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Original Article

Optimization of reducing sugar production from enzymatic hydrolysis of banana peels using response surface methodology

Suthida Akkarachaneeyakorn*, Archara Suwakrai, and Duangporn Pewngam

Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bang Sue, Bangkok, 10800 Thailand

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Abstract

Banana varieties have a significant effect on the reducing sugar content in the peel. The *Musa* ABB cv. Kluai 'Hom Thong' banana variety produced the most reducing sugar in the peel (52.45 mg/g), followed by cv. Kluai 'Namwa' (44.80 mg/g), cv. Kluai 'Leb Mu Nang' (39.13 mg/g) and cv. Kluai 'Khai' (36.98 mg/g). A 4-factor-5-level central composite design (x_1 and x_2 = value of temperature (70-90 °C) and period of time (15-75 min) by hydrolysis with α-amylase; x_3 and x_4 = value of temperature (50-70 °C) and period of time (75-135 min) by hydrolysis with glucoamylase), was applied to determine the optimal conditions for reducing sugar production from banana peel (cv. Kluai 'Hom Thong') by enzymatic hydrolysis using α-amylase and glucoamylase. The optimal conditions for the highest reducing sugar yield (60.65 mg/g) were α-amylase at 86 °C for 75 minutes and glucoamylase for 60 °C for 75 minutes.

Keywords: banana peel, central composite design, optimization, reducing sugar, response surface method

1. Introduction

Banana is a plant that grows easily with all parts having a beneficial economic use and being processed into many products. Several varieties of banana are popular for consumption: *Musa* ABB cv. Kluai 'Namwa', *Musa* ABB cv. Kluai 'Hom Thong', *Musa* ABB cv. Kluai 'Khai', and *Musa* ABB cv. Kluai 'Leb Mu Nang'. After processing into banana products the unwanted banana peels can create a negative environmental impact. Banana peel is rich in carbohydrates as well as potassium, calcium, and phosphorus (Emaga *et al*., 2007; Nagarajaiah & Prakash, 2011). Banana peel waste can be reduced by digestion into reducing sugar, which can then be used as a raw material for producing foods, such as vinegar, and fermentation into ethanol (Arumugam & Manikandan, 2011; Kocher & Dhillon, 2013; Narkprasom, 2013; Singh & Singh, 2007). However, because the starch in banana peel is insoluble in water, the peel must first be dried in an oven and ground into powder to increase its surface area and the efficiency of digestion by the enzyme (Borglum, 1980). The advantage of enzymatic starch digestion is the higher purity of products compared with using acid digestion, because enzymes are more specific to the substrate. Enzymes are also proteins, which makes the products obtained safe for consumers. The present study considered two enzymes, α-amylase and glucoamylase. First, α-amylase (endoenzyme) breaks down starch to oligosaccharide and α-limit dextrin, a process known as liquefaction (Wong and Robertson, 2003). The α-amylase cannot break down α-1,6-glucosidic bonds, so another type of enzyme, a "debranching enzyme", such as α-1,6-glucosidase or glucoamylase (exoenzyme) is required. This enzyme cleaves both the α-1,4-glucosidic and α-1,6-glucosidic bonds of the dextrins to glucose or another reducing sugar from the ends of the polymer chains. Factors affecting the reducing sugar yield were the hydrolysis temperature and time, pH, substrate concentration, and enzyme concentration. Every enzyme has a temperature range of optimum activity. Increasing the temperature increases the kinetic energy of the reactants and consequently increases the rate of reaction, forming products. As the temperature increases, the enzyme is denatured and no longer functioning,

*Corresponding author
Email address: suthida.a@sci.kmutnb.ac.th

which decreases the rate of reaction (Cavalieri & Corcuera, 2003). Changes in pH (above or below the optimum pH) affect the rate of reaction because the enzyme molecules have active sites whose shapes are not or less complementary to the shapes of their substrate. Small changes in pH do not cause a permanent change to the enzyme because the bonds can be reformed. However, extreme changes in pH can cause enzymes to denature and permanently lose enzyme function. Increasing the substrate concentration increases the rate of reaction because more substrate molecules collide with enzyme molecules to form products. However, after a certain concentration, an increase has no effect on the rate of reaction. Increasing the enzyme concentration increases the rate of reaction because more enzymes collide with substrate molecules. However, this phenomenon only has an effect up to a certain concentration, where the enzyme concentration is no longer the limiting factor (Fennema, 1996).

