Philippine Journal of Science
152 (2): 571-580, April 2023
ISSN 0031 - 7683
Date Received: 06 Sep 2022

Production of High-maltose Syrup from Selected Rice (*Oryza sativa* L.) Bran by Enzymatic Method

Garry A. Diopol[1,4*], Francisco B. Elegado[2], Katherine Ann T. Castillo-Israel[3], Mary Ann O. Torio[4], and Lawrence Yves C. Uy[5,6,7]

[1]Environment and Biotechnology Division, Industrial Technology Development Institute, Department of Science and Technology, Taguig City 1631 Philippines
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, College, Laguna 4031 Philippines
[3]Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines
[4]Institute of Chemistry, University of the Philippines Los Baños, College, Laguna 4031 Philippines
[5]Southeast Asian Regional Center for Graduate Study and Research in Agriculture, College, Los Baños 4031 Laguna, Philippines
[6]University of the Philippines Los Baños Graduate School, College, Laguna 4031 Philippines
[7]German Academic Exchange Service (DAAD), Postfach 200404 D-53134 Bonn, Germany

Rice bran is an underutilized rice by-product. This study develops a methodology to enzymatically produce high-maltose syrup from rice bran of PSB Rc18. Rice bran liquefaction was conducted sequentially by screening, and optimization using response surface methodology (RSM) to determine the optimum condition for dextrose equivalents (DE). The DE upon liquefaction was affected by α-amylase concentration, rice bran concentration, temperature, and pH. The highest DE of 15.6% was attained by a combination of 30% of rice bran concentration, 0.12% of α-amylase concentration, 80 °C, and pH 5 for 60 min. These optimum conditions of DE acquired in liquefaction were carried over in the saccharification process for maltose conversion of rice bran using RSM. The maltose conversion was affected by DE, temperature, and pH. The saccharification process yielded the highest maltose conversion of 47.78% acquired in combination with 15.6% DE, 335 units of β-amylase, 50 °C, and pH 5 for 24 hr.

Keywords: high-maltose syrup, *Oryza sativa* L., response surface methodology, rice bran

INTRODUCTION

Milling of rice paddy would yield products such as rice endosperm, rice bran, rice husk, and rice germ [Wells (1993), de Deckere and Korver (1996), and van Hoed *et al*. (2006) in Sharma *et al*. (2015)]. Among these by-products, except for rice endosperm, rice bran is the most promising for utilization in novel products (Grist 1959). International Rice Research Institute (IRRI) reported in 2015 that the whole rice grain would yield around 8–12% of rice bran after milling. Sohail *et al*. (2016) reported that rice bran has a global production of 29.3 million metric tons annually.

Rice bran is composed of carbohydrates (43.5–54.3%), protein (14.1–18.2%), fat (1.6–20.9%), ash (12.8–15.3%),

*Corresponding author: gadiopol@itdi.dost.gov.ph; gadiopol@gmail.com

and fiber (8.4–10.5%) [Prakash and Ramaswamy (1996); Hu (1996), Hernandez *et al*. (2000), Piironen *et al*. (2000), and Jiang and Wang (2005) in Sharma *et al*. (2015)]. This variation in rice bran composition depends on the rice type, climatic conditions, and milling process of rice (Grist 1985).

Rice bran has been already applied in snack foods, bakery products, cereals, crackers, pasta products, dough conditioners, beverages, gluten-free foods, and medical foods (Kahlon 2009; Gul *et al.* 2015). Rice bran-based beverages such as isotonic drinks were also developed (Mitchell 2009; Isarra and Rawdkuen 2016). In addition, healthy meal replacement drinks made from stabilized rice bran were introduced in the market (Isarra and Rawdkuen 2016). So far, the major product made from rice bran is rice bran oil [Barber *et al*. (1974), Hammond (1994), and van Hoed *et al*. (2006) in Sharma *et al*. (2015)].

In particular, the carbohydrates of rice bran – which is around 50% of its composition [Hu (1996), Hernandez *et al*. (2000), Piironen *et al*. (2000), and Jiang and Wang (2005) in Sharma *et al*. (2015)], if utilized – may produce significant products such as maltose-containing syrups. High-maltose syrups have mild sweetness, low viscosity in solution, low hygroscopicity, and good thermal stability – thus deemed good for several food applications (Shaw and Sheu 1992). Maltose-containing syrups have several applications and are typically used in brewing, baking, canning, confectionery, and other food industries (Saha *et al*. 2009).

At present, there is no known study that hydrolyzed the carbohydrates of rice bran to produce maltose-containing syrup using enzymes. Therefore, this study aimed to produce high-maltose syrup from selected rice (*Oryza sativa* L.) bran by enzymatic method.

