Preparation of Isoflavone glucosides from Soy germ and β-Glucosidase from *Bacillus coagulans* PR03 for Isoflavone aglycones Production

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ABSTRACT

This research optimized isoflavone aglycones production from soy germ. The processes included isoflavone glucosides extraction from soy germ to use as a precursor for isoflavone aglycones production. β-glucosidase was produced from B. coagulans PR03 and used to convert glucosides form to aglycones. For the β-glucosidase production using Plackett and Burman Design (n=8) and 2^2 factorial experiment with central composite design, the suitable medium containing peptone (2.00%), beef extract (14.84%), glucose (2.00%) and magnesium sulphate (0.10%) with pH 7.96 was used and incubated at 30 °C. The resulting β-glucosidase activity was 4.01 mU/ml. However, the isoflavone glucosides was extracted by use of soy germ in 80% ethanol at a ratio of 1:5 with high power ultrasonication technique at 80 °C for 160 min using a completely randomized design. The extracted isoflavone glucosides contained daidzin, genistin and glycitin of 307.47, 214.84 and 73.63 mg/100 g. (dry basis), respectively. Finally, isoflavone aglycones production

was optimized using 2^2 factorial experiment with central composite design, the total isoflavone glucosides (595.95 mg/100 g. dry basis) was convert to isoflavone aglycones (112.90 µg/ml) by β-glucosidase at 37.5 ^oC for 120 hours.

Keywords: Isoflavone aglycones, Isoflavone glucosides, Soy germ, β-glucosidase, *B. coagulans* PR03

INTRODUCTION

The significance of healthy food consumption has become more prominent as many consumers are gaining more awareness of good eating habit such as eating more fruits and vegetables. It is known that bioactive compounds in those foods can reduce the risk of many severe diseases such as cardiovascular diseases, diabetes and cancers (Crozier et al., 2009). One of the most acknowledged bioactive compounds is isoflavone. It is a secondary metabolite from the plant with a limited taxonomic distribution such as soybean (*Glycine max*). Isoflavones have been associated with biological properties such as estrogenic and anti-estrogenic activities against hypocholesterolemia (Murphy, 1982), atherosclerosis, osteoporosis, anti-carcinogenic activity against breast cancer (Ohta et al., 2002) and reduced hot flush in postmenopausal women (Han et al., 2002). It can bind with estrogen receptors, and it is known as phytoestrogen (Sarkar et al., 2002). Suggested dosage of isoflavone consumption is approximately 18-20 mg for one serving size (4 time to 80 mg/day) (Barnes et al., 1995).

The isoflavones in soybean are generally in forms of glucosides (daidzin, genistin, glycitin) more than aglycones (daidzein, genistein and glycitein). The whole soybean seed contains only 2% soy germ, and the highest quantity of isoflavones is found in the soy germ. Isoflavones found in soy germ are 5-6 times higher than the other parts, for instance, seed coat and cotyledon (Nahas et al., 2004). There is an investigation of isoflavone and antioxidant activity in cotyledon, seed coat and soy germ from four varieties of soybean. It was found that the total isoflavones in cotyledon, seed coat and soy germ were 2.73-9.71, 5.56-16.94 and 27.76-81.43 mg/ fresh weight, respectively (Yue et al., 2009).

Isoflavone is a flavonoid substance that is soluble in various polar solvents. Wiriyacharee et al. (2012) studied the extraction of isoflavone from soy germ with different solvents: water, ethanol, methanol, acetone and acetonitrile. It was found that the isoflavone glucosides was efficiently extracted with polar solvents from aqueous acetone and acetonitrile. It can be seen that isoflavones is well soluble. Moreover, high power ultrasonic technique allows polar solvents to easily penetrate extracted materials by destroying the plant cell membrane. The technique can reduce the time of the extraction with

significantly higher yield. Ultrasound causes cavitation, which is a process that occurs in the middle of ultrasound sound wave by chemical and physical changes due to the bubbles that are produced by the compression and decompression in the liquid. Rostagno et al. (2002) investigated the effect of extraction of isoflavones from frozen soybeans compared to conventional extraction and ultrasonic extraction . It was found that the optimal condition for extraction of isoflavones was using ultrasound with 50% ethanol solution at 60 °C for 20 min.

