

Hydrolysis Optimization of Isoflavone Conversion in Soy Germ (Chiang Mai 60) Using Response Surface Methodology (RSM)

Sriwiang Tipkanon*, **Ratchanee Charoen**

Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachin Buri, Thailand

Wanticha Savedboworn

Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachin Buri, Thailand

Ahmed Moursy Abdallah

Department of Food and Dairy Sciences and Technology, Faculty of Environmental Agricultural Science, Suez Canal University, El-Arish, Egypt

* Corresponding author. E-mail: swtn@kmutnb.ac.th

Received: 29 August 2014; Accepted: 3 February 2015; Published online: 19 February 2015

© 2015 King Mongkut's University of Technology North Bangkok. All Rights Reserved.

Abstract

Response surface methodology (RSM) by using central composite design (CCD) of three factors was employed to optimize the hydrolysis conditions of isoflavone conversion in soy germ. A five-level, three variables central composite design with six replicates at central point was employed. The three independent variables investigated in this experiment were pH (X_1) 3-8; reaction temperature (X_2) 25-75°C; and reaction time (X_3) 2-12 h. Dependent variables were highly fitted to the regression equation for both total amount of isoflavone glucosides (Y_1) and isoflavone aglycones (Y_2). The high regression coefficient values indicated that the variables were fitted to the regression equation for the total isoflavone glucosides ($R^2 = 0.937$) and total isoflavone aglycones ($R^2 = 0.956$). Optimum hydrolysis conditions for maximizing the determination of total isoflavone aglycones content were: at pH = 5.3, reaction temperature = 51°C, and reaction time = 7.5 h.

Keywords: Hydrolysis, Soy germ, Isoflavone, RSM, CCD

1 Introduction

Soybeans (*Glycine max* (L.) Merrill) have been incorporated into daily diets in many Asian countries for centuries [1]. Each soybean seed consists of three basic parts: seed coat, cotyledons, and embryo. The embryo consists of three parts: radicle, hypocotyl and epicotyls; radicle and hypocotyl together are known as germ. Soy germ is only 2% of the whole soybean seed [2,3]. Soybean seeds often have the hulls removed

prior to oil extraction to both increase the processing capacity of the plant and protein content of the meal. Following the process, soy germ (byproduct) is mechanically separated from soybean during the process of dehulling and cracking [4], but few investigations using this germ have been conducted. The germ considered to be rich source of isoflavones and other nutrients. On average, soy germ contained about 40% protein, essential fatty acids, omega 3 and 6, lecithin, vitamin E, and saponin. Soy germ is by far the most

Please cite this article as: S. Tipkanon, R. Charoen, W. Savedboworn, and A. M. Abdallah, "Hydrolysis Optimization of Isoflavone Conversion in Soy Germ (Chiang Mai 60) Using Response Surface Methodology (RSM)," *KMUTNB Int J Appl Sci Technol*, Vol. 8, No. 2, pp. 127-135, Apr.-June 2015, <http://dx.doi.org/10.14416/j.ijast.2015.02.001>

nutrient-dense soy ingredient one can consume to obtain all of the proven health benefits of the soybean [4]. Soybean contains high amount of isoflavone, with structure similar to human estrogen. However, the isoflavone content in soy germ is 5-6 times higher than in soy cotyledons [2,3]. Soy isoflavones are present in 12 chemical forms: three aglycones (daidzein, genistein, glycitein) and three conjugated forms to each aglycone called glucosides. The conjugated forms have an additional glucose moiety, which could be free of the other groups (daidzin, genistin, glycitin) or could be bound to either an acetyl group (6'-*O*-acetyldaizin, -genistin, -glycitin) or a malonyl group (6'-*O*-malonyldaizin, -genistin, -glycitin) [1,5].

The isoflavones in soy occur primarily as *β -glucoside* forms with a small percentage as the principal bioactive aglycone and the percentage of each differs depending on varieties, crop year, growing conditions, and food processing conditions [1,5-7]. Liu et al. [8] reported that isoflavone glucosides (daidzin, glycitin, and genistin) were the major soy isoflavones and comprised up to 70% of the total soy isoflavone. Numerous studies have revealed that isoflavone aglycones are superior to isoflavone glucosides in various bioactivities [9,10]. There is interest in increasing the amounts of isoflavone aglycones in soy germ, soybean, and soy product. Some authors have reported that the isoflavone glucosides were hydrolyzed into their corresponding aglycones during soaking by the *β -glucosidase* enzyme [11-14]. During soybean processing, isoflavone glucosides can be converted to isoflavone aglycones. When soy germ are soaked in water, the endogenous *β -glucosidase* enzyme hydrolyze the glucoside forms to their aglycone forms [14]. Under acidic conditions, the isoflavone glucosides can be deconjugated to give aglycones. In addition, under acidic or basic conditions, the acetyl and malonyl groups can also be removed [15]. Isoflavone are quite heat stable. Some loss of total isoflavone content in soy product is most likely due to its leaching during aqueous processing of soybeans [5]. Although isoflavones are not destroyed by heat in conventional food processing operations, heat treatments cause a change in the conjugation profile of the isoflavones in soy product [6,16]. Tipkanon et al. [9] investigated conversion of isoflavone glucosides to isoflavone aglycones in non-defatted soy germ flour by added *β -glucosidase* enzyme (1-4 unit/g of

