of 300 °C. The initial column temperature was 60 °C for 3 minutes, gradually increasing at 8°C per minute to 180 °C, where it remained for 5 minutes. The injected volume was 1 µL in split-injection system, at a ratio of 1/15.

Co-injection was performed with PE authentic standard (Sigma Aldrich, chromatographic pattern), which constituted an identification technique. The PE analysis and quantification were made by calculating calculating the compound peak areas based on PE concentrations, analyzed under the same conditions.

Determination of L-Phenylalanine

The L-phenylalanine determination was carried out in supernatant sample, filtered through 0,22µm filter, by HPLC (high-performance liquid chromatography), reverse phase column C18 (4,6nmx250nm, 100S-ODS, Shimadzu, Japan) equipped with UV/VIS SPD-10A detector (Shimadzu, Japan) at a wavelength of 250 nm. Mobile phase was composed by methanol and water solution (20/80, v/v) using isocratic method in a flow rate of 1 ml min^{-1}. As standard, it was used L-phenylalanine (Sigma Aldrich, chromatographic pattern). The L-phenylalanine concentrations were calculated from L-phenylalanine standard calibration curve, analyzed under identical conditions.

Statistical analysis

The statistical method used was full factorial design. In order to verify influence of glucose (GLI) and phenylalanine (PHE) independent variables in PE production , factorial designs were made (2^2) with three repetitions at the centre point, using the STATISTICAL software version 7.1 (Statsoft^{TM}) for analysis and obtaining surface response and contour plot, at the 5% significance level, to obtain the best glucose / PE yield.

RESULTS AND DISCUSSION

Initial planning: factorial $(2)^2$ and maximum inclination path

The factorial design $(2)^2$ was performed in seven assays randomly conducted varying glucose and PHE factors, which provide variations in the PE production (Table 2).

TABLE 2. Factorial design $(2)^2$ with levels (coded and real) and 2-phenylethanol (PE) production.

Assay	Factors		Variable response
	Glucose ($g\ L^{-1}$)	PHE ($g\ L^{-1}$)	PE Production ($g\ L^{-1}$)
1	+1 (50)	-1 (0)	0.04
2	-1 (0)	- 1 (0)	0.01
3	+1 (50)	+1 (3)	0.64
4	-1 (0)	+1 (3)	0.10
5	0 (25)	0 (1.5)	0.22
6	0 (25)	0 (1.5)	0.21
7	0 (25)	0 (1.5)	0.28

The linear regression model obtained with the process results (Table 2) shows that PE production in the studied system tends to increase with increasing amount of glucose and PHE (Equation 1), presenting $R^2 = 0.979$ and where was considered only p- value <0.05 factors. This fact was also observed by GARAVAGLIA et al. (2007).

$$PE = 0.21 + 0.14\ GLI + 0.17\ PHE + 0.13\ GLI*PHE \quad (1)$$

In this RCCD contour plot (Figure 2) it is observed that the best production value for PE, 0.64 $g.L^{-1}$, was obtained with glucose 50 $g.L^{-1}$ and PHE 3.0 $g.L^{-1}$. ESHCOL et al. (2009) used resistant and thermo tolerant *S. cerevisiae* chains and obtained 0.85 $g.L^{-1}$ of PE in shaken flasks.

The treatment with cassava wastewater without supplementation almost did not produced PE (Figure 2). Treatment with PHE and without glucose produced more PE than treatment with glucose and without PHE, demonstrating the importance of precursor presence to obtain PE. This fact was also observed by GARAVAGLIA et al. (2007) who with low PHE values obtained PE low concentrations and with high precursor values obtained 0.39 $g.L^{-1}$ of PE after 84h of cultivation.