Large molecular size of isoflavone in the glucosides form usually slows the absorption rate in the human body. However, soybean fermentation process can reduce the molecular size of the glucosides to aglycones by β-glucosidase. Previous investigations show that β-glucosidases from various microorganisms such as *Aspergillus oryzae* (Horii et al., 2009), *Bacillus subtilis* (Kuo and Lee, 2008), *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *L. casei* (Donkor and Shah, 2008), *Escherichia coli* (Ismail and Hayes, 2005), *Paecilomyces thermophila* (Yang et al., 2009), *Pseudomonas* sp. (Yang et al., 2004) *Thermotoga maritima* (Xue et al., 2009) and *B. coagulans* (Phongphisutthinant et al., 2015) were used. Especially, the source of β-glucosidases from *B. coagulans* PR03 is able to convert isoflavones from glucosides to aglycones (Wiriyacharee et al., 2011 and 2012) and easily isolated from Thai fermented soybean (tua-nao). Phongphisutthinant et al. (2015) reported that *B. coagulans* PR03 was predominant bacteria enriching isoflavone aglycones in tua-nao production. The well-absorbed structure of isoflavone aglycones would have higher potency as estrogen than isoflavone glucosides. The chemical forms of these isoflavones affect their biological activities (Izumi et al., 2000). Among them, isoflavone aglycones, especially the genistein and daidzein, exhibit higher biological activity than isoflavone glucosides (Kuo et al., 2006). Enzymatic hydrolysis especially β-glucosidase has become the method more specific than chemical hydrolysis (Song et al., 2011).

The scope of this study is to investigate the isoflavone glucosides extraction from soy germ using with ethanol in combination with ultrasonication technique. Additionally, β-glucosidases production from *B. coagulans* PR03 is optimized and it is applied to produce isoflavone aglycones from isoflavone glucosides.

MATERIALS AND METHODS

Materials

Soy germ is a by-product of the tofu industry. Normally, soy germ was used in the animal feed industry. It was obtained from the tofu factory in Chiang Mai province (Chiang Mai field crop research center). Soy germ was packaged in High Density Polyethylene (HDPE) plastic bags and stored at -18 ^{o}C until further used (not exceed 4 months storage).

Isoflavone glucosides extraction

Isoflavone glucosides were extracted from soy germ using a completely randomized design (CRD) with three replicates which were 40, 60, 80% and absolute (99.8%) ethanol as a solvent (ratio of 1:5 by weight) with high power ultrasonication technique at high power and low frequencies (Elma Schmidbauer GmbH, Singen, Germany) were fixed at 40 kHz and power levels 550 W/cm^2 (Fan et al., 2012). The study was 2^2 factorial design with 2 center points was employed. The process variables included: extraction temperature (x_1) ranging from 30 to 80 ^{o}C; extraction time (x_2) from 0 to 110 min were on daidzin, genistin, glycitin and total glucosides while the response variables. Lastly, the different time (60-180 min) for isoflavone glucosides extraction were continuously examined using a completely randomized design (CRD) with three replicates (Wiriyacharee, 2012).

β-Glucosidase production from *B. coagulans* PR03

For β-glucosidase production, the activated condition of *B. coagulans* PR03 (isolated from fresh tua-nao) was investigated by inoculating the culture in 10 ml nutrient broth and incubated at 30 ^{o}C for 24 hours. A medium development was also studied by Plackett and Burman Design ($n=8$) which was used to identify important factors affecting β-glucosidase activity of *B. coagulans* PR03 and used to find the suitable medium for cultivation. The optimal β-glucosidase production was studied using 2^2 factorial with central composite design (Wiriyacharee, 2012). The process variables included: beef extract (x_1) from 8 to 15 % ; pH value (x_2) ranging from 6 to 8 and the response variable was measured for β-glucosidases activity. *B. coagulans* PR03 was prepared in 90 ml of the developed medium at 30 ^{o}C for 24 hours. Furthermore, the β-glucosidase activity was measured every 3 hours to predict the optimal harvesting time of β-glucosidase.

