



Syrup production via enzymatic conversion of a byproduct (broken rice) from rice industry

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ABSTRACT. In a hydrothermal process for the gelatinization of the rice starch, it was studied the rate of hydration, temperature and time. An incomplete 3^3 factorial design was used to evaluate the effects of temperature, enzyme/substrate relation and liquefaction time, with dextrose equivalent (DE) as the response variable. The maximum value obtained was 14.69% DE at 89°C, with 0.21% (w/w) of enzyme to substrate in 22 minutes. For saccharification, it was studied enzyme/substrate ratios (0.6, 2.4, 4.2 and 6.0% (w/w)) at 60°C at a pH of 4.30, yielding 49.23, 69.65, 65.12 and 74.67% DE, respectively, after 72 hours. The syrup had a content of 12.78, 16.87, 15.96 and 17.87% of glucose (w/v), pH 2.60 - 2.71. The starch content converted into glucose after liquefaction was 61.80% and after saccharification ranged from 75.16 to 77.05% for the relation enzymes substrate tested.

Keyword: gelatinization, liquefaction, enzymatic hydrolysis, response surface methodology (RSM).

Produção de xarope por conversão enzimática a partir do coproduto (quirera) da indústria de arroz

RESUMO. No processo hidrotérmico para gelatinização do amido de arroz, estudou-se a taxa de hidratação, a temperatura e o tempo. Empregou-se um delineamento fatorial incompleto 3^3 para avaliar os efeitos da temperatura, relação enzima/substrato e tempo na liquefação, sendo a variável resposta medida em dextrose equivalente (DE). O valor máximo obtido foi de 14,69% DE para 89°C, 0,21% (m/m) de enzima por substrato, em 22 minutos. Para a sacarificação, foram estudadas relações de enzima/substrato (0,6; 2,4; 4,2; e 6,0% (m/m) a 60°C, em um pH de 4,30, obtendo-se de 49,23; 69,65; 65,12 e 74,67% DE, respectivamente, após 72 horas. Os xaropes obtidos apresentaram teores de 12,78; 16,87; 15,96 e 17,87% de glicose (m/v), pH de 2,60 a 2,71. O teor de amido convertido em glicose após a liquefação foi de 61,80% e após a sacarificação variou de 75,16 a 77,05% para as relações de enzima substrato testadas.

Palavras-chaves: gelatinização, liquefação, hidrólise enzimática, metodologia de superfície de resposta (MSR).

Introduction

Sugar and syrup production from rice starch hydrolysates using enzymes has increased in recent years. This syrup is widely used in foods, and may also be used as a source of fermentable sugar in beverage production. Rice syrup is a versatile sweetener and is considered healthy; it is widely used in food and beverages such as soft drinks, fruit drinks, breads, cakes, canned fruits, jams, breakfast cereals and bars as well as dairy products (Johnson, Padmaja, & Moorthy, 2009; Van Der Maarel & Leemhuis, 2013).

In Brazil, rice is a typical product of the domestic market, and only 5% of the national production is exported. The annual consumption per capita of rice in Brazil is approximately 25 kg. Projections of production and consumption of rice show that

Brazil will harvest 14.12 million tons of rice in 2019/2020, equivalent to an annual increase in production of 1.15% in the next ten years. Consumption should grow at an average annual rate of 0.86% to 14.37 million tons in 2019/2020 (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2015). For the crop year 2014/2015, data supplied until the month of September 2015 showed production of 12.449 million tons (Companhia Nacional de Abastecimento [CONAB], 2015).

The constituent parts of the rice grain are the hull, germ, pericarp, seed coat, aleurone and endosperm. To be consumed, the grain is subjected to a post-harvest beneficiation process and drying. The beneficiation process consists of stripping and polishing the grain, obtaining as byproducts of this

operation the bark, bran and broken rice. Depending on the processing conditions employed in beneficiation, this can be detrimental to the producer, since broken rice has a lower value, considering that the market demands whole grains (Carvalho et al., 2012; Chen, Wang, & Ouwerkerk, 2012).

Broken rice is formed by fragments of grains that are broken during the beneficiation process, with a size less than a $\frac{1}{4}$ of its original length; these grain fragments pass through a circular sieve with holes 1.6 mm in diameter. Studies have shown that this byproduct (broken rice) is composed to a large extent of residual endosperm, with high starch content, reaching values up to 88% (dry basis w/w). Literature data indicate that during the rice beneficiation process, on average 14% of the grains are broken and classified as broken rice. Broken rice is the most important co-product obtained from cereal beneficiation. It is an important raw material in fermentation industries such as breweries and distilleries of fine alcohols (Limberger, Silva, Emanuelli, Comarela, & Patias, 2008; Limberger et al., 2009; Carvalho et al., 2012).

The production of syrup with a high content of dextrose equivalent (DE) from hydrolyzed starch requires the application of amylolytic enzymes such as amylases (α -amylase (EC 3.2.2.1) and amyloglucosidase (EC 3.2.1.3). α -amylase is an endoamylase that hydrolyses 1,4- α -glycoside linkages of amylose and amylopectin chains, decomposing starch into soluble dextrans and oligosaccharides. Amyloglucosidase is an α -1-4 D exoglycosidase (glucoamylase). The enzyme catalyzes the hydrolysis of α -1-4 and α -1,6 starch linkages forming glucose from the non-reducing and of amylose and amylopectin chains (Tawil, Viksø-Nielsen, Rolland-Sabaté, Colonna, & Buléon, 2012; Wani et al., 2012; Foresti, Williams, García, & Vázquez, 2014).

Taking into account the production viewpoint and the amount of byproduct generated, this study aimed at adding value to fragments from the rice industry by producing syrup with a high DE content by starch hydrolysis with amylolytic enzymes.