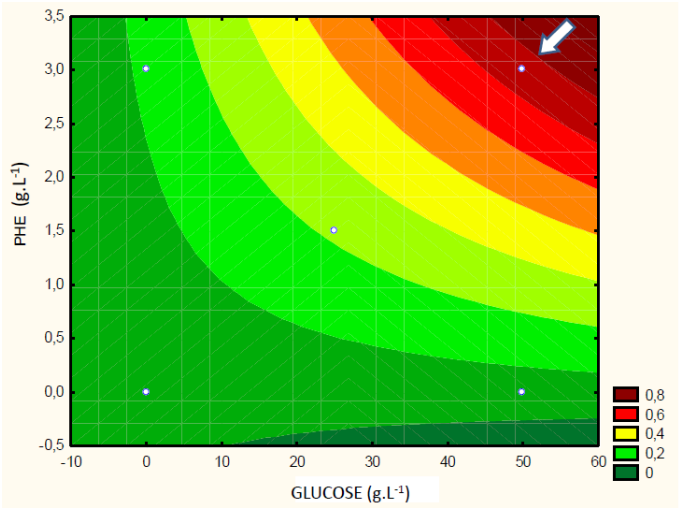

FIGURE 1. Contour plot showing PE production ($g\ L^{-1}$) as a function of glucose and L-phenylalanine concentration.

Aiming to expedite identification of the nearest point of PE greater production as a function of glucose and PHE concentration, it was made the Maximum inclination Path, which aims to reach optimal point faster and more economically.

The concentrations tested were 30, 40, 50 and 60 g L^{-1} of glucose and 3.0, 4.5, 5.1 and 6.6 g.L^{-1} of PHE, obtaining 0.35, 0.33, 1.20, and 0.71 g.L^{-1} of PE, respectively. The highest PE yield obtained was 1.20 g L^{-1}, using 50 g L^{-1} of glucose and 5.1 g L^{-1} of PHE, being this the center point of the following RCCD. Glucose and PHE concentrations were increased based on data of positive effects in increase on PE in initial planning (Figure 2).

Rotatable Central Composite Design

From the results obtained with the factorial design (2^2) and taking into account PE higher production to choose the center point, experimental matrix was formed for the first Rotatable central composed design (RCCD). It was also considered the greatest PE production from the first RCCD to form the second RCCD experimental matrix (Table 3).

TABLE 3. First and second Rotatable Central Composite Design (RCCD) with glucose and phenylalanine factors (coded and real) and 2-phenylethanol (PE) concentrations and PE yield concerning to glucose concentration (PE / glucose).

	1ºRCCD			
Assay	Glucose (GLU) (g.L^{-1})	L- Phenylalanine (g.L^{-1})	2- Phenylethanol (PE) (g.L^{-1})	Yield PE/GLU (g.g^{-1})
1	1 (60)	-1 (3.6)	2.33	0.042
2	-1 (40)	-1 (3.6)	0.77	0.019
3	1 (60)	1 (6.6)	0.70	0.012
4	-1 (40)	1 (6.6)	1.03	0.026
5	-1.414 (36)	0 (5.1)	0.95	0.026
6	+1.414 (64)	0 (5.1)	1.35	0.024
7	0 (50)	-1.414 (3.0)	0.69	0.014
8	0 (50)	+1.414(7.2)	0.96	0.019
9	0 (50)	0 (5.1)	1.19	0.024
10	0 (50)	0 (5.1)	1.20	0.024
11	0 (50)	0 (5.1)	1.17	0.023
Assay	2º RCCD			
1	-1 (31.6)	-1 (1.9)	1.38	0.043
2	1 (88.4)	-1 (1.9)	1.93	0.028
3	-1 (31.6)	1 (6.1)	1.99	0.063
4	1 (88.4)	1 (6.1)	2.41	0.029
5	-1.414 (20)	0 (4)	1.41	0.070
6	+1.414 (100)	0 (4)	1.95	0.025
7	0 (60)	-1.414 (1)	0.27	0.005
8	0 (60)	+1.414(7)	2.22	0.037
9	0 (60)	0 (4)	2.37	0.039
10	0 (60)	0 (4)	2.28	0.038
11	0 (60)	0 (4)	1.97	0.033

With the experimental results of PE production in the first RCCD, a regression model was obtained (Equation 2), demonstrating that PE production in the system studied tends to increase with increment of glucose concentration and decrease of PHE concentration. The coefficient of determination (R^2) of the obtained model was 0.744, relatively low, but is a characteristic of biological processes.

$$PE = 1.18 + 0.23\ GLI + 0.04\ GLI^2 - 0.12\ PHE - 0.12\ PHE^2 - 0.47\ GLI*PHE \quad (2)$$

The highest concentration of PE (2.33 g L^{-1}) was obtained with glucose 60 g.L^{-1} and PHE 3.6 g L^{-1} in the first RCCD (Figure 3a).