non-defatted soy germ flour at pH 5-6, 35-55°C incubation temperature, and 1-6 h incubation time) and reported 79-100% conversion yield. They concluded that non-defatted soy germ flour treated by 1 unit *β -glucosidase* enzyme at pH 5 for 5 h at 45°C were the optimum condition for hydrolysis of isoflavone glucosides. Pandjaitan et al. [13] investigated conversion of genistin to genistein in soy protein concentrate by added *β -glucosidase* enzyme (0-4 unit/g of defatted soy flour at pH 2-10, 37-70°C incubation temperature, and 0.5-4 h incubation time) and reported 41-100% conversion yield. They reported that the optimum conditions were pH 5, 50°C incubation temperature, 2 unit of enzyme/g soy flour, 1 h incubation period, and 1:10 (w/v) defatted soy flour to water ratio. The conversion of isoflavone glucoside to aglycone form results in an increased amount of health-promoting compound in soy germ, soybean, and soy products [11-14]. Isoflavone aglycones, especially genistein and daidzein have attracted considerable attention for their health benefits in the prevention of several cancers such as breast and colon cancer, osteoporosis, postmenopausal symptoms, hypercholesterolemia, and cardiovascular disease [9,10,17,18]. For health benefit of soy germ isoflavone, Nahas et al. [19] reported that soy germ isoflavone exerted favorable effects on vasomotor symptoms and lipid profile in postmenopausal women. Therefore, soy germ can act as a vehicle to deliver isoflavone when incorporated in food and supplementary products for health-promoting purposes [3].

The objective of this research was to study hydrolysis optimization using response surface methodology (RSM) of isoflavone conversion in soy germ in order to develop the isoflavone aglycone-enriched soy germ for basic information in further study of health food product development.

2 Materials and Methods

2.1 Material

Germ (non-defatted) from Thai soybean variety (cv. Chiang Mai 60) was obtained from Juuboonyong Agriculture Co., Ltd., Thailand. This variety was chosen because it is commercially available and on basis of our preliminary study, it contains a high level of total isoflavone content (30.60 $\mu\text{mol/g}$ dry weight

basis). Soy germ was packed in plastic (HDPE) bags, and stored in cardboard boxes at -18°C until further analysis.

2.2 Reagent

Isoflavone glucoside standards (genistin, glycitin and daidzin) and isoflavone aglycone standards (genistein, glycitein, and daidzein) were purchased from LC Laboratories. (Woburn, MA, U.S.A.). Other reagents were analytical grade and purchased from Fisher Scientific (Pittsburgh, PA., U.S.A.) and Sigma Chemical Co. (St. Louis, MO., U.S.A.).

2.3 Experimental design and statistical analysis

Portion of soy germ (~5g) were mixed with 25 mL deionized water and total volume was adjusted to pH with citrate-phosphate buffer according to the conditions of the designed experiment below. Butylated hydroxytoluene, BHT (0.01% w/v) was added to each sample to prevent oxidation. Immediately after the require reaction time was reached, the residue was collected, washed once with deionized water, and dried for HPLC analysis.

The central composite design (CCD) is an experimental design widely used for estimating second order response surfaces [20]. In this research, the central composite design (CCD) was use to study the interaction of hydrolysis variables and to predict the optimum hydrolysis conditions for isoflavone conversion (isoflavone glucosides to isoflavone aglycones) by using response surface methodology (RSM) [20]. The range and coded level of hydrolysis variables studied are listed in Table 1, select based on the preliminary experiments. The three variables were pH ($X_1= 3-8$), reaction temperature ($X_2= 25-75^\circ\text{C}$), and reaction time ($X_3 = 2-12$ h). The coded values of the independent variables were -1.682 (lowest level), -1 (low level), 0 (middle level), 1 (high level), and 1.682 (highest level). Correspondences between these coded and actual values are presented in Table 1. The experimental design consisted of 20 points, including six replications at the central point, and was carried out in a random order (Table 2). The concentrations of total isoflavone glucosides (Y_1) and total isoflavone aglycones (Y_2) were taken as response of design experiment. The full quadratic model [20] for isoflavone

conversion were established by using the following mathematical equation (Eq.1)