There have been several studies on starch digestion using enzymes: Jaiswal *et al*. (2011) studied the optimal conditions for starch digestion using α-amylase by varying the pH and temperature and found that the enzyme worked best at pH 5.5 and 75 °C. Cereia *et al*. (2000) found the optimal conditions for glucoamylase activity produced by the *Scytalidium thermophilum* to be pH 5.0 and 55 °C. Arumugam and Manikandan (2011) studied ethanol production from banana peel by digesting dried banana peel with α-amylase and glucoamylase. They found that the optimal production used α-amylase at pH 6.0 ± 0.05 and 93 °C for 1 hr and glucoamylase for subsequent digestion at pH 5.0 ± 0.05 and 60 °C for 1 hr. These studies have shown that the optimal conditions for α-amylase are pH 5.5-6 and 75-93 °C, and for glucoamylase the optimal conditions are pH 5.0 and 55-60 °C. The optimal pH for α-amylase and glucoamylase varied slightly, whereas the optimal temperatures varied much more. If the temperature is lower than the optimum, enzymes work slowly and at a higher than the optimum temperature the enzyme denatures and is unable to bind with the substrate (Nithiya 2014). Therefore, the present study aims to determine the optimal conditions for banana peel digestion using α-amylase and glucoamylase based on varying the digestion temperatures and times at pH 6.0 ± 0.05 and 5.0 ± 0.05. The raw material is banana peel ripened to level 6 (yellow peel) (Tapre & Jain, 2012), the industry level for processing. Experiments are designed using a central composite design (CCD), a proven method for finding the optimal conditions for chemical and biological processes. CCD also requires fewer experiments than a full factorial method, which helps to reduce the project costs (Montgomery, 2001).

The objectives of this research were to study the effect of banana varieties on the amount of reducing sugar obtained from the digestion of banana peel using α-amylase and glucoamylase. This study is to determine the optimal conditions that enable banana varieties producing the maximum amount of reducing sugar by using CCD and response surface methodology (RSM). From the results, an equation for predicting the amount of reducing sugar obtained from the digestion of banana peel by α-amylase and glucoamylase is developed and tested.

2. Materials and Methods

2.1 Materials

Fresh bananas (*Musa* ABB cv. Kluai 'Namwa', *Musa* ABB cv. Kluai 'Hom Thong', *Musa* ABB cv. Kluai 'Khai' and *Musa* ABB cv. Kluai 'Leb Mu Nang') at a maturity level of 6 (yellow peel) (Tapre & Jain, 2012) were purchased in January 2014 from the local market of Nonthaburi, Thailand. The peels were carefully removed without damaging them and were washed manually using tap water to remove dirt. The peels were reduced in size to pieces 2 cm × 2 cm and blanched in boiling water for 5 minutes, cooled quickly using cold water, and the water was drained. The pieces were dried at 65 °C in a hot-air oven (Memmert 100-800, Schwabach, Germany) for 24 hours, were then allowed to cool to room temperature, ground using a cross beater mill (SK100, Retsch, Haan, Germany), screened to select particles with a size between 37-1,000 μm and kept in zip lock polypropylene bags at room temperature until needed. The enzymes α-amylase, from *Bacillus subtilis,* and glucoamylase, from *Aspergillus niger,* were both present in Termamyl SC purchased from the Brenntag Company (Bangkok, Thailand).