MATERIALS AND METHODS

Rice Bran Materials and Laboratory Reagent

Five hundred (500) kg of rice bran of Philippine Seed Board Rice cultivar 18 (PSB Rc18) "Ala" was obtained from a reputable rice miller (JD Aguilar Rice Mill, San Leonardo, Nueva Ecija, Philippines). This was packed in an aluminum foil bag, transported by vehicle, and kept in a 4–5˚C storage chiller, the experiment was immediately conducted. The α-amylase for liquefaction was purchased from Sigma-Aldrich (A3306 SIGMA; produced from *Bacillus licheniformis*) with an enzymatic activity of 20,000–60,000 U/mL. The β-amylase for saccharification was also purchased from Sigma-Aldrich (A7005-10KU; produced from sweet potato) with an enzymatic activity of ≥ 750 U/mg of protein. Other reagents, unless mentioned, were analytical grade or better.

Rice Bran Stabilization

The method was developed by the author. Rice bran was sieved in a mechanical shaker (AR403, Erweka GmbH, Pittlerstraβe 45 63225 Langen, Germany) with a mesh size of 750 μm, then mixed with water (1:3 rice bran-water ration) before being passed through a colloid mill (Loher and Sohne: VDE 0530 and Colloid Mill: JM-65, Biobase group, Jinan, Shandong, China) for 15 min. After milling, the rice bran mixture was centrifuged (Z 383K, Hermle, *Siemensstrasse* 25, Wehingen, Germany) for 5 min at 1500 revolutions per minute (rpm) at 30 °C. The stabilized rice bran was collected and dried at 70 °C in a cabinet dryer (UF750, Memmert GmbH+ Co. KG, Aeussere Rittersbacher Strass 38, Schwabach, Germany) until 3.5–8.5% moisture was achieved. The dried stabilized rice bran was kept and stored at a chilled condition (4–5 °C) until used.

Liquefaction

The liquefaction process was based on the studies of Chen and Chang (1984), Shaw and Sheu (1992), Pontoh and Low (1995), and Saha *et al*. (2009). A stabilized rice bran was weighed directly into a 10-L stainless stock pot that serves as a reactor vessel, then purified water was added to bring the total weight of slurry (rice bran: water ration) to 30% concentration. The slurry was stirred and gelatinized for 15–20 min at 70 ± 2 °C. The pH of the slurry was maintained at 5.0 by the addition of either 1N HCl or 1N NaOH. Afterward, before heating, a 0.12% (v/w) of α-amylase was added, then heated and maintained at 80 °C for 60 min. A fabricated mechanized mixer with a thermal regulator was used. The slurry was cooled and then centrifuged (HERMLE: Z 383K, Hermle, Siemensstrasse 25, Wehingen, Germany) for 5 min at 1500 rpm at 30 °C, producing precipitate and 75–80% of supernatant. The supernatant was later analyzed for percent (%) dextrose equivalents (DE).

Saccharification

The saccharification was based on the studies of Chen and Chang (1984), Shaw and Sheu (1992), Pontoh and Low (1995), and Saha *et al*. (2009). The supernatant from liquefaction with 15.6% DE was transferred to the reaction bath and treated with 335 units of β-amylase at pH 5, then heated and maintained at 50 °C for 24 h. The profile of high-maltose syrup was later determined by high-performance liquid chromatography (Prominence 20A, Shimadzu, Europa GmbH).

Analytical Methods

Proximate analysis and starch analysis. The proximate composition of rice bran (total carbohydrates, ash, moisture, protein, and total fat) were analyzed based on the Official Methods of Analysis of the Association of Official Analytical Chemists (International 19th Edition, 2012). Starch analysis was analyzed based on AOAC 920.44A method of Official Methods of Analysis of the Association of Official Analytical Chemists (International 20th Edition, 2016).

Dextrose equivalents (DE) analysis. The DE values were analyzed using the Official Methods of Analysis of the Association of Official Analytical Chemists (16th edition, 1995) method 930.44 (Lane-Eynon Method). The modified method was based on the study of Pontoh and Low (1995). A 0.5-mL sample aliquot was transferred to a 100-mL volumetric flask and made up to volume by the addition of distilled water. Five (5) mL of Fehling A solution (34.639 g $CuSO_4.5H_2O$ in 500 mL distilled water), and 5 mL of Fehling B solution (173 g $KNaC_4H_4O_6.4H_2O$ in 50 g NaOH in 500 mL distilled water) was added to a 250-mL Erlenmeyer flask, then three drops of 1% (w/v) methylene blue solution was added. The resulting solution was then rapidly titrated (< 1 min) with the sample solution until the blue color disappeared. Three (3) parallel titrations were conducted, and the mean was calculated.