Material and methods

Feedstock materials

Broken rice (*Oryza sativa* L.), a byproduct of rice milling, were used as the raw material. The enzymes used for the hydrolysis of starch were α -amylase (Termamyl 120L) and amyloglucosidase (AMG 200L), provided by Novozymes® (Denmark). Termamyl® 120L is thermostable, produced by a

selected strain of *Bacillus licheniformis*. Amyloglucosidase (AMG 200L) was obtained by submerged fermentation from a selected strain of *Aspergillus niger*.

Determination of physicochemical and microbiological characteristics of broken rice

The broken rice were characterized for their particle size, moisture, protein, ether extract, ash, fiber and starch according to the official methodology of the Association of Official Analytical Chemists (AOAC, 2005). Amylose and amylopectin were assessed according to the methodology of the American Association of Cereal Chemists (AACC, 2001). The determination of the mineral content was performed by nitroperchloric digestion of the ashes and the minerals contained in the sample were determined by atomic absorption spectrometry (AAS; Perkin-Elmer® 2380, USA) using an air-acetylene flame. The total count of strict and facultative aerobic microorganisms and viable fungi was performed in all the necessary steps according compendium of methods for the microbiological examination of foods of American Public Health Association (APHA, 2001).

Determination of enzyme activity

α -Amylase (Termamyl 120L)

The determination of the units of bacterial α -amylase was based on the time required to obtain a degree of pattern hydrolysis with a starch solution 2% at $30 \pm 0.1^\circ\text{C}$. A Bacterial Amylase Unit (BAU) is defined as the amount of enzyme that dextrinizes 1 mg of starch per minute, under standard conditions, according to the manufacturer's recommendation.

Amyloglucosidase (AMG 200L)

The determination of the activity of amyloglucosidase was based on the conversion of starch to dextrose by an enzymatic essay, in which dextrose is quantitatively determined by the Somogyi-Nelson method. One amyloglucosidase unit (AGU) is defined as the amount of enzyme which produces 1 g of dextrose from starch in 1 hour, according to the manufacturer's recommendation.

Specific Enzyme Activity

The specific enzyme activity was found through the ratio between enzyme activity and milligrams of protein. The protein content of Termamyl 120L and AMG 200L enzymes were determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

Hydrothermal process for maceration of rice

The material was milled in a multi-purpose laboratory mill (TECNAL®, Brazil) and run through a 60 mesh sieve. Samples with 20% (w/w) solids were macerated with distilled water and kept for 24 hours at temperatures of 20, 50, 60, and 70°C. The steep water was also subjected to these temperatures before being added. The rate of water absorption was measured as grams of water absorbed per gram of solid at certain time points, i.e. every 5 minutes for 20 minutes, then every 10 minutes for the first hour, and finally in 24 hours.

Enzymatic hydrolysis

Experimental method for liquefaction with α -Amylase (EC 3.2.2.1)

Broken rice milled flour was weighed out (5.68 g), corresponding to 5 g d.b. and added to 15.95 g of a salt solution (9.36 g NaCl, 69.00 g KH_2PO_4 and 4.80 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1 L; pH 5.2), according to the manufacturer's recommendation. The total volume was of 20 mL. This paste was macerated for 15 minutes at 70°C, based on previous results (section Hydrothermal Process for Maceration of Rice), in a Dubnoff water bath with orbital shaking (TECNAL®, Brazil).

A weighed amount of α -amylase (Termamyl 120) was diluted in 0.43 M CaCl_2 (pH 7.0), according to the manufacturer's recommendation, so that the desired amount of enzyme was present in 5 mL of this solution, in accordance with the levels of variation of the experimental design (Table 3). 20 mL of the flour dispersion was added to 5 mL of the enzyme solution. Thus, the resulting paste had a 20% concentration of dry solids. The paste were homogenized, brought back to the Dubnoff water bath and then kept under constant agitation. At the end of hydrolysis, samples were quickly removed from the Dubnoff bath and immersed in an ice water bath. Then, 1 g of liquefied broken rice flour paste was added to 100 mL of ice-cold distilled water. Samples were diluted when necessary and reducing sugars were determined by the methodology recommended by Somogyi-Nelson. The determination of the pH of the rice flour paste was performed before and after the liquefaction process.

Experimental Design

To optimize the liquefaction process, an incomplete factorial Box-Behnken design was used (3^3), containing 15 experiments with three replications at the center point. Each independent variable (X_1 , X_2 and X_3) was transformed into coded variables (x_1 , x_2 and x_3) (Table 3). The independent

variables studied in the response surface methodology (RSM) were: temperature (X_1), the enzyme-substrate ratio (X_2) and time (X_3) analyzed at three equidistant levels of variation and coded as -1, 0 and +1, using as the dependent variable (Y) dextrose equivalent (DE). Independent parameters, as well as the focal point and levels, were based on preliminary experimental data and the literature (Kolusheva & Marinova, 2007). The optimization of the conditions, the regression coefficients and analysis of variance (ANOVA) were determined by the software Statistica v.9.0 (Statsoft®, USA).

Mathematical model

The mathematical model employed is shown in Equation (1):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \beta_{ii} X_i^2 \quad (1)$$

where Y is the response variable (%DE) and the terms β_0 , β_i , β_{ii} and β_{ij} are the coefficients of the determined regression equation. X_i and X_j are values of the factors codified to x_i and x_j .

Evaluation of the extent of liquefaction

The extent of liquefaction was evaluated by the amount of reducing sugars produced, such as glucose, expressed in DE, which represents a measure of reducing sugars present in the product as a percentage of the total dry substances (Lin et al., 2013). The content of hydrolysis DE in each test was obtained by subtracting the DE of the sample from the DE of raw material.