It is observed that there are two trends for obtaining higher PE production (Figure 3), one that uses high glucose and low PHE levels and the other that uses low glucose and high PHE values. It was opted for the first due to the higher PE values in this cultivation. Both trends can be explained by yeast biochemistry, which presentes two PE formation mechanisms, according to ETSCHMANN et al. (2002). The first is by Ehrlich Pathway, which uses PHE as raw material for PE formation, which should justify productivity with low glucose and high PHE values. The second is from phenylpyruvate, where PE can be produced by the Shikimate Pathway, without the participation of PHE (ETSCHMANN et al., 2002).

The results obtained in the contour plot with PE/glucose yield factors (Figure 3b) were similar to PE production, with higher glucose / 2-phenylethanol conversion rate (0.042 g g^{-1}), found at point 60.0 g.L^{-1} of glucose and 3.6 g.L^{-1} of PHE. In other words, the trend is to increase the conversion efficiency in the same direction of the increase and decrease of glucose and PHE concentration, respectively. Figure 3b also demonstrates the trend on increased bioconversion rate, although less significant, to low glucose concentration and high concentration of PHE, showing the use of Erlich Pathway for PE obtainment.

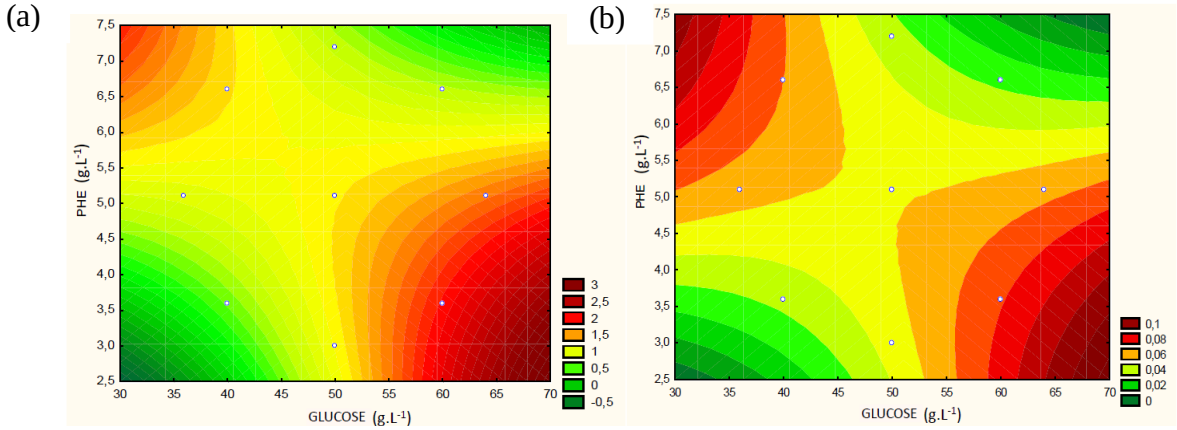

FIGURE 2. Contour plot of 1st RCCD with 2-phenylethanol (PE) production (g.L^{-1}) (a) and glucose/PE bioconversion (g.g^{-1}) (b) as a function of glucose and L-phenylalanine (PHE) concentration.

As the first RCCD has not reached the best conditions for PE obtainment, it was accomplished the second RCCD, whose experimental matrix has already been presented (Table 3).

With the experimental results of PE production of the second RCCD, was obtained a regression model (Equation 3) which demonstrates that the production of PE was favored with the highest PHE values. The linear model, shown in Equation 3, was predictive by F test and presented $R^2 = 0.776$.

$$PE = 2.21 + 0.48\ PHE \quad (3)$$

It was observed that PE higher concentration (2.42 g.L^{-1}) in the second RCCD was obtained with glucose 88.4 g.L^{-1} and PHE 6.1 g.L^{-1} (Figure 4a). This value is very close to the PE average production obtained at the centre point of the RCCD, which was 2.37 g.L^{-1} with the values of 60.0 and 4.0 g L^{-1} of glucose and PHE, respectively.

There was a trend towards increasing PE production with higher PHE values (Figure 4a), while increasing glucose amount had no effect. GARAVAGLIA et al. (2007) used *Kluyveromices marxianus*, and at optimized conditions, pH 7.0 and 3.0 g.L^{-1} of PHE, obtained 0.77 g L^{-1} of PE and

also noted that, when there was increased PHE consumption, there was also higher PE production.