β-Glucosidase activity analysis

One ml of inoculum was centrifuged at 12,000 g for 10 min to collected cells of *B. coagulans* PR03 and washed with a sodium phosphate buffer solution at pH 7.0. The β-glucosidases activity was measured by adding 0.5 mM *p*-nitrophenyl-beta-D-pyranoside (*p*-NPG) in an amount of 0.5 ml and incubated at 37 °C for 30 min. Finally, the reaction was stopped with 0.5 ml of 2M Na_2CO_3 and measured the absorbance at 620 nm. The β-glucosidase 1 unit was defined as the amount of enzyme required to hydrolyze 1 mol of *p*-Nitrophenol / 1 min (Fujita et al., 2010).

Isoflavone aglycones production

The production of isoflavone aglycones was investigated by extracted isoflavone glucosides solution (100 ml), β-Glucosidase from *B. coagulans* PR03 (100ml ; 0.4 Unit) and deionized water (800 ml) with the ratio of 1: 1: 8, time and temperature of isoflavone aglycones production were studied using 2^2 factorial experiment with central composite design. The process variables included: production time time (x_1) from 72 to 168 hours ; production temperature (x_2) ranging from 30 to 45 °C and the response variables were measured for daidzein, genistein, glycitein and total aglycones. During the experiment, the solution was shaked at 200 rpm.

HPLC Analysis of isoflavone glucosides and aglycones

The analysis of isoflavones were conducted by High Performance Liquid Chromatography (HPLC) technique as per Klejdus et al., (2005) with Diode array detector (DAD) using Column from Inersil ODS3 size 250x4.6 mm., injection volume of 20 µL, flow rate at 1.0 ml/min, mobile phase A as 0.1% acetic acid, mobile phase B as methanol, gradient separation at 5 min 35% B, 8 min 42% B, 22 min 90% B, 28 min 100% B, 29.5 min 30% B and held at that level for 2.5 min, a total run time was 32 min, column oven at 40 °C, total run time was 32 min and 255 nm. of detection wavelength and isoflavone glucosides and aglycones contents as daidzein, daidzin, glycitein, glycitin, genistein and genistin were obtained by comparison with isoflavone standards solution.

Statistical Analysis

The experimental design and result analysis particularly Plackett and Burman Design and completely randomized design (CRD) were established using SPSS 11.0 (SPSS Inc., Chicago, USA) and Design-Expert software version 7.1 (Statease Inc., Minneapolis, USA). One-way analysis of variance

(ANOVA) and multiple-range test were used for comparison of the results. Homogenous groups and the least significant difference (LSD) at a significant level ($P \leq 0.05$) were determined. To obtain interaction between process variables and responses, 2^2 factorial design with 2 center points and central composite design were employed. The variables included: factor1 (x_1) and factor2 (x_2) with 2 center points. The interaction between each response and process variables were estimated by a second order polynomial equation (1). Analysis of variance (ANOVA) was also used to examined the statistical significant of the models.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \quad (1)$$

where Y is a response, x is a process variable and β is a coefficient of the term.

RESULTS

The optimal concentration of ethanol on isoflavone glucosides extraction from soy germ

The effect of ethanol on isoflavone glucosides extraction from soy germ was examined. The result showed that 80% ethanol extract had the highest amount of isoflavone glucosides (280.80 mg/100 g. dry basis) (Table 1). This result coincides with a previous study of Seung et al., (2010) which demonstratedthe maximum amount of isoflavones was obtained from cotyledons (2.18 mg/g.) when extracted with 80-90% (v/v) ethanol above 90 °C for 100 min.

Table 1. Effect of ethanol on isoflavone glucosides extraction from soy germ.

Concentration of ethanol (%)	Isoflavone glucosides (mg/100 g. dry basis)			
	Daidzin	Genistin	Glycitin	Total glucosides
Absolute (99.8%)	124.63±0.11^b	128.32±0.06^b	12.19±0.53^b	265.17±0.70^b
80	130.24±0.45^a	137.59±0.49^a	16.07±1.27^a	280.70±0.43^a
60	117.72±0.19^c	121.86±0.28^c	11.57±0.53^c	251.15±0.62^c
40	98.45±0.52^d	102.76±0.13^d	8.04±0.06^d	209.25±0.59^d

Note: Different superscripts in the same column denoted by small letter are significantly different ($P \leq 0.05$).

Effect of temperature and time on isoflavone glucosides extraction from soy germ

The effect of temperature (30-80 °C) and time (0-110 min) on the extraction efficiency of isoflavone glucosides extraction from soy germ was studied. The relationship of isoflavone glucosides with extraction time and temperature are shown in Table 2.