The starch content was determined in the liquefied paste under the optimal conditions for the evaluation of the extent of its conversion. The starch fraction in the paste, at the optimal point coordinates obtained by RSM, was obtained by centrifuging the suspension for 15 minutes at 3,000 g, thereby separating the supernatant and the paste. The paste was treated with successive washes with different solvents and centrifuged for 5 minutes at 3,000 g. The solvents used and their sequence of treatment were: 1) distilled water; 2) ethanol; 3) petroleum ether; 4) 0.15% NaOH; 5) distilled water. The last wash was performed only when the absence of alkalinity was determined and sugar was qualitatively tested. Then, the paste was dried in an oven with forced air circulation at 40°C and crushed, producing flour. The starch content of the flour was evaluated as prescribed by AOAC methods (2005). In the remaining steps in which the resulting starch content was evaluated after enzymatic treatment, the same procedure was used.

Saccharification of the liquefied paste with amyloglucosidase (EC 3.2.1.3.)

Starting from the optimal point determined by the RSM coordinates, tests were conducted to study saccharification behavior with amyloglucosidase (AMG 200L) at 60°C and pH 4.3, as indicated by the manufacturer's ideal working conditions, the pH was adjusted with phosphoric acid 6 M. The enzyme to substrate ratios in which the saccharification experiments were conducted were 0.6, 2.4, 4.2 and 6.0% (w/w). The extent of saccharification was evaluated by assessing glucose production over time. The determination of glucose was performed spectrophotometrically using an enzymatic method (GOD-PAP, Biolabo®, France). The results are expressed in terms of the content of hydrolysis DE, done by subtracting this value from DE at the maximum point of liquefaction.

The determination of starch after saccharification of the liquefied paste was performed under the studied conditions (section Evaluation of the Extent of Liquefaction). The syrup was characterized by its pH and glucose.

Scanning electron microscopy (SEM)

The starch rice was observed under SEM for both control and hydrolyzed starch. Starch granules were mounted on aluminum specimen stubs with double-sided adhesive tape, with a 20-30 nm layer of gold in a KSE 24M high vacuum evaporator and scanned in a scanning electron microscope (LEO 435VP, England) was used, at an accelerating voltage of 10 kV and the selected regions depicting distinct morphological features were photographed for taking micrographs of specimens before and after their fracture.

Results and discussion

Characterization of raw materials

The particle size analysis of the broken rice showed 63.40% (w/w) of particles with dimensions larger than 1.0 mm, 36.19% between 0.5 and 1.0 mm and 0.41% less than 0.500 mm in size. The results of chemical analysis of the broken rice, expressed on a dry basis (88.93 of dry solids) are shown in Table 1. The component present in the highest proportion in the raw material was starch. The amylose fraction (21) and amylopectin (79) are consistent with data from Limberger et al. (2008) and Lin et al. (2013). Taking into account the ether extract, fiber and ash, it can be assumed the presence of the rice embryo and pieces of hull in the studied product.

Table 1. Composition of the rice byproduct.

Composition ^A	% (w/w)	mg g ⁻¹
Starch	81.13 (± 1.03)	-
Ash	1.65 (± 0.21)	-
Ether extract	2.21 (± 0.06)	-
Total fiber	0.60 (± 0.17)	-
Protein	9.65 (± 0.08)	-
Dry solids	88.93 (± 0.63)	-
Humidity	11.07 (± 0.63)	-
Nitrogen	1.14 (± 0.01)	-
Calcium	-	446.70 (± 14.21)
Phosphorus	0.36 (± 0.09)	-
Magnesium	-	1340.10 (± 127.13)
Potassium	0.26 (± 0.09)	-
Silica	-	317.10 (± 0.05)
Boron	3.60 (± 0.01)	-
Copper	-	12.40 (± 5.57)
Sulfur	-	900.50 (± 85.56)
Iron	-	126.20 (± 23.04)
Manganese	23.80 (± 6.52)	-
Sodium	-	173.80 (± 10.61)
Zinc	24.60 (± 1.69)	-

^A triplicate values.

The composition of minerals in rice and, consequently, in the broken rice, presents a wider range of variation of the values when compared to the literature; however, these data may be regarded as coherent, because the values of minerals are directly related to different compositions of nutrients present in the soil where the rice was grown (Limberger et al., 2009; Carvalho et al., 2012; Surendiran, Alsaif, Kapourchali, & Moghadasian, 2014).

For the total count of strict aerobic and facultative viable microorganisms, the raw material presented an estimated value of 3.91 e 1.70 log UFC g⁻¹ for fungi.

Enzymes

Table 2 shows the results for the enzymatic activity of α -amylase (Termamyl 120L) and amyloglucosidase (AMG 200L). The enzymes maintained their biocatalytic activity unchanged throughout the experimental work.

Table 2. Enzymatic activity.

Enzymes	^A BAU g ⁻¹ ^B UGA g ⁻¹	Protein (mg g ⁻¹ enzyme)	Specific activity ^A BAU mg ⁻¹ protein ^B UGA g ⁻¹ of protein
α -Amylase	7843.14 ^A	31.5	249 ^A
Amyloglucosidase	133 ^B	175	703 ^B

^A Bacterial Amylase Unit (BAU); ^B Amyloglucosidase Unit (UGA).

The standard count of microorganisms and fungi was always lower than that recommended by the Food and Agriculture Organization (FAO) specifications for food enzymes.

Maceration Rice

The equilibrium moisture content of the broken rice in 20% solids, which represents the hydration level of rice at a temperature of 20°C, absorbed

water rapidly after 10 minutes and reached a plateau value of 0.24 w/w of solid. It appears that this level, besides being influenced by the amount of amylose and gelatinization temperature, was also affected by the silica (317.10 mg g⁻¹) content of the rice.

The results of hydration at temperatures of 50 and 60°C for 24h were 0.40 and 0.42 w/w respectively. At the temperature of 70°C showed an increase in hydration of 0.12 to 0.74 w/w after 24 hours. This increase in moisture at 70°C can likely be attributed to the gelatinization of the starch. The hydration behavior of rice is seen as an important quality parameter for cooking. It is expressed in terms of grams of water absorbed per gram of rice for a certain cooking time, and may be further defined as the swelling ratio.