Table 1: Hydrolysis variables and their coded and actual levels of three variables

Variable	Symbol	Coded level of variable				
		-1.682	-1	0	1	1.682
pH	X_1	3	4	5.5	7	8
Reaction temperature (°C)	X_2	25	35	50	65	75
Reaction time (h)	X_3	2	4	7	10	12

Table 2: Coded level combinations for a three-variable central composite orthogonal and rotatable design (CCD)

Test run ^a no.	Coded level of variable ^b		
	pH	RT (°C)	Rt (h)
1	-1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	-1	1	1
5	1	-1	-1
6	1	-1	1
7	1	1	-1
8	1	1	1
9	0	0	-1.682
10	0	0	1.682
11	0	-1.682	0
12	0	1.682	0
13	-1.682	0	0
14	1.682	0	0
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Note: ^a Test runs were performed in a random order.

^b RT, reaction temperature; Rt, reaction time.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where Y_i is a response variables of isoflavone content (total isoflavone glucosides and total isoflavone aglycones), the b_i are regression coefficients for linear effects; b_{ik} are regression coefficients for effects from interaction; b_{ii} are regression coefficients for quadratic effects; and X_i are coded experimental levels of the variables. Minitab Statistical software (Minitab Pty Ltd., Inc. Sydney, NSW) and SPSS software (Statistical Software Package for Social Sciences, SPSS Inc., and IBM Co., Chicago, IL) were used to design

of experiment and fit the second order polynomial equation of the experimental data, and STATISTICA (StatSoft, Inc. Tulsa, OK) was used to plot the response surface graphs. The quality of fit of the second order polynomial model equation was expressed in the form of contour plot, in order to illustrate the relationship between the responses and the experimental levels of each of the variables utilized in this study.

2.4 High-performance liquid chromatography (HPLC) analysis of isoflavone

Each test sample (~0.5 g) was separately extracted at 65°C for 2 h in 80% aqueous methanol solvent and then saponified with 3 mL of 2 M NaOH with constant shaking on an orbital shaker at ambient temperature for 10 min. Each mixture was then acidified by 1 mL of glacial acetic acid, filtered, diluted with water to 50% methanol and centrifuged to clarify. The supernatant was collected and then injected into a reversed-phase HPLC system [21].

A high performance liquid chromatography (HPLC) solvent gradient system was used according to the method of Griffith and Collison [22]. The HPLC system consisted of a Hewlett Packard® 1100 series HPLC (Agilent Technologies, Forest Hill, Vic., Australia) with an autosampler, a quaternary pump, a diode array ultraviolet (UV) visible detector, Lichrospher® 100RP-18 (250 mm × 4.6 mm internal diameter, 5 µm) reversed phase column. For the linear HPLC gradient, mobile phase A was combined water + methanol + 0.1% glacial acetic acid (88 + 10 + 2), and mobile phase B was combined methanol + 0.1% glacial acetic acid (98 + 2). An injection of a 10-µL of sample was followed by an increase of mobile phase B from 20 to 100% and a decrease of mobile phase A from 80% to 0% in 25 min. The solvent flow rate was 0.8 ml/min. The chromatograms obtained at a wavelength of 260 nm were used to quantify isoflavone concentration. In this study, isoflavone glucosides (daidzin, genistin, and glycitin) content and isoflavone aglycones (daidzein, genistein and glycitein) content were performed in triplicate, and results were reported as µmol/g.

2.5 Verification of model

Optimization of hydrolysis conditions, including pH of hydrolysis, reaction temperature, and reaction time

for maximize a quantitative HPLC determination of isoflavone aglycones in soy germ were calculated by using the predictive equation from RSM. The actual determination of isoflavone aglycones was carried out by HPLC after hydrolysis at the optimum conditions, and the result (observed value) was compared to the predicted value.

3 Results and Discussion

3.1 Model fitting for response surface methodology (RSM)

The experimental design was employed by using a five-level three-factor central composite design with six replicates at the central point. Table 3 showed the treatments with coded levels and their experimental results of isoflavone glucosides (daidzin, genistin, and glycitin), and isoflavone aglycones (daidzein, genistein, and glycitein) in soy germ.