2.2 Production of reducing sugar

Reducing sugars were obtained from the dried powder of peels of the *Musa* ABB cv. Kluai 'Namwa', *Musa* ABB cv. Kluai 'Hom Thong', *Musa* ABB cv. Kluai 'Khai' and *Musa* ABB cv. Kluai 'Leb Mu Nang' bananas by a two-step enzymatic hydrolysis, as shown in Figure 1. Ten grams of dried banana peel powder was added to 90 grams of distilled water in an Erlenmeyer flask, and the contents were mixed by vortex. The suspension was autoclaved (ES 315, Tomy, Tokyo, Japan) at 121 °C and 15 psi for 15 minutes. Then, the suspension was cooled to room temperature, and the pH was adjusted to 6.0 ± 0.05 using calcium hydroxide or citric acid. Thereafter, 6% (w/w) α-amylase was added, the suspension was heated in a shaking water bath at a pressure of 110 psi at 78 °C for 44 minutes, and then the hydrolysate was cooled to room temperature. The pH was adjusted to 5.0 ± 0.05 using calcium hydroxide or citric acid, 9% (w/w) glucoamylase was added, and the suspension was heated in a shaking water bath (WNB 22, Memmert, Schwabach, Germany) at 110 psi at 50 °C for 123 minutes. The hydrolysate was then cooled to room temperature. The suspended solids were separated from the liquid by centrifuge (Suprema 21, TOMY, Tokyo, Japan). The supernatant was analyzed for reducing sugar content.

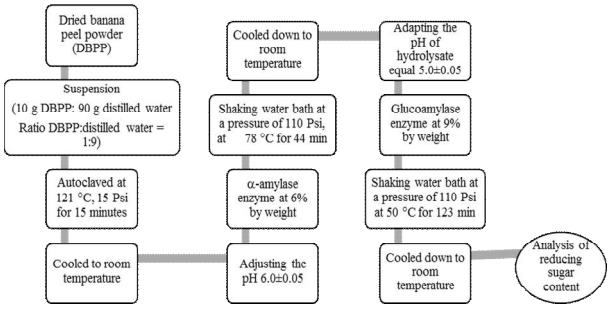

Figure 1. Enzymatic hydrolysis of dried banana peel powder.

2.3 Determination of the reducing sugar content

The content of reducing sugar was determined by the dinitrosalicylic acid (DNS) method adapted from Miller (1959). One ml of DNS solution was added to 1.0 ml of sample. The mixture was placed in boiling water for 10 minutes, cooled and then 9 ml of distilled water was added. After homogenizing the reaction mixture, the absorbance at 540 nm was measured (GENESYS 20 Spectrophotometer, Thermo Fisher Scientific, California, USA). A standard curve for reducing sugar was prepared using serial concentrations of glucose (0-700 mg/g). The results were expressed as reducing sugar concentration (mg) per sample weight (g).

2.4 Determination of the chemical composition of banana peel

The fresh peel and dried banana peel powder from *Musa* ABB cv. Kluai 'Hom Thong' were analyzed as follows: The moisture content was analyzed using the AOAC method (2012) with nine replicates. The average moisture was recorded. The protein, fat, ash, and fiber contents were analyzed using a modified AOAC method (2012).The protein, fat, ash, and fiber contents were determined in triplicate, and the average values were recorded.

2.5 Determination of the effect of banana varieties on the amount of reducing sugar

Determinations of the amount of reducing sugar from the peel of different varieties were performed in triplicate. One-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test was carried out using the software program Minitab 16. Values of $P \leq 0.05$ were considered statistically significant.

2.6 Determination of the optimum conditions for enzymatic hydrolysis

The hydrolysate obtained from the pretreatment of banana peel was subjected to a two-step enzymatic hydrolysis (Figure 1). The temperature and time for hydrolysis with α-amylase or glucoamylase enzyme were varied, as shown in Table 1. After the set time, the samples were cooled to room temperature, and the reducing sugar yield was measured.

Table 1. CCD for optimizing the yield of reducing sugar with four independent variables (coded/actual level) of enzymatic hydrolysis