The statistical analysis of the results allowed fitting a coded empirical model relating daidzin (equation 2), genistin (equation 3), glycitin (equation 4) and total glucosides (equation 5) to extraction temperature and extraction time. The ANOVA analyses show a high correlation coefficient and a good performance of the *F* test for regressions. Therefore, equation 2, 3, 4 and 5 are predictive of daidzin, genistin, glycitin and total glucosides in the investigated range of factors.

Daidzin = 105.94-3.75(extraction temperature) +2.33(extraction time) +0.02(extraction temperature)(extraction time) +0.04 (extraction temperature)^2 -0.02(extraction time)^2 ; R^2= 0.9766 (2)

Genistin = 133.00-4.81(extraction temperature) +1.20(extraction time) +0.03(extraction temperature)(extraction time) +0.04 (extraction temperature)^2 -0.01(extraction time)^2 ; R^2=0.9468 (3)

Glycitin = 3.07-0.11(extraction temperature)+0.03 (extraction time) +4.85x10^{-4}(extraction temperature)(extraction time) +1.12x10^{-3}(extraction temperature)^2 -2.61x10^{-4}(extraction time)^2 ; R^2=0.9343 (4)

Total glucosides = 243.27-8.69(extraction temperature) +3.5(extraction time) +0.05(extraction temperature)(extraction time) +0.09(extraction temperature)^2 +0.03(extraction time)^2 ; R^2=0.9666 (5)

Table 2. Effects of temperature and time on isoflavone glucosides extraction from soy germ extracted with 80% ethanol.

Extraction temperature (ºC)	Extraction time (min)	Isoflavone glucosides (mg/100g. dry basis)			
		Daidzin	Genistin	Glycitin	Total glucosides
30	0	17.72±2.39	9.00±0.39	0.35±0.01	27.55±3.43
80	0	59.76±2.39	38.40±0.79	1.36±0.15	99.52±3.22
30	110	149.96±11.44	104.04±1.33	3.11±0.10	257.11±11.78
80	110	270.23±0.18	247.43±32.32	6.06±0.11	523.72±32.37
30	55	122.74±0.89	115.33±12.20	2.54±0.01	240.61±11.39
80	55	189.09±1.51	148.14±6.46	3.45±0.01	340.68±4.95
55	0	43.07±4.16	31.88±6.67	0.89±0.01	75.84±10.83
55	110	157.68±1.87	130.29±10.00	2.86±0.08	290.83±11.90
55	55	128.34±0.28	92.45±0.70	2.43±0.01	223.22±0.99
55	55	128.44±0.39	86.92±0.81	2.55±0.01	217.90±0.85