Without being treated (control), raw soy germ contained the following concentrations of isoflavones: daidzin (13.21 µmol/g), genistin (4.35 µmol/g), glycitin (6.12 µmol/g), daidzein (1.47 µmol/g), genistein (0.56 µmol/g), and glycitein (0.58 µmol/g). The concentrations of the three glucosides (daidzin, 3.11-7.63 µmol/g; genistin, 1.37-2.90 µmol/g; and glycitin, 1.36-4.75 µmol/g) decreased comparing with control. In contrast, the three aglycones (daidzein, 9.56-14.34 µmol/g; genistein, 2.05-3.75 µmol/g; and glycitein, 2.77-5.97 µmol/g) increased as a result of conversion of their corresponding glucosides. The results revealed that the hydrolysis conditions (pH, reaction temperature, and reaction time) affected to isoflavone glucosides and isoflavone aglycones concentration.

Several researchers also demonstrated that isoflavone glucosides (in soybean seed, soy germ, soy protein concentrate, and soy food) can be converted to isoflavone aglycones depending on enzyme concentration, substrate concentration, pH, soaking temperature, and soaking time during soaking [11-14,23,24].

The coefficients of the regression model for the determination of isoflavone glucosides and isoflavone aglycones. The full quadratics model was established using mathematical equation in Eq. 1. The predicted concentrations of total isoflavone glucosides and total isoflavone aglycones models are showed in Eq. 2 and Eq. 3, respectively.

Table 3: Central composite design arrangement and the experimental value for response variables of hydrolysis of soy germ

Test run ^a no.	Hydrolysis condition ^b			Isoflavone glucosides (μmol/g dry weight basis)*			TIG ^c (μmol/g)	Isoflavone aglycones (μmol/g dry weight basis)*			TIA ^c (μmol/g)
	pH (X ₁)	RT (°C) (X ₂)	Rt (h) (X ₃)	Daidzin	Genistin	Glycitin		Daidzein	Genistein	Glycitein	
Raw soy germ (control)				13.21	4.35	6.12	23.68	1.47	0.56	0.58	2.61
1	4	35	4	5.93	2.53	3.90	12.36	10.30	2.58	3.50	16.38
2	4	35	10	5.7	2.42	3.06	11.18	11.61	2.85	3.88	18.34
3	4	65	4	6.50	2.24	4.01	12.75	10.64	2.81	3.22	16.67
4	4	65	10	5.81	2.4	3.44	11.65	11.55	3.06	3.95	18.56
5	7	35	4	7.03	2.89	4.75	14.67	9.56	2.53	2.77	14.86
6	7	35	10	6.86	2.65	4.31	13.82	10.14	2.63	3.12	15.89
7	7	65	4	7.63	2.89	4.75	15.27	9.59	2.05	2.89	14.53
8	7	65	10	5.91	2.35	3.89	12.15	10.97	2.93	3.77	17.67
9	3	50	7	6.23	2.90	4.18	13.31	10.64	2.69	3.35	16.68
10	8	50	7	6.35	2.85	4.43	13.63	10.15	2.44	3.32	15.91
11	5.5	25	7	6.01	2.29	3.73	12.03	11.43	3.13	3.42	17.98
12	5.5	75	7	5.73	2.22	3.26	11.21	11.56	3.46	3.98	19.00
13	5.5	50	2	6.43	2.55	4.31	13.29	10.37	2.78	2.99	16.14
14	5.5	50	12	5.54	2.45	3.76	11.75	11.31	2.89	3.48	17.68
15	5.5	50	7	3.29	1.38	1.48	6.15	14.42	3.59	5.79	23.80
16	5.5	50	7	3.38	1.37	1.36	6.11	14.20	3.53	5.82	23.55
17	5.5	50	7	3.15	1.50	1.68	6.33	14.33	3.40	5.97	23.70
18	5.5	50	7	3.11	1.42	1.65	6.18	14.34	3.64	5.82	23.80
19	5.5	50	7	3.17	1.53	1.72	6.42	14.32	3.52	5.76	23.60
20	5.5	50	7	3.21	1.45	1.61	6.27	14.11	3.75	5.82	23.68

Note: ^a Test runs were performed in a random order.
^b RT, reaction temperature; Rt, reaction time.
^c TIG, total isoflavone glucosides; TIA, total isoflavone aglycones.
* Standard deviation (SD) range was 0.05-2.13.