Run No.	α-amylase		Glucoamylase		Reducing sugar content (mg/g)
	Temperature (x_1, °C)	Time (x_2, min)	Temperature (x_3, °C)	Time (x_4, min)	
1	+1(85)	-1(30)	-1(55)	-1(90)	32.45
2	+1(85)	+1(60)	-1(55)	+1(120)	21.79
3	0(80)	0(45)	0(60)	0(105)	28.96
4	0(80)	0(45)	0(60)	0(105)	26.74
5	-1(75)	+1(60)	-1(55)	+1(120)	19.92
6	+1(85)	-1(30)	-1(55)	+1(120)	31.68
7	+1(85)	-1(30)	+1(65)	-1(90)	13.87
8	-1(75)	-1(30)	-1(55)	+1(120)	35.77
9	-1(75)	-1(30)	-1(55)	-1(90)	25.88
10	+1(85)	+1(60)	+1(65)	+1(120)	24.27
11	-1(75)	+1(60)	+1(65)	+1(120)	33.13
12	-1(75)	-1(30)	+1(65)	-1(90)	16.17
13	-1(75)	+1(60)	-1(55)	-1(90)	32.28
14	+1(85)	+1(60)	-1(55)	-1(90)	35.01
15	-1(75)	-1(30)	+1(65)	+1(120)	36.80
16	0(80)	0(45)	0(60)	0(105)	29.72
17	0(80)	0(45)	0(60)	0(105)	31.94
18	+1(85)	+1(60)	+1(65)	-1(90)	33.83
19	+1(85)	-1(30)	+1(65)	+1(120)	17.96
20	-1(75)	+1(60)	+1(65)	-1(90)	32.54
21	0(80)	-2(15)	0(60)	0(105)	30.92
22	0(80)	+2(75)	0(60)	0(105)	40.04
23	0(80)	0(45)	+2(70)	0(105)	12.85
24	0(80)	0(45)	0(60)	0(105)	25.80
25	-2(70)	0(45)	0(60)	0(105)	23.76
26	0(80)	0(45)	0(60)	0(105)	30.92
27	0(80)	0(45)	0(60)	-2(75)	29.13
28	0(80)	0(45)	-2(50)	0(105)	19.32
29	+2(90)	0(45)	0(60)	0(105)	15.57
30	0(80)	0(45)	0(60)	+2(135)	24.01

Note: x_1 and x_2 = value of temperature (°C) and period of time (min) by hydrolysis with α-amylase; x_3 and x_4 = value of temperature (°C) and period of time (min) by hydrolysis with glucoamylase.

2.7 Response surface methodology

Response surface methodology is an empirical statistical technique. The graphical representation of the function is called the response surface, which is used to describe the individual and cumulative effects of the test variables and their subsequent effects on the response. Four variables in the enzyme hydrolysis process (x_1 and x_2 = actual value of temperature (70-90 °C) and period of time (15-75 min) by hydrolysis with α-amylase; x_3 and x_4 = actual value of temperature (50-70 °C) and period of time (75-135 min) by hydrolysis with glucoamylase) were used for the CCD. Five levels of the four factors were coded to values -2, -1, 0, +1, and +2. The four independent variables (coded/actual level) of enzymatic hydrolysis as introduced in Table 1. A total of 30 runs for the CCD (6 center points) were used to optimize the four parameters and five levels. Analysis of variance (ANOVA), response surface regression and the determination of the optimum conditions were performed using Minitab 16. Factors were rejected when their significance levels were less than 95% or P >0.05.

A quadratic model was used to correlate the relationship between the independent variables and the response to predict the optimal hydrolysis conditions.

$$Y = (\beta_{11}x_1^2) + (\beta_{22}x_2^2) + (\beta_{33}x_3^2) + (\beta_{44}x_4^2) + (\beta_1x_1) + (\beta_2x_2) + (\beta_3x_3) + (\beta_4{*}x_4) + (\beta_{12}x_1x_2) + (\beta_{13}x_1x_3) + (\beta_{14}x_1x_4) + (\beta_{23}x_2x_3) + (\beta_{24}x_2x_4) + (\beta_{34}x_3x_4) + \beta_0 \quad (1)$$

where Y = the predicted reducing sugar content; x_1, x_2, x_3 and x_4 = independent variables for enzymatic hydrolysis; β_{11}, β_{22}, β_{33} and β_{44} = the second order coefficients; β_1, β_2, β_3 and β_4 = the linear coefficients; and β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} = the cross-product coefficients.

3. Results and Discussion

3.1 Moisture content of banana peel powder

The average initial moisture content of the dried banana peel powder used in this study was 5.72 ± 0.13% (dry basis), higher than the results from Nagarajaiah and Prakash (2011) of 1.45 to 1.71% (dry basis). The difference between the moisture content was due to difference of the drying temperature and time. However, the moisture of dried banana peel powder from this experiment was classified as dried food because the dry basis of the moisture content was lower than 15%, which inhibits the growth of bacteria, yeasts and molds (Jay, 1998).