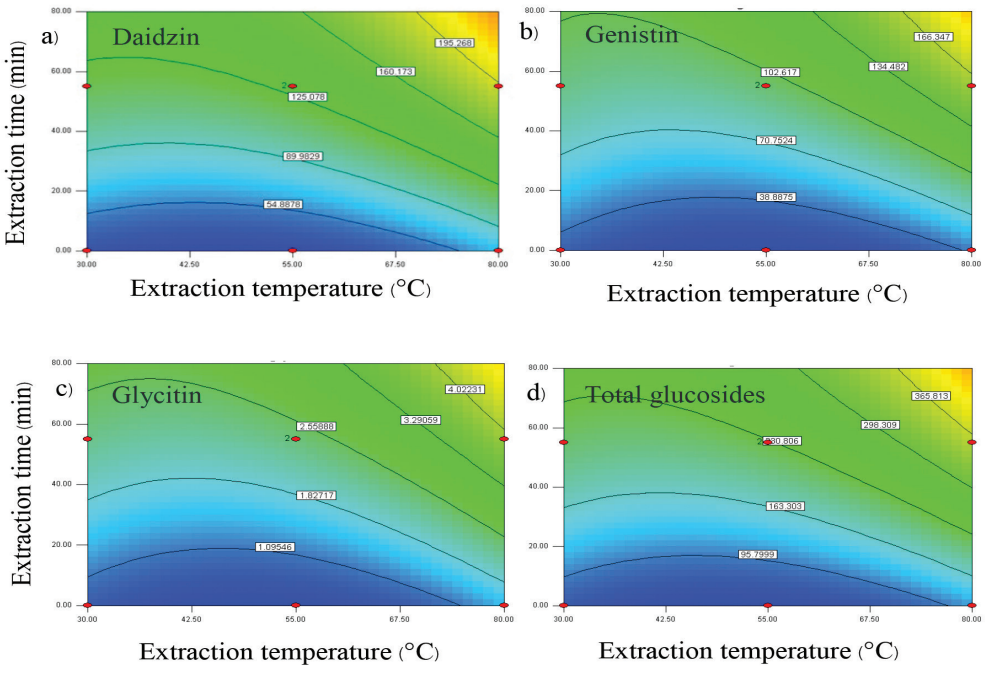

Figure 1. Response surfaces of the correlation between extraction time and temperature on a) Daidzin, b) Genistin, c) Glycitin and d) Total glucosides.

The response surfaces for daidzin, genistin, glycitin and total glucosides, are shown in Figure 1. This surface indicates that the conditions that maximize yield are 80 °C and 110 min. It is also shows an interaction between extraction temperature and time. With the increasing extraction time the amounts of daidzin, genistin, glycitin and total glucosides are increased. Therefore, the extraction temperature should be 80 °C and the further extraction time should be continuously investigated.

Effect of time on isoflavone glucosides extraction from soy germ

The results in Table 3 show that an extraction time of 160 min could extract the maximum amount of isoflavone glucosides (595.93 mg/100 g. dry basis). It could be concluded that the suitable extraction condition for isoflavone glucosides from soy germ was the high power ultrasonication technique with 80% ethanol at 80 °C for 160 min. This trend was similarly observed in the previous study, 90 °C for 100 min (Wiriyacharee et al., 2012).

Table 3. Effect of time on isoflavone glucosides extraction from soy germ by 80% ethanol at 80 °C.

Time (min)	Isoflavone glucosides (mg/100g. dry basis)			
	Daidzin	Genistin	Glycitin	Total glucosides
60	196.55±0.11^{f}	139.32±0.21^{g}	45.09±0.44^{g}	380.96±0.20^{g}
80	216.28±2.89^{e}	152.83±0.31^{f}	49.51±0.22^{f}	418.61±2.42^{f}
100	243.58±3.85^{d}	166.72±0.84^{e}	54.13±0.09^{e}	464.44±4.36^{e}
120	262.87±0.23^{c}	186.34±0.14^{c}	62.25±0.82^{c}	511.45±0.96^{c}
140	268.80±0.89^{b}	190.73±0.59^{b}	63.75±0.48^{b}	523.28±1.87^{b}
160	307.47±0.64^{a}	214.84±0.40^{a}	73.63±0.08^{a}	595.93±1.09^{a}
180	245.16±0.36^{d}	171.12±0.21^{d}	58.46±0.06^{d}	474.74±0.62^{d}

Note: Different superscript in the same column denoted by small letter are significantly different ($P \leq 0.05$).

Study on medium formulation and condition for β-glucosidase production by *B. coagulans* PR03

Plackett and Burman Design ($n=8$) was used to screen factors affecting β-glucosidase activity of *B. coagulans* PR03. Table 4 shows that beef extract and pH were significant factors that positively affected β-glucosidase activity. Thus, they were optimized in the next experiment while other factors were fixed

according to their effect. The factor with positive impact such as magnesium sulfate was fixed at a high level. On the other hand, negative impact factors including peptone, glucose and temperature were fixed at a low level.

Table 4. Effect of cultivation factors on β-glucosidase activity of *B. coagulans* PR03.

Factors	Low level (-)	High level (+)	β-glucosidase activity (mU/ml)	
			Effect	Calculate-test
A = Beef extract (%)	2.00	10.00	0.010	3.536*
B = Peptone (%)	2.00	10.00	-0.003	-1.179
C = Magnesium sulfate (%)	0.02	0.10	0.009	3.071
D = Glucose (%)	2.00	6.00	-0.001	-0.500
E = pH	5.00	7.00	0.016	5.500*
F = Temperature (°C)	30.00	50.00	-0.004	-1.393

Note: * significantly different with 80% confident interval (t-table = 3.078).