Studies have shown that the water absorbed by the rice during cooking is better explained by the proportionality of the surface area of the grain per unit mass (as a function of size and shape) than for amylose. The swelling and solubility of rice flour are also properties related to hydration (Sowbhagya, Ramesh, & Ali, 1994). The time required for maceration varies with temperature, cultivar and the degree of polishing of the raw materials, justifying our assessment of the performance of broken rice in terms of these parameters (Lai, Chang, & Luh, 1980).

The ratio employed was 20% (w/w) total solids of broken rice and mashing at 70°C for 15 minutes, yielding an absorption of 0.43 g water per g of solid. It is known that the value of 0.40 g of water per g of solid, after mashing, is sufficient for the complete gelatinization of rice starch when subsequently cooked (Oyashiki, Uchida, Hanai, Obayashi, & Oka, 1987; Briffaz, Mestres, Escoute, Lartaud, & Dornier, 2012). Thus, the conditions to perform the maceration of the broken rice were determined, and the broken rice was subsequently subjected to enzymatic hydrolysis.

Enzymatic Hydrolysis

Liquefaction – Experimental Design

The results of the experiment based on the experimental design for the liquefaction of starch in broken rice are shown in Table 3.

The mathematical statistical quadratic model, which represents the response function, is equivalent to Equation (2). In the canonical equation fitted to the experimental data, the dependent variable Y is the % of DE, for which significant terms at the 5% level are indicated by an asterisk.

Table 3. Uncoded and coded levels of independent variables used in the RSM design for enzymatic hydrolysis – liquefaction.

Independent variables	Symbol	Levels coded		
		-1	0	+1
Temperature (°C)	X_1	85	90	95
Relation E/S (% w/w)	X_2	0.05	0.15	0.25
Time (minutes)	X_3	10	20	30
Experiments	x_1	x_2	x_3	Y (% DE)
1	-1	-1	0	2.71
2	+1	-1	0	1.44
3	-1	+1	0	10.58
4	+1	+1	0	4.91
5	-1	0	-1	8.16
6	+1	0	-1	2.83
7	-1	0	+1	8.60
8	+1	0	+1	2.83
9	0	-1	-1	5.06
10	0	+1	-1	10.52
11	0	-1	+1	7.74
12	0	+1	+1	12.88
13 ^A	0	0	0	13.48
14 ^A	0	0	0	13.48
15 ^A	0	0	0	13.48

^A Central point.

$$\begin{aligned}
 Y = & 13.480^* - 2.255^* x_1 + 2.742^* x_2 + 0.685 x_3 \\
 & \pm 0.57 \quad \pm 0.35 \quad \pm 0.35 \quad \pm 0.35 \\
 & - 6.007^* x_1^2 - 2.562^* x_2^2 - 1.867^* x_3^2 - 1.100 x_1 x_2 \\
 & \pm 0.51 \quad \pm 0.51 \quad \pm 0.51 \quad \pm 0.49 \quad (2) \\
 & - 0.110 x_1 x_3 - 0.080 x_2 x_3 \\
 & \pm 0.49 \quad \pm 0.49
 \end{aligned}$$

According to Equation (2), all linear and quadratic terms were significant ($p < 0.015$), except the terms x_3 linear (time) and interactions terms. Among the significant terms, only linear x_2 (enzyme/substrate ratio) positively influenced the response DE, while the other terms influenced it negatively.

The quality of the equation was verified through ANOVA (Table 4), in which the model was significant at the 5% level, with experimental and adjusted coefficients of determination equal to 98.19 and 94.94%, respectively, and a significant regression deviation. However, Waszczynskyj, Rao, and Silva (1981) suggest that the test of lack of fit may be considered irrelevant when the mean square of the experimental error is extremely low, which occurred in the present work, confirming the validity of the model for predictive analytics.

Table 4. ANOVA for the response variable DE.

	Degrees of freedom (df)	Sum of Squares	Mean Square	F-value	p-value
Regression	9	265.043	29.449	30.194	< 0.001
Linear	3	104.604	34.868	35.750	0.008
Square	3	155.525	51.841	53.153	< 0.001
Interaction	3	4.914	1.638	1.679	0.285
Lack of fit	3	4.876	1.625	2.34 x 10 ¹³	0.000
Pure error	2	1.39 x 10 ⁻¹³	6.96 x 10 ⁻¹⁴		
Total	14	269.920			

$$R^2 = 0.9819; R^2_{(Adj)} = 0.9494$$

The three-dimensional response surface for the canonical Equation (2) demonstrates that the optimal value of DE in the process occurs when applying a temperature of 88.79°C, with an enzyme substrate ratio of 0.208 (% w/w) and a time of 21.78 min., indicating a maximum estimated response of 14.47% (Figure 1). The hydrolysis of the starch is not stable, and a DE below 13% occurs with the formation of insoluble starch particles, which are removed by filtration (BeMiller & Whistler, 2009).

The optimum conditions shown in Figure 1 were experimentally validated in triplicate, under the following conditions: temperature of 89°C, enzyme/substrate ratio of 0.21% (w/w) and time of 22 min., yielding an average value of 14.69% DE. The *t*-test for single samples applied to the average showed no significant difference between the average value obtained experimentally and that estimated by the model of 14.47% DE, with a *p*-value of 8.81%. Moreover, Levene's test showed that the condition of homogeneity of variance can be accepted because the *p*-value was equal to 32.87%.