High-performance liquid chromatography (HPLC) analysis. The rice bran sugar syrups were analyzed by HPLC (Prominence 20A, Shimadzu, Europa GmbH) for the concentration of maltose and other sugars (*i.e.* glucose, fructose, and sucrose). Samples were analyzed using the following settings: [1] detector: refractive index detector (RID); [2] column: NH_2-column Zorbax (250 mm x 4.6 mm); [3] mobile phase: acetonitrile to water (80:20); [4] flow rate: 1.0 mL/min; [5] injection volume: 20 µL; [6] column temperature: 180 °C; [7] detector temperature: 200 °C; [8] injector temperature: 150 °C.

Statistical Analyses

All statistical analyses were conducted in duplicate. All analyses were done using R Software version 3.3.1, R Studio version 1.1.453 (R Core Team 2013) under the Institute of Statistics, University of the Philippines Los Baños.

The liquefaction of rice bran was intended to determine the optimum condition of the response variable, DE. It was determined by RSM using R Software version 3.3.1, R Studio version 1.1.453 (R Core Team 2013). The liquefaction of rice bran was composed of a two-stage sequential experiment: [1] screening and [2] optimization. The impact of several variables in DE was studied in the screening of liquefaction using 2^4 factorials in a randomized complete block design. The experimental design generated 16 runs (each run in duplicates), and the variables were: [1] α-amylase concentration, [2] rice bran concentration, [3] temperature, and [4] pH (Table 2). The data obtained from the optimization of DE were fitted to a second-order polynomial model, and the regression coefficients were obtained by multiple linear regression. The data was computed at α equals 5%. To further optimize the parameters in getting the maximum DE, the optimized α-amylase concentration and pH derived from Table 2 were used as constants in the succeeding optimization of liquefaction using a central composite design (CCD). The experimental design of a two-factor factorial using CCD generated 13 runs with variables: [1] rice bran concentration and [2] temperature (Table 3).

Second-order polynomial model:

$$y = b_0 + b_1x_1 + b_2x_2 + \ldots b_nx_n$$

The saccharification was intended to determine the optimum condition to obtain the high-maltose syrup conversion from rice bran. The optimum condition of high-maltose syrup from rice bran was determined by RSM using CCD and processed by R Software version 3.3.1, R Studio version 1.1.453 (R Core Team 2013). The optimization of maltose conversion was a three-stage sequential process that involved the following: [1] screening, [2] optimization, and [3] validation.

RESULTS

Proximate and Starch Analysis

The initial proximate composition of bran from rice variety PSB Rc18 is shown in Table 1. The starch content of bran from PSB Rc18 was 26.0 g/ 100 g of rice bran. Upon the application of the colloid milling to stabilize the rice bran, there were changes in composition which showed a marked increase in total carbohydrates and a decrease in total fat.

Liquefaction

The screening of liquefaction obtained a DE of 7.30–24.70%. The lowest DE of 7.30% was obtained from the combination of 20% rice bran concentration, 0.12% α-amylase concentration, 95 °C temperature, and pH 5 for 60 min. On the other hand, the highest DE of 24.70% was obtained from the combination of 30% rice bran concentration, 0.12% of α-amylase concentration, 80 °C, and pH 5 in 60 min (Table 2).

The optimization of liquefaction of rice bran obtained DE in the range of 5.06–15.6%. The lowest DE of 5.06% was obtained from the combination of 17.9% rice bran

Table 1. Effect of the colloid mill process in the stabilization of bran from PSB Rc18.

Parameters	Colloid mill		Difference (%)
	Before	After	
Total carbohydrates, g/ 100 g	55.3 ± 0.03	69.2 ± 0.24	+25.14
Ash, g/ 100 g	7.3 ± 0.08	8.6 ± 0.04	+17.81
Moisture, g/ 100 g	9.6 ± 0.16	3.5 ± 0.08	–63.54
Protein (N x 6.25), g/ 100 g	13.4 ± 0.08	13.1 ± 0.07	–2.23
Total fat, g/ 100 g	14.4 ± 0.09	11.9 ± 0.06	–17.36

Table 2. Experimental design of screening of liquefaction used in RSM studies using four independent variables, and DE value after the analysis.