Study on optimal conditions affecting the β-glucosidase production from screened factors

The results from Table 4 showed that beef extract and pH affected on β-glucosidase activity. The effects of beef extract and pH were further examined and the results are shown in Table 5.

The statistical analysis of the results allowed fitting a coded empirical model relating β-glucosidase activity (equation 6) to extraction temperature and time. The ANOVA analyses showed a high correlation coefficient and a good performance of the *F* test for regressions. Equation 6 is predictive of β-glucosidase activity in the investigated range of factors.

$$\begin{aligned}\text{β-glucosidase activity} = {} & -25.38-0.68(\text{beef extract}) \\ & +7.96(\text{pH}) \\ & +0.20(\text{beef extract})(\text{pH}) \\ & -0.03(\text{beef extract})^2-0.66(\text{pH})^2\end{aligned} \quad ; R^2 = 0.9577 \qquad (6)$$

Table 5. The β-glucosidase activities from varied beef extract and pH value.

Beef extract (%)	pH value	β-glucosidase activity (mU/ml)
9.03	6.29	1.68
13.97	6.29	1.74
9.03	7.71	2.35
13.97	7.71	3.80
8.00	7.00	2.15
15.00	7.00	2.53
11.50	6.00	0.92
11.50	8.00	3.09
11.50	7.00	2.87
11.50	7.00	2.67

Note: The β -Glucosidase 1 unit was defined as the amount of enzyme required to hydrolyze 1 mol of *P*- Nitrophenol / 1 min.

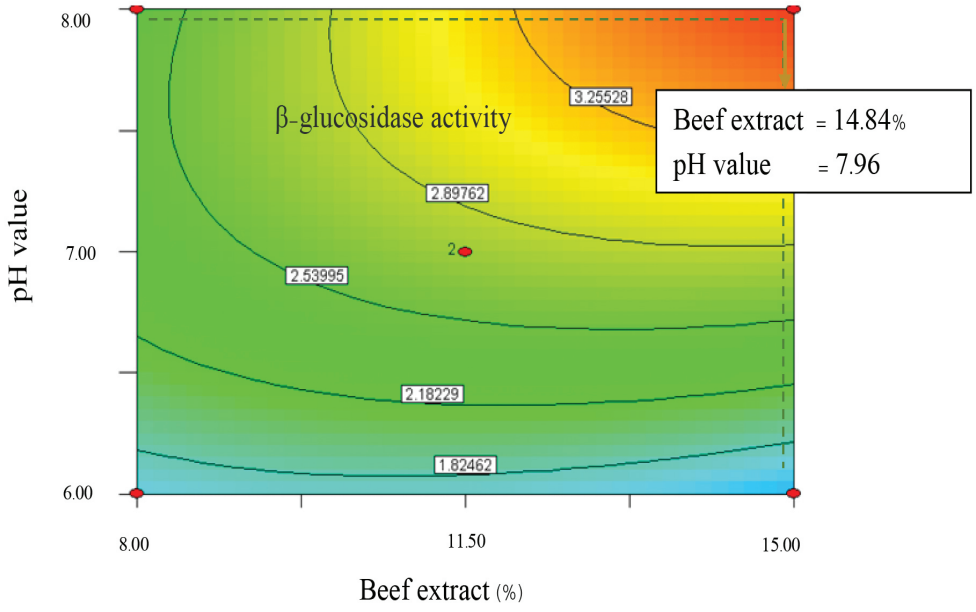

Figure 2. Response surface of the correlation between beef extract and pH value on β-glucosidase activity.

From the equation 6 and Figure 2, the predicted optimal model showed that the optimal condition for *B. coagulans* PR03 cultivation was beef extract 14.84% with the fixed level of 2.00% peptone, 2.00% glucose and, 0.10% magnesium sulfate, then incubated at 30 °C and pH 7.96. However, the suitable harvesting time for the highest β-glucosidase activity from *B. coagulans* PR03 was 18 hours with the maximum activity of 4.01 mU/ml (Figure 3).