3.2 Effect of banana variety on the amount of reducing sugar

Yields of reducing sugar of the samples were calculated with a linear equation based on a standard curve using glucose (y = 2573.7 x + 23.139, R^2 = 0.996 where y = reducing sugar (mg/g) and x = absorbance) (Figure 2). Figure 3 shows that the variety of banana had a significant effect on the amount of reducing sugar in the peel (P <0.05). The *Musa* ABB cv. Kluai 'Hom Thong' banana variety produced the most reducing sugar in the peel (52.45 mg/g), followed by cv. Kluai 'Namwa' (44.80 mg/g), cv. Kluai 'Leb Mu Nang' (39.13 mg/g) and cv. Kluai 'Khai' (36.98 mg/g).

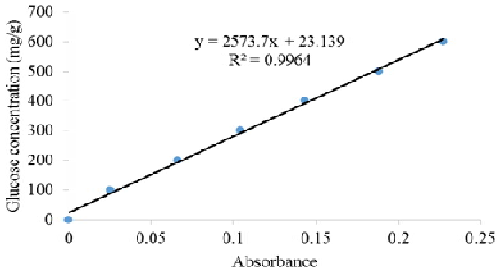

Figure 2. Calibration curve for relationship between glucose concentration and absorbance value.

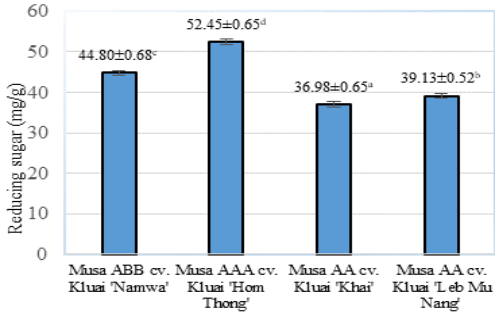

Figure 3. The amount of reducing sugar from the peel of different varieties of banana. Values are expressed as mean±SD of triplicate measurement. a-d mean significant at the confidence level of 95 %.

For *Musa* ABB cv. Kluai 'Khai', the reducing sugar content was similar to that found by Arumugam and Manikandan (2011), where the content was 36.67 mg/g. For the peels of other banana varieties, Emaga *et al*. (2007) found that the amount of reducing sugar in Grande Naine *(Musa Cavendish*) was 22.00-32.40 mg/g, and Oberoi *et al*. (2012) found 48.00 mg/g in the *Musa acuminate* variety. These differing contents of reducing sugar in the peel are due to the different varieties of banana used in the experiments and differences in the geography of the banana plantations and storage methods. Based on these facts, the peel of the *Musa* ABB cv. Kluai 'Hom Thong' variety was selected for the next step of the experiment because it had the highest amount of reducing sugar. The chemical composition of the peel of *Musa* ABB cv. Kluai 'Hom Thong' in fresh and dried forms is shown in Table 2. Hot air drying had a significant ($P \leq 0.05$) effect on the moisture, carbohydrate, fiber, protein and lipid contents but had no significant effect on the ash content ($P > 0.05$). Heat-drying by a hot-air oven at 65 °C for 24 hours can cause thermo-chemical degradation of the *Musa* ABB cv. Kluai 'Hom Thong' banana peel. The amount of carbohydrate and ash was consistent with the research by Essien *et al.* (2005), who found that the carbohydrate and ash contents in waste banana peel were 59.59 and 13.44% dry basis.

3.3 Optimization of the reducing sugar yield

Table 1 shows the experimental conditions used according to the CCD and the resulting reducing sugar contents, which varied from 12.85 to 40.04 mg/g, which is consistent with the research by Oberoi *et al.* (2012). Run numbers 3-4, 16-17, 24, and 26 at the center point were used to determine the experiment error.

Table 2. Chemical composition of the peel of *Musa* ABB cv. Kluai 'Hom Thong' in fresh and dried forms.