Run #	Alpha α-amylase concentration (%)	Rice bran concentration (%)	Temperature (°C)	pH	Dextrose equivalents (%)
1	0.12	20.0	95.0	5.0	7.45 ± 0.00
2	0.12	20.0	95.0	5.0	7.30 ± 0.00
3	0.03	30.0	95.0	5.0	17.48 ± 0.03
4	0.12	20.0	80.0	6.5	11.32 ± 0.01
5	0.12	30.0	80.0	5.0	24.09 ± 0.02
6	0.03	20.0	80.0	5.0	10.30 ± 0.00
7	0.03	20.0	95.0	6.5	12.59 ± 0.02
8	0.12	30.0	95.0	6.5	14.61 ± 0.01
9	0.12	20.0	80.0	6.5	11.45 ± 0.00
10	0.12	30.0	95.0	6.5	14.24 ± 0.00
11	0.03	30.0	95.0	5.0	15.95 ± 0.02
12	0.03	30.0	80.0	6.5	10.84 ± 0.01
13	0.12	30.0	80.0	5.0	24.70 ± 0.00
14	0.03	30.0	80.0	6.5	11.08 ± 0.01
15	0.03	20.0	95.0	6.5	14.05 ± 0.01
16	0.03	20.0	80.0	5.0	14.88 ± 0.03

concentration, 0.12% α-amylase concentration, 87.5 °C temperature, and pH 5 in 60 min. On the other hand, the highest DE of 15.6% was obtained from the combination of 30% rice bran concentration, 0.12% of α-amylase concentration, 80 °C temperature, and pH 5 for 60 min (Table 3).

Saccharification

The screening of saccharification obtained the highest maltose of 41.73% from the combination of 15.6% of DE, 335 units of β-amylase, 50 °C temperature, and pH 5 for 24 h (Table 4). The optimization of saccharification obtained the highest maltose of 47.78% from DE of 15.6% in combination with 335 units of β-amylase, 50 °C temperature, and pH 5 for 24 h (Table 5). The validation of saccharification obtained a mean absolute error (MAE) of 0.051 and a mean percentage absolute error (MAPE) of 3.77% (data not shown).

DISCUSSION

Stabilization of Rice Bran

Rice bran is prone to oxidation due to rapid lipase activity, which occurs within seconds after its removal from the kernel, making it rancid and inedible. Rice bran contains several types of lipases that are site-specific and cleave the *sn*1,3 – site of triacylglycerol [Aizano *et al*. (1973), Prabhu *et al*. (1999), and Ramezanzadeh *et al*. (1999a) in Fabian and Ju (2011)]. Moreover, rice bran also contains amylases, catalase, ascorbic acid oxidase, cytochrome oxidase, lipoxygenases, polyphenol oxidases, dehydrogenase, and esterase that may contribute to oxidative rancidity of the bran [Aizano *et al.* (1973), Prabhu *et al*. (1999), Ramezanzadeh *et al*. (1999a), and Ramezanzadeh *et al*. (1999b) in Fabian and Ju (2011)]. Therefore, stabilization of rice bran was required prior to its use. The stabilization process typically involves the

Table 3. Experimental design of optimization of liquefaction used in RSM studies using two independent variables, and DE value after the analysis.

Run#	Alpha (α) amylase concentration (%)	Rice bran concentration (%)	Temperature (ºC)	pH	Dextrose equivalents (%)
1	0.12	20.0	95.0	5.0	9.68 ± 0.01
2	0.12	32.1	87.5	5.0	8.32 ± 0.01
3	0.12	25.0	87.5	5.0	10.58 ± 0.01
4	0.12	30.0	80.0	5.0	15.6 ± 0.06
5	0.12	17.9	87.5	5.0	5.06 ± 0.00
6	0.12	25.0	87.5	5.0	7.30 ± 0.01
7	0.12	25.0	87.5	5.0	7.76 ± 0.01
8	0.12	25.0	87.5	5.0	8.09 ± 0.01
9	0.12	25.0	87.5	5.0	9.04 ± 0.02
10	0.12	30.0	95.0	5.0	10.32 ± 0.02
11	0.12	25.0	98.1	5.0	8.59 ± 0.01
12	0.12	20.0	80.0	5.0	8.44 ± 0.01
13	0.12	25.0	76.9	5.0	7.31 ± 0.00

Table 4. Experimental design in screening of saccharification used in RSM studies using three independent variables, and maltose value after the analysis.

Run #	Beta (β)-amylase (U)	Dextrose equivalent (%)	Temperature (ºC)	pH	Maltose (%)
1	335	15.60	35.0	5.4	36.71 ± 0.28
2	335	5.06	13.8	4.0	8.15 ± 0.13
3	335	5.06	35.0	4.0	23.20 ± 0.18
4	335	5.06	56.2	4.0	21.85 ± 0.20
5	335	15.60	50.0	5.0	41.73 ± 0.23
6	335	5.06	35.0	4.0	23.11 ± 0.29
7	335	15.60	35.0	4.0	1.07 ± 0.00
8	335	5.06	35.0	4.0	13.43 ± 0.27
9	335	5.06	50.0	5.0	15.16 ± 0.04
10	335	15.60	13.8	4.0	21.88 ± 0.66
11	335	5.06	35.0	5.4	15.24 ± 0.18
12	335	5.06	20.0	5.0	23.38 ± 0.33
13	335	5.06	20.0	3.0	41.10 ± 0.57
14	335	15.60	35.0	2.6	1.07 ± 0.46
15	335	5.06	50.0	3.0	2.06 ± 0.17
16	335	15.60	35.0	4.0	14.69 ± 0.22
17	335	15.60	35.0	4.0	14.75 ± 0.00
18	335	15.60	20.0	5.0	11.69 ± 0.15
19	335	5.06	35.0	2.6	2.15 ± 0.04
20	335	15.60	20.0	3.0	16.42 ± 0.44
21	335	15.60	56.2	4.0	10.28 ± 0.39
22	335	15.60	50.0	3.0	3.29 ± 0.07