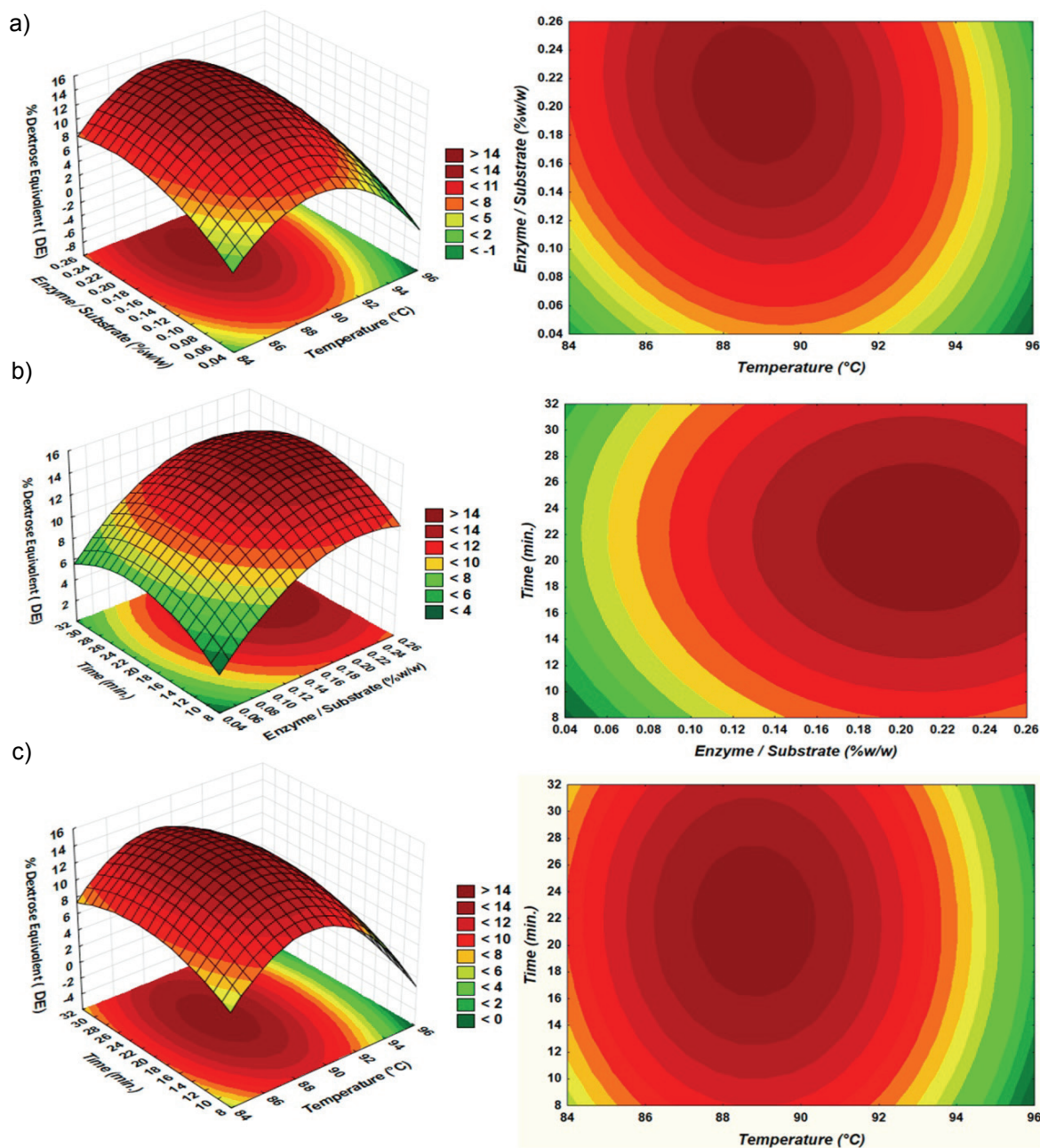


Figure 1. Response surfaces and contour plots for the % DE (Y) with the independent variables: (a) temperature (X_1) vs. enzyme/substrate ratio (X_2), with time (X_3) fixed at its optimum; (b) time (X_3) vs. enzyme/substrate ratio (X_2), with temperature (X_1) fixed at its optimum; (c) temperature (X_1) vs. time (X_3), with enzyme/substrate ratio (X_2) fixed at its optimum.

The parameters determined in the experimental design reduced the viscosity of the suspension. The pH was 5.62 in the broken rice flour paste; after the liquefaction process, the pH was 5.72. The aim of the liquefaction process is to convert a suspension of starch to dextrins and reduce the viscosity, thus to facilitate the conversion to glucose through amyloglucosidase.

After the gelatinizing process, α -amylase increased the rate of hydrolysis, in a multiple attack system where part of the chain is released and part remains complexed with the enzyme when multiple α -1,4 bonds are hydrolyzed until dissociation of the enzyme's active site (Silva, Quintino, Monteiro, & Asquiere, 2010).

As shown in Figure 1, with the time determined as ideal, there was a decrease in DE, possibly due to saturation of the enzyme's active site. According to Kolusheva and Marinova (2007), the extent of starch hydrolysis is inhibited with an increase in the concentration of reducing sugars in the medium. The increase in temperature also led to decrease in DE probably due to denaturation of the enzyme's active site. Amylases of fungal origin generally have reduced activity at temperatures higher than 50°C, although, in the presence of an excess of calcium ions deactivation can be decreased (Soares et al., 2010).

It is well known that, although the action of α -amylase is limited to breaking the α -1,4 bonds of amylose and amylopectin, different saccharides and dextrins are formed, such as maltoheptoses, maltohexoses, maltopentoses, maltotrioses, maltose and glucose therefore, the DE was less than 30% (Ruiz, Sanchez, Torres, & Molina, 2011). The DE value of 14.69% represents, approximately, 50% of the maximum value cited by Ruiz et al. (2011) and higher than the DE value of 13% found by BeMiller and Whistler (2009). The total count of aerobic strict and facultative viable microorganisms in the suspension after liquefaction was 2.60 log UFC mL⁻¹, with no fungal growth.