In this cultivation, glucose/2-phenylethanol conversion presented a different contour plot (Figure 4b) of PE production, showing that bioconversion yield, valued by industrial sector and in which is wanted maximum yield without raw materials waste, did not increase with the same trend of PE production. The highest conversion PE/glucose yield was 0.070 $g.g^{-1}$ in cultures containing 20.0 $g.L^{-1}$ of glucose and 4.0 $g.L^{-1}$ of PHE.

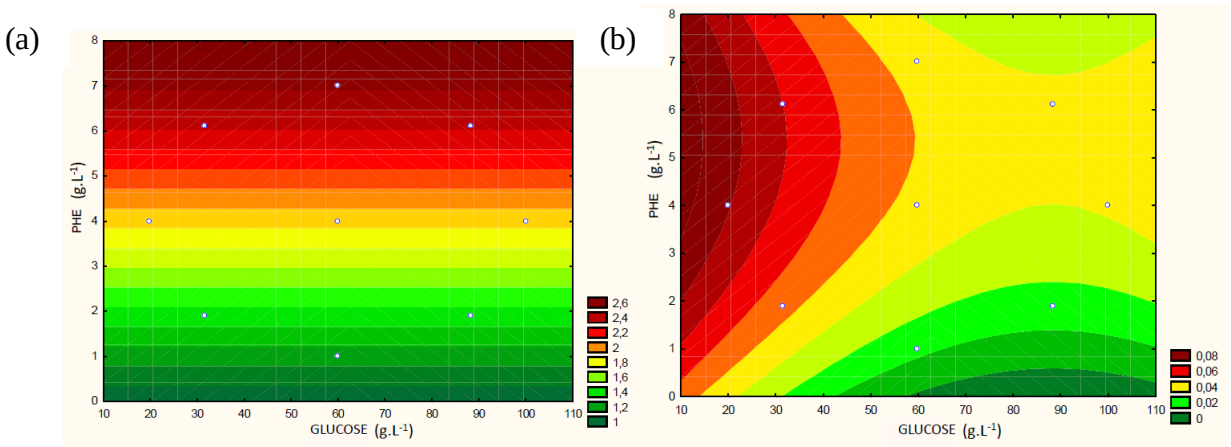

FIGURE 3. Contour plot of 2nd RCCD with 2-Phenylethanol (PE) production ($g.L^{-1}$) (a) and PE/glucose yield ($g.g^{-1}$) (b) as a function of glucose and L-phenylalanine (PHE) concentration.

Applying desirability function to maximize PE production and PE/glucose yield, it was obtained desirability graph shown in Figure 5.