The coefficient of determination or (R²) for both total isoflavone glucosides and total isoflavone aglycones, which is an indicator of how well the model fits the data, were 0.937 and 0.956, respectively, suggesting an adequate model for all response variables.

$$\text{Total isoflavone glucosides } (Y_1) = 67.287 - 11.359X_1 - 0.759X_2 - 3.145X_3 - 0.011X_1X_2 - 0.047X_1X_3 - 0.006X_2X_3 + 1.149X_1^2 + 0.009X_2^2 + 0.249X_3^2 \quad (2)$$

$$\text{Total isoflavone aglycones } (Y_2) = -43.769 + 12.428X_1 + 0.795X_2 + 3.771X_3 + 0.005X_1X_2 + 0.009X_1X_3 + 0.006X_2X_3 - 1.196X_1^2 - 0.008X_2^2 - 0.274X_3^2 \quad (3)$$

3.2 Effect of hydrolysis conditions on total isoflavone glucosides and total isoflavone aglycones concentration in soy germ

The empirical model is plotted as a three dimension surface representing the response (total isoflavone glucosides and total isoflavone aglycones concentration) as a function of 2 factors with in tested hydrolysis parameters (Figure 1-3).

Contour plot at Figure 1 illustrates the interaction of pH and reaction temperature on total isoflavone glucosides and total isoflavone aglycones concentration in soy germ. The decrement of total isoflavone glucosides with the increase of pH and temperature up to a critical point can be observed from the Figure 1A.

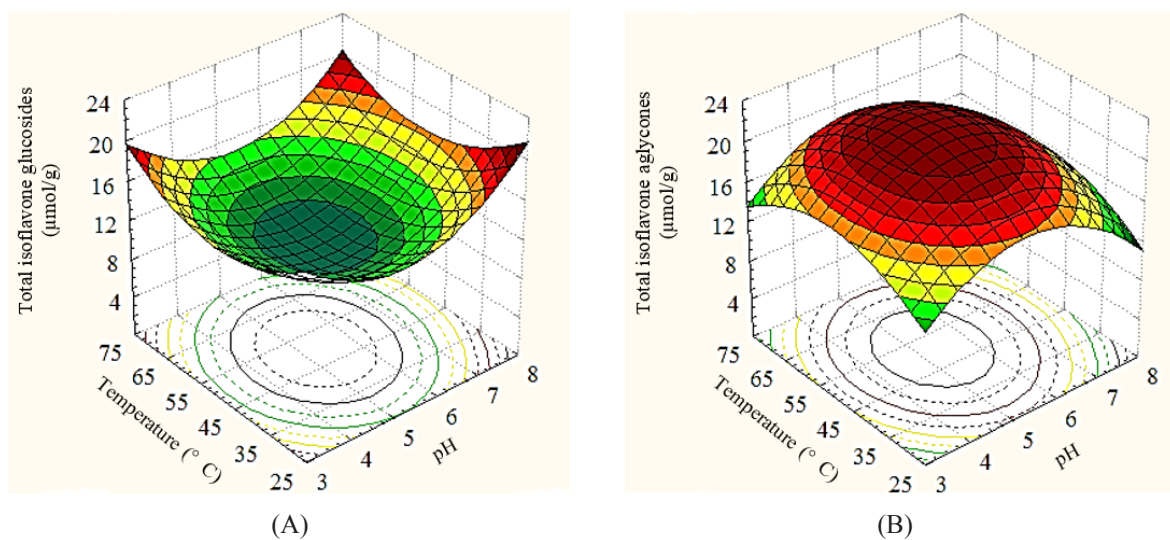


Figure 1: Effects of pH and reaction temperature (RT) on total isoflavone glucosides (A) and total isoflavone aglycones (B) at a fixed reaction time (7 h).