Table 5. Experimental design in optimization of saccharification used in RSM studies using two independent variables, and maltose value after the analysis.

Run #	Beta (β)-amylase (U)	Dextrose equivalent (%)	Temperature (°C)	pH	Maltose (%)
1	335	15.6	60.0	5.0	35.26±0.02
2	335	15.6	55.0	5.5	42.60±0.04
3	335	15.6	60.0	6.0	32.03±0.12
4	335	15.6	55.0	5.5	41.18±0.07
5	335	15.6	50.0	5.0	47.78±0.03
6	335	15.6	50.0	6.0	29.63±0.09
1	335	15.6	55.0	5.5	41.52±0.16
2	335	15.6	62.1	5.5	25.12±0.06
3	335	15.6	55.0	4.8	29.63±0.10
4	335	15.6	55.0	6.2	28.17±0.08
5	335	15.6	55.0	5.5	39.71±0.19
6	335	15.6	47.9	5.5	23.35±0.14

combination of either heat, water, or pressure to deactivate the enzyme (Sharma *et al*. 2015); other studies also used gamma-irradiation, microwave treatment, ohmic heating, dry heat treatment, extrusion, toasting, and parboiling and autoclaving [Shin and Godber (1996), Ramezanzadeh *et al*. (1999), Lakkakula *et al*. (2004), Sharma *et al*. (2004), Agrawal *et al*. (2004), Amente *et al*. (2006), and Rosniyana *et al*. (2009) in Gul *et al*. 2015)].

Rice bran was stabilized by applying pretreatment using a colloid mill process. Colloid mills are widely used in the food industry, primarily in homogenization (McClements 2004). Colloid mill is one of several mechanical ways of disrupting cell envelopes, which are mainly composed of the cytoplasmic membrane and cell wall (Garver and Epstein 1959; Jahanshahi and Najafpour 2007) to facilitate the release of biomolecules such as nucleic acids, proteins, carbohydrates, lipids, enzymes, inorganic ions, vitamins, pigments, inclusion bodies, and water (Tangtua 2014).

The colloid mill process of bran from PSB Rc18 increased the yield of carbohydrates by 25.14% and was able to remove 17.36% of lipids. Removal of lipids prevents oxidation and subsequent rancidity of rice bran.

Liquefaction

Starch conversion to sugars required the action of enzymes. Amylases hydrolyzed starch through liquefaction and saccharification (Saha *et al*. 2009). Liquefaction is the process that converts granular starch into soluble dextrins of lower molecular weights expressed as DE (Akoh *et al*. 2008). The duration of liquefaction depends on the plant source of starch and the molecular weight of starch (Pontoh and Low 1995). The liquefaction of starch usually starts by applying the thermostable α-amylase to the starch, followed by heating to gelatinize at a specified temperature for a specified time. Holding at the required temperature is done until the desired DE is met. The design matrix of liquefaction for the screening and the corresponding responses are shown in Table 2.

The screening of liquefaction identified that rice bran concentration ($P < 0.0001$), pH ($P = 0.0023$), and temperature ($P = 0.0172$), have significant effects on DE at $\alpha = 5\%$. Interaction among variables such as α-amylase *vs.* rice bran concentration ($P < 0.0001$), and α-amylase *vs.* temperature ($P < 0.0001$) was also significantly affecting the DE. However, pH has no significant interaction with other variables such as α-amylase (Table 6). Factors with significant effect with DE (P value < 0.05) and significant interaction with α-amylase were further selected for further optimization studies. After the screening, optimization of DE was conducted *via* RSM.

The result shows that the interaction of rice bran concentration and temperature ($P = 0.06579$) has a significant effect on the liquefaction process, and the P value of the overall model ($P = 0.08425$) was significant (data not shown). Hence, the temperature has been carried over in saccharification. However, rice bran concentration as another variable was replaced by DE as the parameter. According to the study of Shaw and Sheu (1992), the maltose level of sugar syrup can be greatly affected by the DE value of the initial rice starch supernatant (hydrolysates). Moreover, since another enzyme (β-amylase) was also introduced in the hydrolysates, the pH – which commonly affects the activity of the enzyme – was also included as another variable in saccharification.