Treatment	Maturity level	Moisture content (%)	Carbohydrate (%)	Fiber (%)	Protein (%)	Lipid (%)	Ash[ns] (%)
Fresh *Musa* ABB cv. Kluai 'Hom Thong' peel	6	75.19±1.45 [b]	65.81±0.1 [b]	4.40±0.15 [a]	10.95±0.01 [b]	9.34±0.02 [b]	9.50±0.28
Dried *Musa* ABB cv. Kluai 'Hom Thong' peel powder[***]	6	6.02±0.06 [a]	62.33±0.8 [a]	11.50±0.72[b]	4.20±0.05 [a]	6.42±0.07 [a]	9.50±0.00

Note: Values are expressed as mean ± SD of triplicate measurement. [***] % dry basis Numbers on the same column with differing superscripts are significantly different at $P \leq 0.05$. [ns] indicates numbers on the same column are not significantly different at $P > 0.05$.

Table 3. Analysis of variance of regression model in terms of four independent variables (actual value) for yield of reducing sugar.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	1363.90	1363.90	97.422	12.73	<0.001*
Linear	4	217.11	308.76	77.190	10.09	<0.001*
x_1	1	48.31	176.58	176.577	23.08	<0.001*
x_2	1	82.10	52.20	52.202	6.82	0.020*
x_3	1	77.44	127.48	127.480	16.66	0.001*
x_4	1	9.26	20.71	20.714	2.71	0.121
Square	4	469.39	469.39	117.349	15.34	<0.001*
$x_1 * x_1$	1	88.71	92.37	92.369	12.07	0.003*
$x_2 * x_2$	1	174.03	123.12	123.118	16.09	0.001*
$x_3 * x_3$	1	206.33	204.44	204.438	26.72	<0.001*
$x_4 * x_4$	1	0.33	0.33	0.325	0.04	0.839
Interaction	6	677.40	677.40	112.900	14.75	0.000*
$x_1 * x_2$	1	8.60	8.60	8.600	1.12	0.306
$x_1 * x_3$	1	63.32	63.32	63.322	8.28	0.012*
$x_1 * x_4$	1	73.32	73.32	73.316	9.58	0.007*
$x_2 * x_3$	1	222.83	222.83	222.830	29.12	<0.001*
$x_2 * x_4$	1	259.45	259.45	259.452	33.91	<0.001*
$x_3 * x_4$	1	49.88	49.88	49.879	6.52	0.022*
Error	15	114.78	114.78	7.652		
Lack-of-Fit	10	86.59	86.59	8.659	1.54	0.332
Pure Error	5	28.20	28.20	5.639		
Total	29	1478.69				

Note: x_1 and x_2 = actual value of temperature (°C) and period of time (min) by hydrolysis with α-amylase; x_3 and x_4 = actual value of temperature (°C) and period of time (min) by hydrolysis with glucoamylase. * Significant at $P \leq 0.05$. DF = The degrees of freedom of an estimate of a parameter. Seq SS = The sequential sum of squares for each term in the model. Adj SS = The adjusted sum of squares for a term in the model. Adj MS = The adjusted mean square = Adj SS/DF.

From the statistical analysis of these results, Table 3 shows that the linear and quadratic effect of the four independent variables involved in enzymatic hydrolysis played a significant role in the yield of reducing sugar ($P \leq 0.05$). Therefore, this equation can be used to predict the yield of reducing sugar from the peel of the *Musa* ABB cv. Kluai 'Hom Thong' variety within the range of the studied variables. Therefore, the yield of reducing sugar could be predicted using Equation 2.

$$Y = (-0.07*x_1^2) + (0.01*x_2^2) - (0.11*x_3^2) + (18.79*x_1) - (2.61*x_2) + (14.40*x_3) + (1.74*x_4) + (0.01*x_1*x_2) - (0.08*x_1*x_3) - (0.03*x_1*x_4) + (0.05*x_2*x_3) - (0.02*x_2*x_4) + (0.02*x_3*x_4) - 1165.54 \quad (2)$$

where x_1 and x_2 = actual value of temperature (70–90 °C) and period of time (15-75 min) of hydrolysis with α-amylase; x_3 and x_4 = actual value of temperature (50–70 °C) and period of time (75 – 135 min) of hydrolysis with glucoamylase; and Y = yield of reducing sugar (mg/g).