Saccharification

The results obtained for saccharification, using the conditions (section Saccharification of the Liquefied Paste with Amyloglucosidase (EC 3.2.1.3.)) previously defined for the liquefaction and mashing steps are shown in Figure 2.

The enzyme/substrate relation that reached the maximum values of DE and glucose in the smallest time of hydrolysis were: 0.6% - 49.23% DE, 12.78% of glucose after 60h; 2.4% - 69.65% DE, 16.87% glucose after 68h; 4.2% - 65.12% DE, 15.96% of glucose after 71h; 6.0% - 74.67% DE, 17.87% of glucose after 65h (Figure 2).

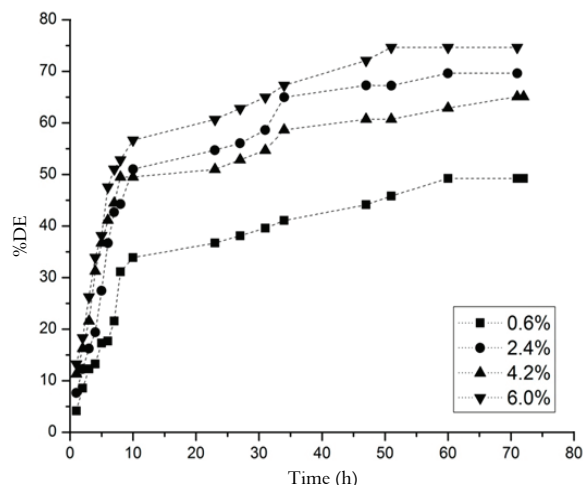


Figure 2. Extension of the saccharification of enzymatic reactions in different relation enzyme/substrate.

The values found for the rate, with the numbers of observations used to describe the linearity of the saccharification progress curve, and the coefficients of determination (R^2) and variation coefficient (VC), are given in Table 5.

Table 5. Initial rate of the reaction under the studied saccharification conditions.

Number of observations	E/S (% w/w)	IR ^A	R ²	VC
8	0.6	3.42	0.9822	8.5171
6	2.4	5.55	0.9501	12.1613
8	4.2	5.62	0.9840	6.0050
6	6.0	6.82	0.9926	4.1577

^A Initial rate: (g.glc) / (g broken rice flour x h).

Based on mass balance, the initial starch content of the raw material was 81.13% and, after liquefaction, it reached a value of 30.99%, in the separate liquefied paste from syrup. This means that 61.80% of the initial amount of starch was converted it. The starch content after each of the studied processes and the conversion to the initial content in the broken rice flour are presented in Table 6.

Table 6. Starch content and conversion rate after the liquefaction and saccharification steps.

	Starch content (%)	Conversion (%)
Broken rice	81.13	-
Liquefaction	30.99	61.80
Saccharification with 0.6% (w/w)	18.62	77.05
Saccharification with 2.4% (w/w)	19.11	76.45
Saccharification with 4.2% (w/w)	20.15	75.16
Saccharification with 6.0% (w/w)	19.91	75.46

The results affecting the velocity (Table 5) and the extension of enzymatic starch hydrolysis (Table 6) can be attributed to various factors such as the granule morphology, the relation amylose amylopectin, crystallinity, degree of gelatinization,

particle size, the extension of retrogradation, the thermal deterioration of the starch to the low moisture content or presence of other components that interact with starch, as the complex amylose/lipid and interactions starch/protein (Leloup, Colonna, & Ring, 1992).

Morphology of starch rice

The SEM micrographs of control starch without the addition of enzyme and hydrolyzed starch with the addition of enzyme α -amylase and amyloglicosidase are shown in Figure 3. The susceptibility of the starch granules can be classified by the intensity and the way in which the granules are eroded or corroded. The control rice starch granules had an irregular shape, probably due to the beneficiation process that rice is subjected; a smooth surface is observed with grain sizes of 1-20 μm . After hydrolysis, there was a noticeable morphological change in grain structure, with highly fragmented topography, high porosity and depth of

the pores when compared to the control, demonstrating the enzymatic activity over the starch granules, with several smaller fragments. Thus, it can be inferred that the degree of starch digestion is greater in the hydrolyzed sample, because of larger surface area (Mohan, Gopal, Malleshi, & Tharanathan, 2005; Uthumporn, Zaidul, & Karim, 2010).

Syrup

The Table 7 shows the glucose contents of the syrups obtained after 72 hours of saccharification, in the enzyme/substrate relations evaluated, under optimal conditions of maceration and liquefaction determined in the previous sections.

Table 7. Glucose, DE and pH after 72 hours of saccharification, in the different ratios E/S.

E/S (% w/w)	Glucose (% w/v)	DE (% w/w)	pH
0.6	12.78	49.23	2.60
2.4	16.87	69.65	2.67
4.2	15.96	65.12	2.66
6.0	17.87	74.67	2.71