Table 6. Analysis of variance (ANOVA) for the parameters affecting the DE in the screening of liquefaction of bran from PSB Rc18.

Source of variation	*DF*	*SS*	*MS*	*f*-value	*P*-value
Alpha (α)-amylase	1	3.9159995	3.9159995	2.48	0.1543[a]
Rice bran concentration	1	118.5215729	118.5215729	74.92	< 0.0001[b]
Temperature	1	14.1946184	14.1946184	8.97	0.0172[c]
pH	1	30.3880919	30.3880919	19.21	0.0023[d]
Alpha (α)-amylase *vs.* rice bran concentration	1	84.0715315	84.0715315	53.14	< 0.0001[e]
Alpha (α)-amylase *vs.* temperature	1	104.1885655	104.1885655	65.86	< 0.0001[f]
Alpha (α)-amylase *vs.* pH	1	0.1965869	0.1965869	0.12	0.7336[a]
Model	7	355.4769666	50.7824238	32.10	< 0.0001[g]
Error	8	12.6563949	1.5820494		
Corrected total	15	368.1333615			

[R2] 0.965620; [CV] 9.047165; [RMSE] 1.257795
[*DF*] degree of freedom, [*SS*] sum of squares, [*MS*] mean square
[a, b, c, d, e, f, g] *P*-value with the same letter is not significantly different based on a 5% level of significance

Therefore, the DE, temperature, and pH were carried over as variables in the saccharification of rice bran.

The liquefaction of rice bran determined that at 30.2% rice bran concentration and 80.47 °C, the highest DE of approximately 14–15% can be achieved. According to Saha *et al.* (2009), the usual DE value after liquefaction using highly thermostable α-amylase is 10–15%.

Saccharification

Saccharification aims to hydrolyze the oligosaccharides (mainly 8–12 glucose units) to form sugars (*i.e.* fructose, glucose, and maltose) syrup catalyzed by enzymes (*i.e.* isomerase, glucoamylase, and β-amylase) [Pandey (1995) in Akoh *et al*. (2008)].

The saccharification was intended to determine the optimum conditions to produce high-maltose syrup from the bran of PSB Rc18. The saccharification involved a three-stage sequential analysis: [1] screening, [2] optimization, and [3] validation. The screening was conducted to determine the effects of factors (DE, temperature, and pH) on the response variable (maltose); optimization to determine the optimum condition in maltose conversion; and validation to verify the optimum condition obtained from the optimization. The saccharification of rice bran was determined by RSM using CCD and analyzed by R Software version 3.3.1, R Studio version 1.1.453 (R Core Team 2013).

In the screening of saccharification, the data was computed based on α = 5%, and factors with a *P* value lower than 0.05 were considered to have a significant effect on maltose. The results determined that temperature (*P* = 0.04349) was significantly affecting the maltose. And the interaction effect of pH *vs.* temperature (*P* = 0.02524) was also significantly affecting the yield of maltose. Moreover, the polynomial regression model's lack of fit (*P* = 0.56718) signified that the model was adequate (data not shown). The lack of fit of the model should have a *P*-value > 0.05 to be adequate (Aydar 2018). Therefore, the variables temperature and pH that affect the maltose were carried over in further studies.

In the optimization of saccharification, after fitting the data in the second-order polynomial model, results show that the quadratic effect of the temperature (*P* = 0.02362) was significantly affecting the maltose at α = 5% (data not shown). Moreover, after testing the lack of fit (*P* = 0.03436) – which is significant – the quadratic combination of pH and temperature (*P* = 0.04548) suggests that it can be used to optimize the maltose yield (Table 7).

The 3D plot of the general quadratic model of maltose response to changes in pH and temperature is shown in Figure 1. Based on the plot, the stationary point of the maltose yield as affected by pH and temperature is at maximum. This means that the optimum condition can be determined by looking at the centroid or central region of the response plots.

The 3D plot (Figure 1) and ridge analysis (data not shown) suggest that the optimum condition for the production of high-maltose syrup from rice bran can be obtained at low temperatures (approximately 53–54 °C), low pH (approximately 5.1–5.3), given that the DE of the liquefied rice bran syrup has a DE of approximately 15%.

The highest concentration of maltose (47.78%) obtained from the optimization of saccharification sufficiently attained the industry classification of high-maltose-

Table 7. Analysis of variance (ANOVA) for the parameters (pH and temperature) affecting the maltose in the optimization of saccharification of bran from PSB Rc18.