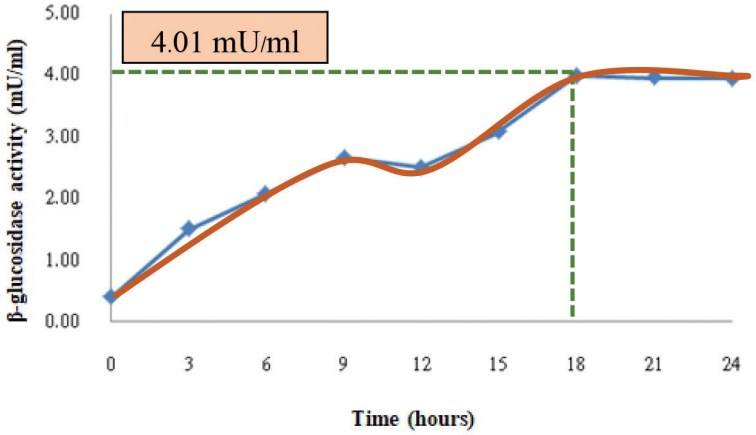

Figure 3. β-glucosidase activity at different incubation time.

The optimal time and temperature of isoflavone aglycones production using β-glucosidase from *B. coagulans* PR03

Isoflavone glucosides preparing from soy germ using high power ultrasonication technique with 80% ethanol at 80 °C for 160 min was applied as a substrate for isoflavone aglycones production. It was necessary to use the β-glucosidase from *B. coagulans* PR03 in order to convert to aglycone form.

The equations 7, 8, 9 and Figure 4 show that the amounts of daidzein, glycitein and total isoflavone aglycones were depended on production time, temperature and interaction between production time and temperature. Increasing production time and temperature, the amount of daidzein, glycitein and total isoflavone aglycones were increased but genistein was not significantly affected ($P \leq 0.05$). Therefore, the suitable time and temperature for isoflavone aglycones production could be 120 hours and 37.50 °C with the total isoflavone aglycones of 112.90 µg/ml.

Daidzein = -759.20 +1.78 (production time) + 39.59 (production temperature) - 0.02 (production time) (production temperature)-0.004 (production time)^2-0.53(production temperature)^2 R^2=0.8515 (7)

Glycitein = -224.70+0.88(production time) +12.9(production temperature) - 0.02 (production time)(production temperature)-0.003(production time)^2 - 0.18 (production temperature)^2 R^2=0.8554 (8)

Total aglycones = -921.19+2.64(production time) - 50.45(production temperature) - 0.02(production time)(production temperature)-0.007(production time)^2 - 0.69(production temperature)^2 R^2=0.8210 (9)

Table 6. Effect of time and temperature on isoflavone aglycones production using β-glucosidase from *B. coagulans* PR03.

Production time (hours)	Production temperature (°C)	Isoflavone aglycones (µg/ml)			
		Daidzein	Genistein	Glycitein	Total aglycones
86.06	32.2	30.79±0.18	42.12±0.72	12.36±0.12	85.27±0.35
153.94	32.2	43.71±0.13	45.68±0.15	16.45±0.28	105.84±0.11
86.06	42.8	10.64±1.07	29.87±3.21	6.07±0.41	46.57±0.21
153.94	42.8	11.25±0.12	31.80±1.02	9.40±0.16	52.44±0.32
72	37.5	45.42±0.13	43.81±3.78	16.61±2.91	105.84±0.75
168	37.5	50.81±0.21	46.65±1.37	13.48±2.46	110.94±0.88
120	30	45.02±0.34	51.24±0.52	19.89±0.66	116.15±0.45
120	45	11.93±0.06	33.02±0.65	11.16±0.18	56.11±0.12
120	37.5	51.26±0.81	50.28±0.81	12.33±0.20	113.88±0.65
120	37.5	51.31±0.06	48.45±0.09	12.15±0.08	111.92±0.33