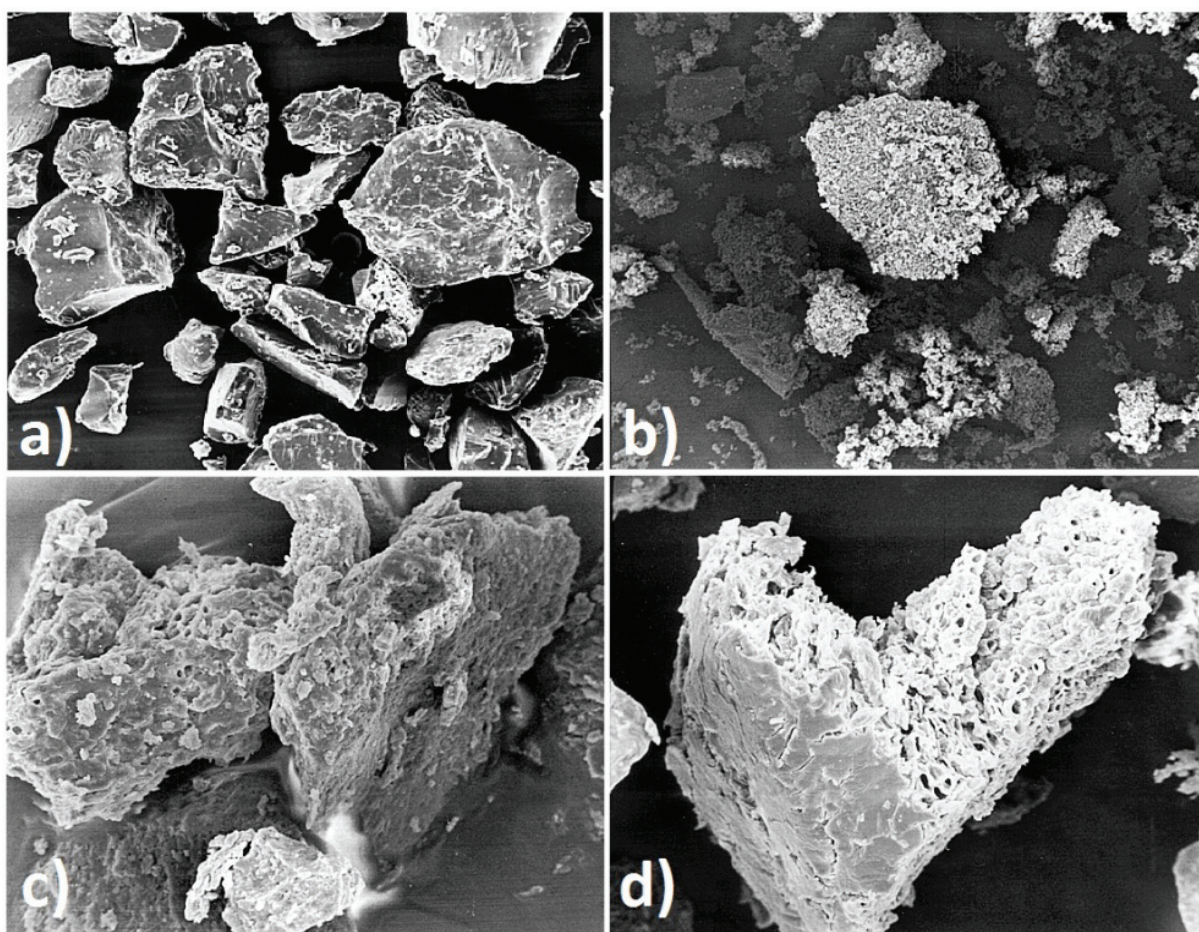


Figure 3. SEM micrographs for control (a) and hydrolyzed starch rice with α -amylase, amyloglicosidase (b) (c) and (d).

The susceptibility of the starch by the amylolytic enzymes, depends beyond the starch source and the enzyme, also depends of the processing conditions. In the reactions catalyzed by enzyme on the starch, in physiological conditions, the hydrolysis constant in disaccharides is around 100%. These are irreversible due to the balance be higher in product formation. In non-physiological conditions, the hydrolysis constant of glycosidic bonds has been around 50% with high concentrations of amylase enzyme. The lipids associated with starch are usually polar and require polar solvent, such as methanol and water, for its extraction. The combination of alcohol and high enzyme concentration, the hydrolysis of glycosidic bonds can reach 85% (BeMiller & Whistler, 2009).

Conclusion

Hydrothermal processing, liquefaction and saccharification of rice starch were optimized at each stage. RSM was useful for the modeling and optimization of microbial α -amylase activity on a paste of broken rice flour, with optimum conditions provided a decrease in the viscosity of the suspension of broken rice flour. It is possible to use byproducts from the rice processing industry for syrup production with high dextrose equivalent. The syrup produced in this study has the potential to be used in the food industry for the production of either yeast-fermented beverages or as a sweetener.