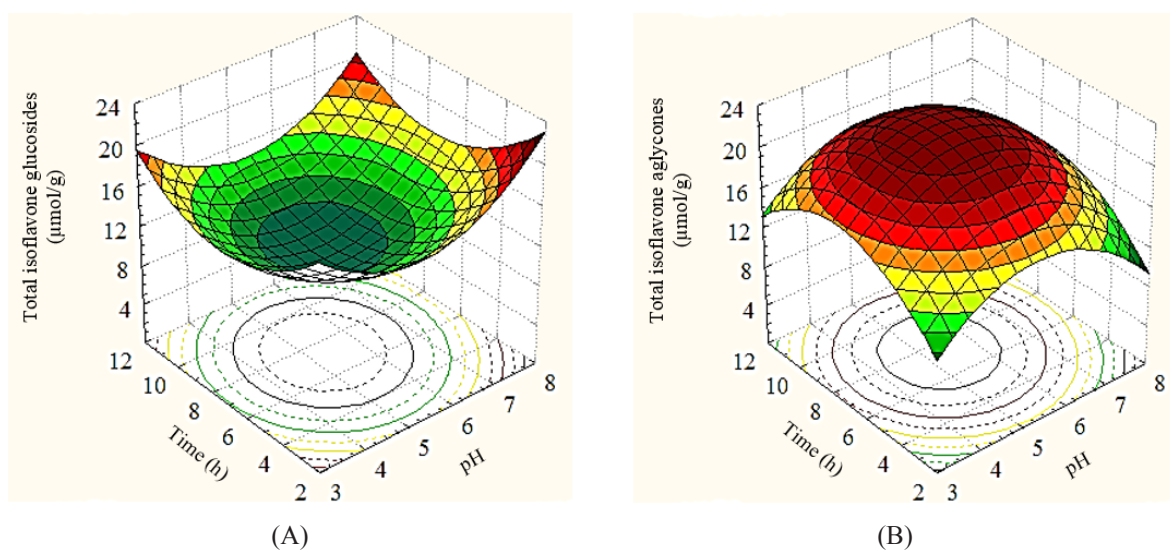


Figure 2: Effects of pH and reaction time (Rt) on total isoflavone glucosides (A) and total isoflavone aglycones (B) at a fixed reaction temperature (50°C).

Meanwhile, as the pH and temperature increased, the concentration of total isoflavone aglycones was increased (Figure 1B). The maximum yields were located in the experimental region. From the graph, the maximum isoflavone aglycones was obtained with pH locating between 4.2 and 6.2 and reaction temperature locating between 40°C and 65°C.

The interactions of pH and reaction time on total

isoflavone glucosides and total isoflavone aglycones are exhibited in Figure 2A and 2B, respectively. Total isoflavone glucosides decreased with increasing pH and reaction time (Figure 2A). The maximum of total isoflavone aglycones was obtained with pH locating between 4.2 and 6.4 and reaction time locating between 5 h and 10 h (Figure 2B). This also could be due to the conversion of isoflavone glucosides to their aglycones.

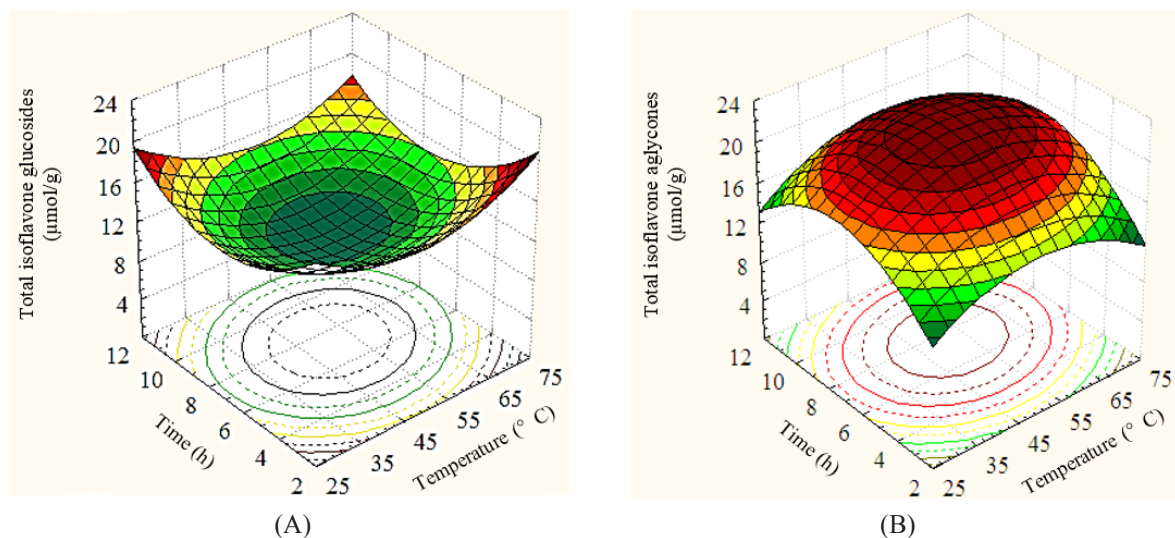


Figure 3: Effects of reaction temperature (RT) and reaction time (Rt) on total isoflavone glucosides (A) and total isoflavone aglycones (B) at a fixed pH (5.5).

In the reaction of temperature and time as showed in Figure 3, again, total isoflavone glucosides decreased with the increment of temperature and time (Figure 3A). Figure 3B illustrates the effect of reaction temperature and time on the total isoflavone aglycones in soy germ. The maximum yield was obtained with reaction temperature locating between 40°C and 62°C and reaction time locating between 5.5 h and 9.0 h.

Isoflavones are not generally destroyed by heat but are rather subject to intra-conversion between different forms [17]. During soybean hydrolysis, isoflavone glucosides (daidzin, genistin, and glycitin) can be converted to isoflavone aglycones (daidzein, genistein, and glycitein), respectively. When soybeans are soaked in water, the endogenous β -glucosidase enzyme hydrolyses the isoflavone glucoside forms to their aglycone forms [14]. Under acidic conditions, the glycones can be deconjugated to give aglycones.