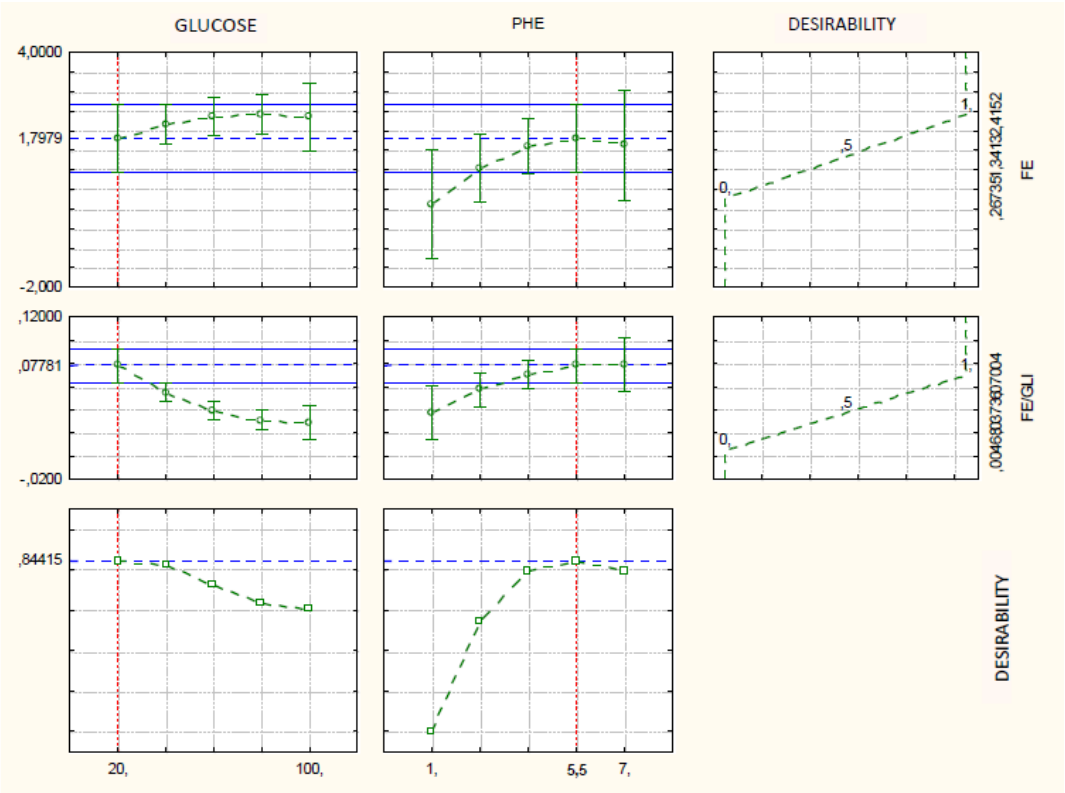

FIGURE 4. Total desirability index of 2-phenylethanol (PE) ($g.L^{-1}$) production and PE/glucose yield ($g.g^{-1}$), according to levels of glucose and L-phenylalanine (PHE).

This method is based on defining desirability function for each answer, with restricted values to the range between 0 and 1. Where zero is an unacceptable value and one is the most desirable value. Glucose points were selected (20.0 $g.L^{-1}$) and PHE (5.5 $g.L^{-1}$) to obtain the highest PE concentrations and PE/glucose yield, with an overall desirability of 84.4% of desired answers (Figure 6).

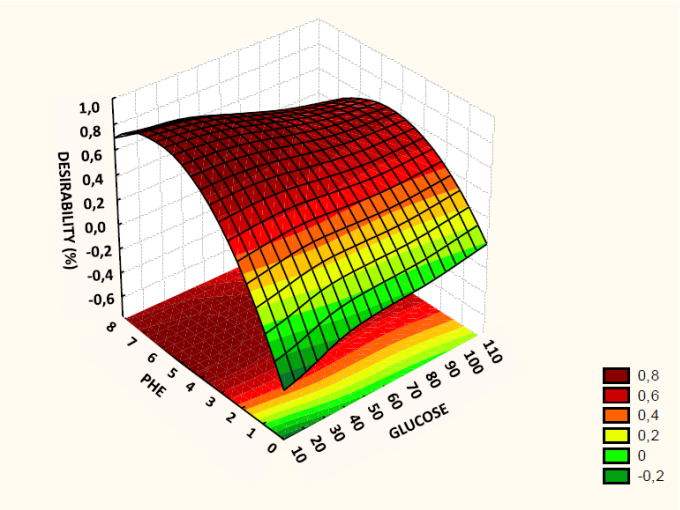

FIGURE 5. Response surface graphic of desirability obtained with 2-phenylethanol (PE) production data ($g.L^{-1}$) and PE/glucose yield factor ($g.g^{-1}$) as a function of glucose and L-phenylalanine (PHE) concentration.

The overall desirability indexes indicate that PE theoretical value to be obtained is 1.79 $g.L^{-1}$ and glucose/2-phenylethanol converted value, of 0.78. SANTOS (2011) applied desirability function in evaluation of the macaúba's (*Acrocomia aculeate*) coconut cake as an input for bioethanol production, generating desirability rates of hydrolysis efficiency of 92%, regarding the quantities of sulfuric acid and cellulase and amyloglucosidase enzymes.

Stage 2 - Validation

The conditions X_1 – Glucose of 20 $g.L^{-1}$ and X_2 - PHE of 5.5 $g.L^{-1}$ were validated, obtaining maximum PE concentration of 1.33 $g.L^{-1}$ (74.3% of the theoretical value of 1.79 $g.L^{-1}$), and higher yield factor of 0.070 $g.g^{-1}$ (89.7% of theoretical value 0.078 $g.g^{-1}$), both obtained at 72 h of cultivation (Figure 7).