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References

- American Association of Cereal Chemists (AACC). (2001). *Approved methods of the American Association of Cereal Chemists*. Minnesota, St Paul: AACC.
- American Public Health Association (APHA). (2001). *Compendium of methods for the microbiological examination of foods*. (4th ed.). Washington, DC: APHA.
- Association of Official Analytical Chemists (AOAC). (2005). *Official methods of analysis: association of official analytical chemists*. Gaithersburg, Maryland: AOAC International.
- BeMiller, J. N., & Whistler, R. (2009). *Starch: chemistry and technology* (3rd ed.). New York, USA: Academic Press.
- Briffaz, A., Mestres, C., Escoute, J., Lartaud, M., & Dornier, M. (2012). Starch gelatinization distribution and peripheral cell disruption in cooking rice grains monitored by microscopy. *Journal of Cereal Science*, 56(3), 699-705. doi: 10.1016/j.jcs.2012.09.001
- Carvalho, A. V., Bassinello, P. Z., Mattietto, R. A., Carvalho, R. N., Rios, A. O., & Seccadio, L. L. (2012). Processing and characterization of an extruded snack made from broken rice flour and broken common bean flour. *Brazilian Journal of Food Technology*, 15(1), 72-83. doi: 10.1590/S1981-67232012000100008
- Chen, Y., Wang, M., & Ouwerkerk, P. B. F. (2012). Molecular and environmental factors determining grain quality in rice. *Food and Energy Security*, 1(2), 111-132. doi: 10.1002/fes3.11
- Companhia Nacional de Abastecimento (CONAB). (2015). Retrived from: <http://www.conab.gov.br>
- Foresti, M. L., Williams, M. P., García, R. M., & Vázquez, A. (2014). Analysis of a preferential action of α -amylase from *B. licheniformis* towards amorphous regions of waxy maize starch. *Carbohydrate Polymers*, 102(1), 80-87. doi: 10.1016/j.carbpol.2013.11.013
- Johnson, R., Padmaja, G., & Moorthy, S. N. (2009). Comparative production of glucose and high fructose syrup from cassava and sweet potato roots by direct conversion techniques. *Innovative Food Science & Emerging Technologies*, 10(4), 616-620. doi: 10.1016/j.ifset.2009.04.001
- Kolusheva, T., & Marinova, A. (2007). A study of the optimal conditions for starch hydrolysis through thermostable α -amylase. *Journal of the University of Chemical Technology and Metallurgy*, 42(1), 93-96.
- Lai, M. H., Chang, W. T. H., & Luh, B. S. (1980). *Rice vinegar fermentation*. In B. S. Luh, (Ed.), *Rice: production and utilization* (p. 712-735). Connecticut, USA: AVI.
- Leloup, V. M., Colonna, P., & Ring, S. G. (1992). Physico-chemical aspects of resistant starch. *Journal of Cereal Science*, 16(3), 253-266. doi:10.1016/S0733-5210(09)80088-X
- Limberger, V. M., Silva, L. P., Emanuelli, T., Comarela, C. G., & Patias, L. D. (2008). Chemical and physical modification of broken rice starch (*Oryza sativa* L.) for use in food industry. *Química Nova*, 31(1), 84-88. doi: 10.1590/S0100-40422008000100018
- Limberger, V. M., Comarela, C. G., Patias, L. D., Brum, F. B., Emanuelli, T., & Silva, L. P. (2009). Extruded snack broken rice production to the food industry usage. *Ciência Rural*, 39(9), 2590-2594. doi: 10.1590/S0103-84782009000900032
- Lin, Q., Xiao, H., Liu, G. Q., Liu, Z., Li, L., & Yu, F. (2013). Production of maltose syrup by enzymatic conversion of rice starch. *Food and Bioprocess Technology*, 6(1), 242-248. doi: 10.1007/s11947-011-0681-9
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin-Phenol reagent. *The Journal of Biological Chemistry*, 193(1), 265-275.
- Ministério da Agricultura, Pecuária e Abastecimento (MAPA). (2015). *Arroz*. Retrived from <http://www.agricultura.gov.br/vegetal/culturas/arroz>
- Mohan, B. H., Gopal, A., Malleshi, N. G., & Tharanathan, R. N. (2005). Characteristics of native and enzymatically hydrolyzed ragi (*Eleusine coracana*) and

- rice (*Oryza sativa*) starches. *Carbohydrate Polymers*, 59(1), 43-50. doi: 10.1016/j.carbpol.2004.08.022
- Oyashiki, H., Uchida, M., Hanai, S., Obayashi, A., & Oka, S. (1987). Relationship between conditions for steaming rice and productivity in mirin-making. *Journal of Fermentation Technology*, 65(5), 543-547. doi: 10.1016/0385-6380(87)90114-2
- Ruiz, M. I., Sanchez, C. I., Torres, R. G., & Molina, D. R. (2011). Enzymatic hydrolysis of cassava starch for production of bioethanol with a colombian wild yeast strain. *Journal of the Brazilian Chemical Society*, 22(12), 2337-2343. doi: 10.1590/S0103-50532011001200014
- Silva, R. N., Quintino, F. P., Monteiro, V. N., & Asquiere, E. R. (2010). Production of glucose and fructose syrups from cassava (*Manihot esculenta* Crantz) starch using enzymes produced by microorganisms isolated from Brazilian Cerrado soil. *Food Science and Technology*, 30(1), 213-217. doi: 10.1590/S0101-20612010005000011
- Soares, I. A., Flores, A. C., Zanettin, L., Pin, H. K., Mendonça, M. M., Barcelos, R. P., ... Baroni, S. (2010). Identificação do potencial amilolítico de linhagens mutantes do fungo filamentoso *Aspergillus nidulans*. *Food Science and Technology*, 30(3), 700-705. doi: 10.1590/S0101-20612010000300021
- Sowbhagya, C. M., Ramesh, B. S., & Ali, S. Z. (1994). Hydration, swelling and solubility behavior of rice in relation to other physicochemical properties. *Journal of the Science of Food and Agriculture*, 64(1), 1-7. doi: 10.1002/jsfa.2740640102
- Surendiran, G., Alsaif, M., Kapourchali, F. R., & Moghadasian, M. H. (2014). Nutritional constituents and health benefits of wild rice (*Zizania* spp.). *Nutrition Reviews*, 72(4), 227-236. doi: 10.1111/nure.12101
- Tawil, G., Viksø-Nielsen, A., Rolland-Sabaté, A., Colonna, P., & Buléon, A. (2012). Hydrolysis of concentrated raw starch: a new very efficient α -amylase from *Anoxybacillus flavothermus*. *Carbohydrate Polymers*, 87(1), 46-52. doi: 10.1016/j.carbpol.2011.07.005
- Uthumporn, U., Zaidul, I. S. M., & Karim, A. A. (2010). Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes. *Food and Bioprocess Processing*, 88(1), 47-54. doi: 10.1016/j.fbp.2009.10.001
- Van Der Maarel, M. J. E. C., & Leemhuis, H. (2013). Starch modification with microbial α -glucanotransferase enzymes. *Carbohydrate Polymers*, 93(1), 116-121. doi: 10.1016/j.carbpol.2012.01.065
- Wani, A. A., Singh, P., Shah, M. A., Schweiggert-Weisz, U., Gul, K., & Wani, I. A. (2012). Rice starch diversity: effects on structural, morphological, thermal, and physicochemical properties – a review. *Comprehensive Reviews in Food Science and Food Safety*, 11(5), 417-436. doi: 10.1111/j.1541-4337.2012.00193.x
- Waszczynskyj, N., Rao, C.S., & Silva, R. S. F. (1981). Extraction of proteins from wheat bran: application of carbohydrases. *Cereal Chemistry*, 58(4), 264-266.

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