Many studies demonstrated the optimum pH for production aglycones. Matsuura et al. [25] observed that the optimum pH for production of aglycones, daidzein and genistein, in soy milk was around 6.0 at 50°C temperature. Two isoforms of β -glucosidase (B, C) brought nearly all the hydrolyzing action into daidzin and genistin [26]. The enzyme showed optimum activity at pH 4.5 and 45°C, and its pH range of action was 3.5-7.0. The enzyme was also stable from pH 4.0-6.0 at 5°C [27]. Furthermore, Pandjaitan et al.

[13] investigated conversion of genistin to genistein in defatted soy flour by added β -glucosidase enzyme (0-4 unit/g defatted soy flour) and reported 41-100% conversion yield. For soy germ flour, Tipkanon et al. [11] reported that the optimal condition to produce soy germ flour with maximized isoflavone aglycones concentration was fixed substrate concentration (a dispersion of 1:5 w/v of soy germ flour:deionized water), β -glucosidase concentration at 1 unit/g of soy germ flour, pH 5 and incubation temperature and time of 45°C and 5 h. This work demonstrated a conversion yield of 79-100% the non-defatted soy germ flour.

3.3 Optimization of isoflavone aglycones concentration in soy germ

In this study, the hydrolysis condition of soy germ was optimized for conversion of isoflavone glucoside forms to their aglycone forms. The response surface analysis denoted that the maximum predicted value of isoflavone aglycones was 25.11 μ mol/g soy germ under the following hydrolysis conditions: pH at 5.3, reaction temperature at 51°C for 7.5 h reaction time. Base on the regression analysis, the model had a satisfactory coefficient of $R^2 = 0.956$. Furthermore, the verification studies have been carried out to validate the optimization result obtained by the response surface analysis. The observed values and predicted

values of total isoflavone aglycones concentration are reported at optimum condition (at given pH, 5.3; reaction temperature, 51°C; and reaction time, 6-9 h). It was suggested that the errors between predicted and verification were to be considered small as the observed values were less than 5% level of significance, means that the model is acceptable for optimization.

4 Conclusions

From this study, the model gained by the experimental design (CCD) was found to be effective in optimizing the hydrolysis conditions for maximizing the HPLC determination of total isoflavone aglycones in soy germ. Optimum conditions for hydrolysis were pH, 5.3; reaction temperature, 51°C; and reaction time, 7.5 h. At this optimal condition, the total isoflavone aglycones to be obtained would be 25.11 µmol/g. Base on the findings, it can be concluded that hydrolysis of soy germ could produce high isoflavone aglycones from conversion of isoflavone glucoside forms to their aglycone forms at an optimum hydrolysis condition. All the variables such as pH, reaction temperature, and reaction time gave a significant effect to isoflavone aglycones production. The RSM was successful in identify the optimum conditions for conversion of isoflavone glucosides to maximum isoflavone aglycones. This study provides a basis for production of isoflavone aglycone-enriched soy germ.

Acknowledgements

This research was supported by grant from Science and Technology Research Institute, King Mongkut's University of Technology North Bangkok.