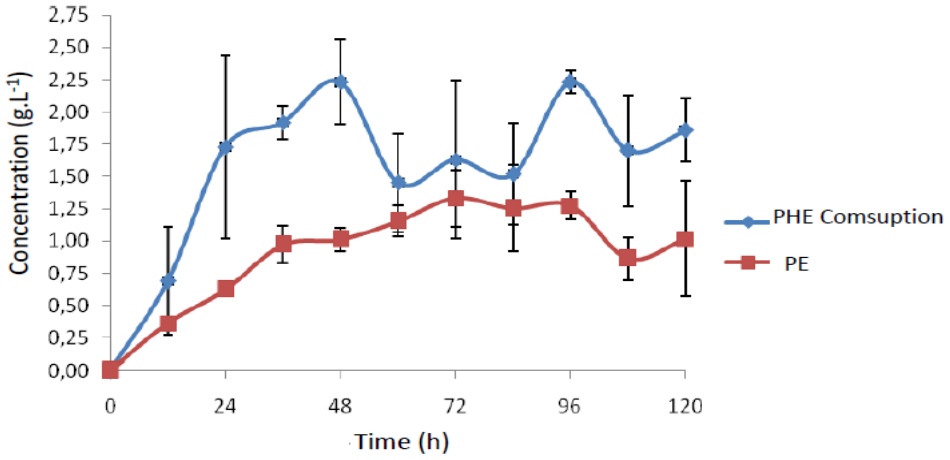

FIGURE 6. Model validation Graph of 2-phenylethanol (PE) production and L-phenylalanine consumption in function of time.

Phenilalanine consumption fluctuated between 30 and 40% of the total (5.5 $g.L^{-1}$). Consumption of PHE in 120 h was 1.77 $g\ L^{-1}$, thus had remained in medium a residual of 3.73 $g\ L^{-1}$ of PHE not consumed. This demonstrates that despite of PE production been increased in the presence of a higher amount of PHE, this was not completely consumed by the yeast. The amount of PE in medium may also be a limiting factor for a higher production. STARK et al. (2003) reported that there is a complete inhibition of yeast growth in the PE concentrations between 2 and 3 $g.L^{-1}$. This was outlined by MEI et al. (2009), which succeeded in obtaining 6.17 $g\ L^{-1}$ of PE, by addition of a macro porous resin responsible for adsorption of part of PE produced, reducing inhibition. MIHAL et al. (2012) also withdrew PE produced in medium that would act as a limiting factor of production using membrane separation technique, reaching a total of 7.1 $g.L^{-1}$ of PE, when used 15.0 $g.L^{-1}$ of PHE and 300.0 $g.L^{-1}$ of glucose.

Observing the results of fermentative parameters as productivity (Qp), biomass yield ($Y_{x/s}$) and product yield ($Y_{p/s}$) for *S. cerevisiae* (Table 4), it can be seen that the higher biomass yield occurred in 84 hours, while the highest product yield in 72 hours. However, the highest productivity was observed at 36 hours, declining over cultivation time. MEI et al. (2009) obtained the highest product yield in 24 hours of cultivation, producing 6.10 $g.L^{-1}$.

TABLE 4. Fermentative parameters of productivity (Q_p), biomass yield ($Y_{x/s}$) and product yield ($Y_{p/s}$) for *S. cerevisiae*.

Time (h)	Q_p ($g\ L^{-1}.h^{-1}$)	$Y_{x/s}$ ($g.g^{-1}$)	$Y_{p/s}$ ($g.g^{-1}$)
12	0.0301	0.0713	0.0189
24	0.0259	0.1516	0.0318
36	0.0270	0.1686	0.0477
48	0.0211	0.1980	0.0497
60	0.0192	0.2065	0.0567
72	0.0185	0.2022	0.0656
84	0.0149	0.2165	0.0618
96	0.0133	0.2037	0.0628
108	0.0080	0.2109	0.0424
120	0.0085	0.2096	0.0501

CONCLUSIONS

The study demonstrated that it is possible to produce PE by *S. cerevisiae* yeast from cassava wastewater of starch industry. However, supplementation of the medium with glucose and L-phenylalanine was needed to obtain the best PE yields. More studies are needed to improve the yield obtained avoiding medium saturation with PE produced itself, which can act as a limiting factor.

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