Source of variation	*DF*	*SS*	*MS*	*f*-value	*P*-value
FO (pH, temperature)	2	0.33714	0.16857	1.6412	0.28317[a]
TWI (pH, temperature)	1	0.24751	0.24751	2.4097	0.18129[a]
PQ (pH, temperature)	2	1.25436	0.62718	6.1061	0.04548[b]
Residuals	5	0.51357	0.10271		
Lack of fit	3	0.50174	0.16725	28.2677	0.03436[c]
Pure error	2	0.01183	0.00592		

[*DF*] degree of freedom, [*SS*] sum of squares, [*MS*] mean square

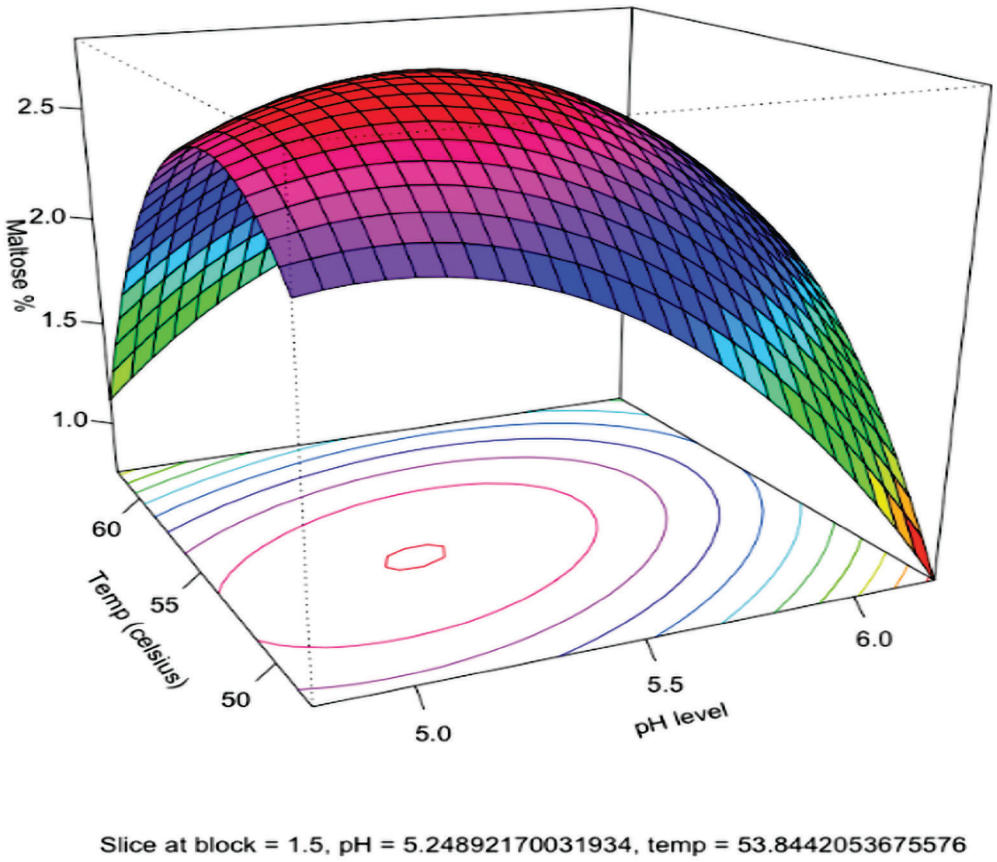

Figure 1. 3D plot of the general quadratic model of maltose response to changes in pH and temperature (R Software version 3.3.1, R Studio version 1.1.453).

containing syrup at 45–60% maltose (Saha *et al*. 2009). Moreover, the optimum condition for temperature and pH obtained in this study was well within the conditions required during the production of maltose syrup (pH 5.0–5.5 and 50–55 °C) using maltogenic enzymes such as β-amylase, *etc.* (Saha *et al*. 2009).

Validation of the model generated in the optimization of saccharification was determined. Using the RSM model, a prediction of desired maltose concentration can be generated; however, an actual experiment was required to verify the model (Lenth 2009; Aydar 2018). The difference between the experimental and predicted value obtained 0.051 in MAE and 3.77% in MAPE. The MAE and MAPE must not exceed 1.0 point and 10 percentage points, respectively, as cited by Khair *et al*. (2017). This means that the data obtained in the experiment were in good agreement with the predicted value of maltose at the optimum working condition identified by the RSM

analysis in the optimization process. Therefore, the RSM model using R Software obtained in this study was valid and reliable.

CONCLUSION

This study successfully produced a high-maltose syrup from the bran of rice variety PSB Rc18. The optimum condition for DE in liquefaction and maltose in saccharification, obtained from the models developed from RSM using R Software, was in good agreement with other related studies.