References

- [1] H. J. Wang and P. A. Murphy, "Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year and location," *J. Agric. Food Chem.*, vol. 42, pp. 1674-1677, 1994.
- [2] K. Liu, *Soybean: chemistry, technology, and utilization*, Gaithersburg, MD: Aspen Publishers, 1997, pp. 3-5.
- [3] T. Schryver, "Increasing health benefits using soy germ," *Cereal Foods World*, vol. 47, pp. 185-188, 2002.
- [4] M. N. Riaz, *Soy applications in food*, Boca Raton, FL: CRC Press of Taylor&Francis group, 2006, pp. 55.
- [5] H. J. Wang and P. A. Murphy, "Mass balance study of isoflavone during soybean processing," *J. Agric. Food Chem.*, vol. 44, pp. 2377-2383, 1996.
- [6] L. Coward, M. Smith, M. Kirk, and S. Barnes, "Chemical modification of isoflavone in soy foods during cooking and processing," *Am. J. Clin Nutr.*, vol. 68(suppl), pp. 1486S-1491S, 1998.
- [7] S. Barnes, M. Kirk, and L. Coward, "Isoflavones and their conjugates in soy foods: Extraction conditions and analysis by HPLC mass spectrometry," *J. Agric. Food Chem.*, vol. 42, pp. 2466-2474, 1994.
- [8] J. Liu, K. Chang, and D. Wiesenborn, "Antioxidant properties of soybean isoflavone extract and tofu in vitro and in vivo," *J. Agric. Food Chem.*, vol. 53, pp. 2333-2340, 2005.
- [9] A. Cassidy, S. Bingham, and K. D. R. Setchell, "Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women," *Am. J. Clin. Nutr.*, vol. 60, pp. 333-340, 1994.
- [10] H. Kim, T. G., Peterson, and S. Barnes, "Mechanisms of action of soy isoflavone genistein: emerging role of its effects through transforming growth factor beta signaling pathways," *Am. J. Clin. Nutr.*, vol. 68, pp. 1418-1425, 1998.
- [11] S. Tipkanon, P. Chompreeda, V. Haruthaithanasan, T. Suwonsichon, W. Prinyawiwatkul, and Z. Xu, "Optimizing time and temperature of enzymatic conversion of isoflavone glucosides to aglycones in soy germ flour," *J. Agric. Food Chem.*, vol. 58, pp. 11340-11345, 2010.
- [12] L. Xie, N. S. Hettiarachchy, R. Cai, K. Tsuruhami, and S. Koikeda, "Conversion of isoflavone glycosides to aglycones in SoyLife and soymeal using β-glycosidase," *J. Food Sci.*, vol. 68, pp. 427-430, 2003.
- [13] N. Pandjaitan, N. Hettiarachchy, and Z. Y. Ju, "Enrichment of genistein in soy protein concentrate with β-glucosidase," *J. Food Sci.*, vol. 65, pp. 403-407, 2000.
- [14] E. Y. W. Ha, C. V. Morr, and A. Seo, "Isoflavone aglucones and volatile organic compounds in soybeans: effect of soaking treatments," *J. Food Sci.*, vol. 57, pp. 414-417, 1992.

- [15] I. Hughes and H. F. Woods, *Phytoestrogens and health*, Holborn, London: The Food Standards Agency, 2003, pp. 29.
- [16] S. Tipkanon, P. Chompreeda, V. Haruthaithanasan, W. Prinyawiwatkul, Hong Kyoong No, and Z. Xu, "Isoflavone content in soy germ flours prepared from two drying method," *Int. J. Food Sci. Tech.*, vol. 46, pp. 2240-2247, 2011.
- [17] K. D. R. Setchell, "Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones," *Am. J. Clin. Nutr.*, vol. 68, pp. 1333-1346, 1998.
- [18] I.L. Nielsen and G. Williamson, "Review of the factors affecting bioavailability of soy isoflavones in humans," *Nutr. Cancer*, vol. 57, pp. 1-10, 2007.
- [19] E. P. Nahas, J. N. Neto, L. D. Luca, P. Traiman, A. Pontes, and I. Dalben, "Benefits of soy germ isoflavones in postmenopausal woman with contraindication for conventional hormone replacement therapy," *Maturitas, The European Menopause Journal*, vol. 48, pp. 372-380, 2004.
- [20] M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, and L.A. Escaleira, "Response surface methodology (RSM) as a tool for optimization in analytical chemistry," *Talanta*, vol. 76, pp. 965-977, 2008.
- [21] AOAC, *Official Method 2001.10*, 17th ed., Gaithersburg, MD: AOAC International, 2000.
- [22] A.P. Griffith and M.W. Collison, "Improved methods for the extraction and analysis of isoflavones from soy-containing foods and nutritional supplements by reversed-phase high-performance liquid chromatography and liquid chromatography-mass spectrometry," *J. Chromatogr A.*, vol. 913, pp 397-413, 2001.
- [23] T. H. Kao, Y. F. Lu, H. C. Hsieh, and B. H. Chen, "Stability of isoflavone glucosides during processing of soymilk and tofu," *Food Res. Int.*, vol. 37, pp. 891-900, 2004.
- [24] A. H. Simonne, M. Smith, D. B. Weaber, T. Vail, S. Barnes, and C. Iwei, "Retention and changes of isoflavones and carotenoids in immature soybean seeds during processing," *J. Agric. Food Chem.*, vol. 48, pp. 6061-6069, 2000.
- [25] M. Matsuura, A. Obata, and D. Fukushima, "Objectionable flavor of soymilk developed during the soaking of soybeans and its control," *J. Food Sci.*, vol. 54, pp. 602-605, 1989.
- [26] M. Matsuura and A. Obata, " β -glucosidases from soybeans hydrolyse daidzin and genistin," *J. Food Sci.*, vol. 58, pp. 144-147, 1993.
- [27] M. Mutsuura, A. Obata, and S. Murao, "Studies on β -glucosidases from soybeans that hydrolyze daidzin and genistin: Isolation and characterization of an isozyme," *Biosci. Biotech. Biochem.*, vol. 59, pp. 1623-1627. 1995.