The 3D response surface from Equation 2 was plotted to illustrate the relationship between the independent and dependent variables, as shown in Figure 4. Different 3D graphs indicate different interactions between the variables. For these four variables, when two variables within the experimental range were shown in the 3D graph, the third and fourth variables were set constant at the center point of the CCD (x_1 = 80 °C, x_2 = 45 min, x_3 = 60 °C and x_4 = 105 min). The factors affecting the reducing sugar yield were the hydrolysis temperature and time for α-amylase and glucoamylase (x_1- x_4). The activity of an enzyme is affected by its environmental conditions. The hydrolysis temperature of α-amylase was the key factor significantly influencing the hydrolysis process. Therefore, experiments were conducted to study the effect of temperature on the reducing sugar yield. The reducing sugar yield increased linearly with increasing hydrolysis temperature of α-amylase from 70 to 86 °C (Figure 4a-c). However, temperature above 86 °C resulted in lower reducing sugar yield. Thus, the liquefaction function of α-amylase to hydrolyze banana peel is effective at temperatures up to 86 °C.

Figures 4b, d, and f show that the reducing sugar yield increased with increasing temperature for glucoamylase hydrolysis up to 60 °C. Thereafter, increasing the temperature of glucoamylase hydrolysis had a negative impact on the reducing sugar yield. Hydrolysis time is one of the important factors for banana peel hydrolysis. To examine its effect on the hydrolysis process, experiments were conducted for various periods. The reducing yield increased rapidly with increasing the hydrolysis time of α-amylase up to 75 minutes and decreased rapidly with increasing the hydrolysis time of glucoamylase up to 135 minutes (Figure 4e).

Figure 4a-f show the hydrolysis temperature and time for the α-amylase and glucoamylase interval (x_1 = 86 °C, x_2 =75 min at pH 6 ±0.05, x_3 = 60 °C and x_4 = 75 min at pH 5 ±0.05) that resulted in the highest amounts of reducing sugar (Y = 60.65 mg/g). This result is consistent with the research of Jaiswal *et al*. (2011) and Cereia *et al*. (2000), who studied the optimal conditions for starch digestion using enzymes and found that α-amylase worked best at pH 5.5 and 75 °C (Jaiswal *et al*., 2011) and glucoamylase worked best at pH 5.0 and 55 °C (Cereia *et al*., 2000).

Minitab 16 was used to solve Equation 2 to find the optimal conditions for the maximum yield of reducing sugar, as shown in Figure 5. The numbers displayed at the top of a column show the current factor level settings and the high and low factor settings in the experimental design. The left of each response row shows the predicted reducing sugar content, y, at the current factor settings and the individual desirability score. The optimal conditions for the maximum yield of reducing sugar were: 86 °C for 75 minutes and 60 °C for 75 minutes for the hydrolysis with α-amylase and glucoamylase, respectively. The predicted yield of reducing sugar is 60.65 mg/g, and the composite desirability is 0.84.

3.4 Model validation

The proposed quadratic model for reducing sugar yield from banana peel (cv. Kluai 'Hom Thong'), shown in Equation 2, was experimentally validated by enzymatic hydrolysis with three replicates to verify the results at the optimal conditions (α-amylase at 86 °C for 75 minutes and glucoamylase at 60 °C for 75 minutes). The average percentage difference between the predicted value (60.65 mg/g) and the experiment (57.76 mg/g) was 5.00 %.