ACKNOWLEDGMENTS

The study would like to acknowledge the DOST-HRDP (Department of Science and Technology–Human Resource Development Program) and the DOST-PCAARRD (Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development) for the financial support, as well as the DOST-ITDI (Industrial Technology Development Institute) for the use of facilities and equipment.

REFERENCES

AKOH CC *et al.* 2008. Review: Biocatalysis for the Production of Industrial Products and Functional Foods from Rice and Other Agricultural Produce. J Agric Food Chem 56(22): 10445–10451. DOI: 10.1021/jf801928e

AYDAR AY. 2018. Utilization of Response Surface Methodology in Optimization of Extraction of Plant Materials. Statistical Approaches with Emphasis on Design of Experiments Applied to Chemical Processes. DOI: 10.5772/intechopen.73690

CHEN W, CHANG Y. 1984. Production of High-Fructose Rice Syrup and High-protein Rice Flour from Broken Rice. J Sci Food Agric 35: 1128–1135.

FABIAN C, JU Y. 2011. A Review on Rice Bran Protein: Its Properties and Extraction Methods Critical. Reviews in Food Science and Nutrition 51(9): 816–827. DOI: 10.1080/10408398.2010.482678

GARVER JC, EPSTEIN RL. 1959. Method of Rupturing Large Quantities of Microorganisms. Applied Microbiology 7(5): 318.

GRIST DH. 1985. Rice, 5th ed. London, UK: Longman.

GRIST DH. 1959. Rice, 3rd ed. London, UK: Longman.

GUL K, YOUSUF B, SINGH AK, SINGH P, WANI AA. 2015. Rice bran: nutritional values and its emerging potential for development of functional food – a review. Bioactive Carbohydrates and Dietary Fiber 6(1): 24–30.

[IRRI] International Rice Research Institute. 2015. Steps to Successful rice production. Retrieved on 05 May 2019 from http://knowledgebank.irri.org/images/docs/12-Steps-Required-for-Successful-Rice-Production.pdf

ISARRA U, RAWDKUEN S. 2016. Rice bran: a potential of main ingredient in healthy beverage. International Food Research Journal 23(6): 2306–2318.

KAHLON TS. 2009. Rice Bran: Production, Composition, Functionality and Food Applications, Physiological Benefits. Fiber Ingredients: Food Applications and Health Benefits. DOI: 10.1201/9781420043853-c14

KHAIR U, FAHMI H, AL HAKIM S, RAHIM R. 2017. Forecasting Error Calculation with Mean Absolute Deviation and Mean Absolute Percentage Error. Journal of Physics: Conf Series 930(2017): 012002. DOI: 10.1088/1742-6596/930/1/012002

LENTH RV. 2009. Response-surface Methods in R, Using RSM. Journal of Statistical Software 32(7).

McCLEMENTS DJ. 2004. Food Emulsions: Principles, Practices, and Techniques, Second Edition. CRC Press. 251p. https://doi.org/10.1201/9781420039436

MITCHELL CR. 2009. Chapter 13 – Rice Starches: Production and Properties, 3rd Ed. Be Miller J, Whistler R eds. San Diego: Academic Press. p. 569–578.

JAHANSHAHI M, NAJAFPOUR G. 2007. Chapter 17 – Advanced Downstream Processing in Biotechnology. Biochemical Engineering and Biotechnology. p. 390–415. DOI: 10.1016/B978-044452845-2/50017-3

PONTOH J, LOW NH. 1995. Glucose syrup production from Indonesian palm and cassava starch. Food Research International 28(4): 379–385.

PRAKASH J, RAMASWAMY HS. 1996. Rice bran proteins: properties and food uses. Critical Reviews in Food Science and Nutrition 36: 537–552. DOI: 10.1080/10408399609527738

R CORE TEAM. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.Rproject.org/

SAHA BC, JORDAN DB, BOTHAST RJ. 2009. Enzymes, Industrial (overview). Encyclopedia of Microbiology 281–294. DOI: 10.1016/b978-012373944-5.00146-2

SHAW J, SHEU J. 1992. Production of High-maltose Syrup and High-protein Flour from Rice by Enzymatic Method. Biosci Biotech Biochem 56(7): 1071–1073.

SHARMA R, SRIVASTAVA T, SAXENA DC. 2015. Studies on Rice Bran and Its Benefits – a Review**.** Int. Journal of Engineering Research and Applications 5(5): 107–112.

SOHAIL M, RAKHA A, BUTT MS, IQBAL MJ, RASHID S. 2016. Rice bran nutraceutics: a comprehensive review. Critical Reviews in Food Science and Nutrition 57(17): 3771–3780. DOI: 10.1080/10408398.2016.1164120

TANGTUA J. 2014. Evaluation and comparison of microbial cells disruption methods for extraction of pyruvate decarboxylase. International Food Research Journal 21(4): 1331–1336.