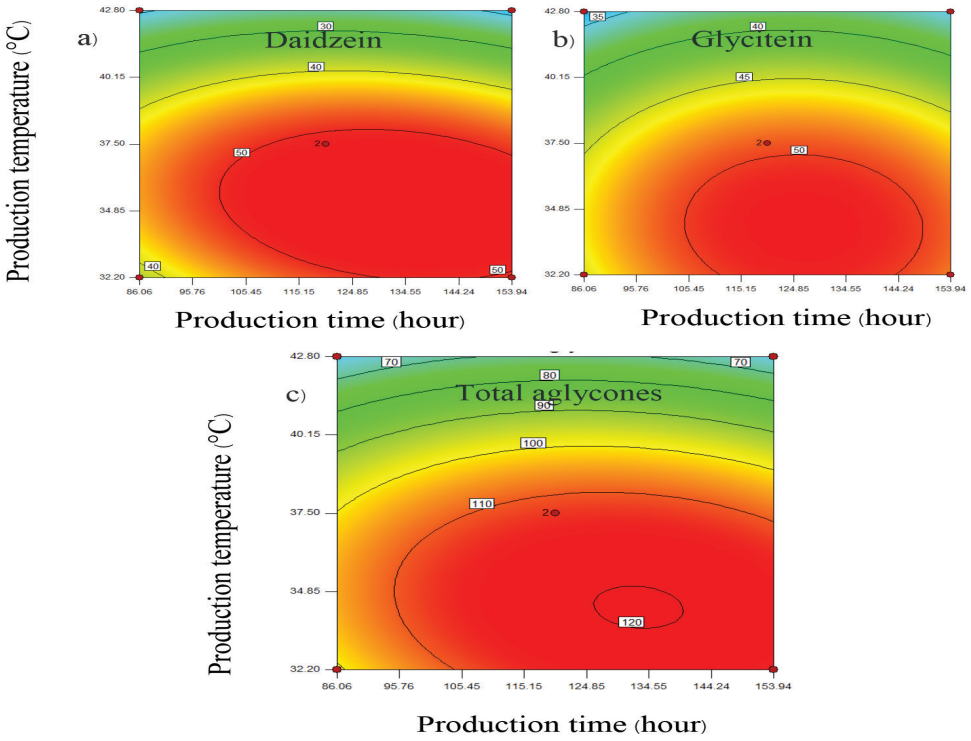

Figure 4. Response surfaces of the correlation between production time and temperature on a) Daidzein b) Glycitein and c) Total aglycones.

DISCUSSION

In this research, soy germ was used as a precursor of isoflavone aglycones production because of rich isoflavone glucosides content (Nahas et al., 2004) and a by-product of the tofu industry. Isoflavone glucosides can be extracted using other polar solvents, such as acetone, acetonitrile and methanol (Wiriyacharee et al., 2012). However, from the safety consumption point of view, this research selected ethanol as main extracting solvent with high power ultrasonication. According to Mauricio et al. (2003) reported isoflavone extraction by ultrasonication gave better results than mix-stiring technique and at a low cost, low toxicity and eco-friendly.

For β-glucosidase from *B. coagulans* PR03 were used incubation pH and temperature at 7.96 and 30 °C. Similar to reported of Zehranur et al. (2017) β-glucosidase belonging to the strains (*L. rhamnosus* EA1 and *L. casei* SC1) exhibited high β-glucosidase activity at pH 7.5 and an optimum temperature at 30 °C.

The isoflavone aglycones were able to produce using isoflavone glucosides with β-glucosidase from *B. coagulans* PR03. According to Park et al. (2003) reported that β-glucosidase from *Paenibacillus xylanilyticus* KJ-03 was capable of hydrolyzing isoflavone daidzin and genistin. Moreover, the application of β-glucosidase and its immobilization could be an alternative choice to promote the bioconversion of the isoflavone glucosides to aglycones such as chitosan was used as stationary phase for immobilized β-glucosidase (Luciana et al., 2014). Lastly, isoflavone aglycones could be applied as bioactive food ingredients for healthy products or supplement food.

CONCLUSION

The suitable method for isoflavone glucosides extraction was high power ultrasonication using 80% ethanol at 80°C for 160 min. The extraction yield of isoflavone glucosides was 595.93 mg/100 g. dry basis. The resulting isoflavone glucosides were converted to isoflavone aglycones by β-glucosidase produced from *B. coagulans* PR03. The optimal media for cultivation of *B. coagulans* PR03 was the mixture of peptone, beef extract, glucose, and magnesium sulfate at amounts of 2.00%, 14.84%, 2.00% and 0.10%, respectively. The incubation condition was at 30°C and pH 7.96 for 18 hours resulting in the highest β-glucosidase activity (4.01 mU/ml). To convert isoflavone glucosides to isoflavone aglycones, 1:1:8 ratio of isoflavone glucosides, *B. coagulans* PR03 and deionized water were incubated at 37.50 °C for 120 hours.

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