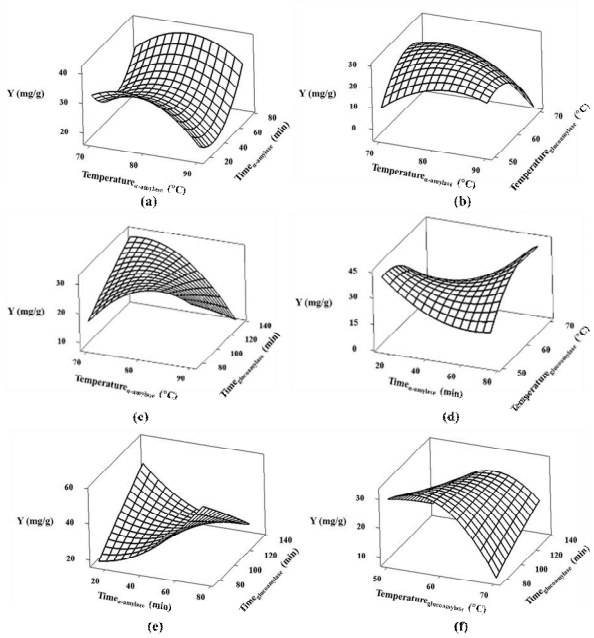

Figure 4. 3D Plots of reducing sugar yield (Y) for hydrolysis by α-amylase and glucoamylase (a) hydrolysis temperature (x_1) and time (x_2) using α-amylase; (b) hydrolysis temperature using α-amylase and glucoamylase (x_1, x_3); (c) hydrolysis temperature using α-amylase (x_1) and hydrolysis time using glucoamylase (x_4) (d) hydrolysis time using α-amylase (x_2) and hydrolysis temperature using glucoamylase (x_3); (e) hydrolysis time using α-amylase and glucoamylase (x_2, x_4); (f) hydrolysis temperature (x_3) and time(x_4) using glucoamylase.

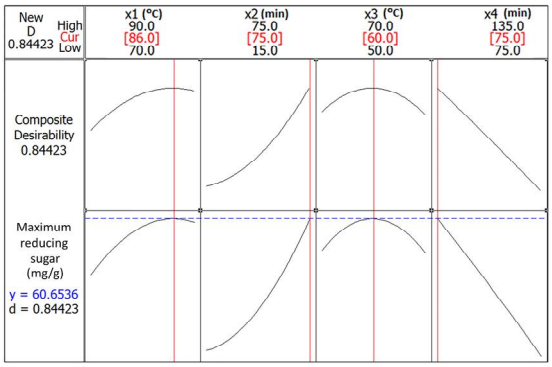

Figure 5. Optimization plot. Note: x_1 and x_2 = actual value of temperature and period of time of hydrolysis with α-amylase; x_3 and x_4 = actual value of temperature and period of time of hydrolysis with glucoamylase.

3.5 Physical properties of the reducing sugar product at the optimal conditions

The optimal conditions for the production of reducing sugar from enzymatic hydrolysis of *Musa* ABB cv. Kluai 'Hom Thong' banana peel was α-amylase at 86 °C for

75 minutes and glucoamylase for 60 °C for 75 minutes. L* indicates brightness, the darkest black at L* = 0, and the brightest white at L* = 100. a* indicates chromaticity on a green (-) to red (+) axis. b* indicates chromaticity on a blue (-) to yellow (+) axis. The hue angle can be calculated from the formula $\tan^{-1}$ (b*/a*). The hue angle formed by a line from the origin to the intercept of a* (x-axis) and b* (y-axis) coordinates, which shows the color, 0° for red, 90° for yellow, 180° for green, and 270° for blue (Choudhury, 2014). The *L**, *a**, *b**, and hue angle of the reducing sugar from banana peel were 29.51, 8.76, 20.29, and 66.64, respectively. The color of the reducing sugar product was brown.

4. Conclusions

The *Musa* ABB cv. Kluai 'Hom Thong' banana peel produced the maximum amount of reducing sugar (52.45 mg/g), followed by cv. Kluai 'Namwa' (44.80 mg/g), cv. Kluai 'Leb Mu Nang' (39.13 mg/g) and cv. Kluai 'Khai' (36.98 mg/g) (The hydrolysis conditions were: 78 °C for 44 minutes and 50 °C for 123 minutes for the hydrolysis with α-amylase and glucoamylase, respectively). The equation for predicting the yield of reducing sugar from the enzymatic hydrolysis of banana peel (*Musa* ABB cv. Kluai 'Hom Thong') using temperature and hydrolysis times for α-amylase and glucoamylase enzymes as dependent variables was valid within the experimental limits and accurately predicted the reducing sugar yield. The optimal conditions for producing the highest reducing sugar yield of 60.65 mg/g were α-amylase at 86 °C for 75 minutes and glucoamylase at 60 °C for 75 minutes.

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