Poster Session | Food Function/Nutrition
[6-1130-P] Functional/Wellness Foods &Nutrition (6th)
Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

[6-1130-P-06] Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

*Arpatsara Seekoompa[1], Pairote Wongputtisin[1], Piyanuch Niamsup[1] (1. Program in Biotechnology, Faculty of science, Maejo University, Chiang mai(Thailand))

Keywords: soybean, antioxidant activity, isoflavones, functional food, optimization

Soybean [*Glycine max* (L.) Merr.] cv. Chiang mai60, a local and popular cultivar of Thailand, plays an important role as source of protein food and phytochemicals contributing many health benefits to consumer. Antioxidant activity is one of the beneficial property obtained from soybean seed. Soy isoflavones, major antioxidants composing in soybean seed, have been isolated and developed into a variety of healthy foods. Therefore, this research aimed to optimize the optimal conditions for antioxidant extraction from seed of Chiang mai 60 for further application in functional food development. Ratio of water to soybean powder, extraction temperature and time were optimized by central composite design (CCD) method, a statistical experimental approach. The results showed that soybean extract with highest ABTS inhibition activity at 85.5% was obtained when the extraction was carried out the ratio of 3.18 ml: 1 g, 45ºC and 4 h (p=0.0004, R-squared = 0.9107). According to HPLC analysis, this soybean extract contained aglycones isoflavones (daidzein, glycitein, genistein) and glucosides isoflavones (daidzin, glycitin, genistin) approximately 0.2985 and 0.2397 mg/g seed, respectively. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of antioxidant and exhibited a potential to be utilized as ingredient for functional food development.

Process optimization for antioxidant extraction from seed of soybean cultivar Chiang mai60

Arpatsara Seekoompa, Pairote Wongputtisin*, Piyanuch Niamsup
Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand

*Corresponding author: pairotewong@gmail.com

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] cv. Chiang mai60, a local and popular cultivar of Thailand, plays an important role as source of protein food and phytochemicals contributing many health benefits to consumer. Antioxidant activity is one of the beneficial property obtained from soybean seed. Soy isoflavones, major antioxidants composing in soybean seed, have been isolated and developed into a variety of healthy foods. Therefore, this research aimed to optimize the optimal conditions for antioxidant extraction from seed of Chiang mai 60 for further application in functional food development. Ratio of water to soybean powder, extraction temperature and time were optimized by central composite design (CCD) method, a statistical experimental approach. The results showed that soybean extract with highest ABTS inhibition activity at 85.5% was obtained when the extraction was carried out the ratio of 3.18 ml: 1 g, 45ºC and 4 h (p=0.0004, R-squared = 0.9107). According to HPLC analysis, this soybean extract contained aglycones isoflavones (daidzein, glycitein, genistein) and glucosides isoflavones (daidzin, glycitin, genistin) approximately 0.2985 and 0.2397 mg/g seed, respectively. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of antioxidant and exhibited a potential to be utilized as ingredient for functional food development.

Keywords: soybean, antioxidant activity, isoflavones, functional food, optimization

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an important leguminous seed crop in many regions of the world. Its seed is rich of high quality protein, oil, saccharides, fiber, vitamins and many phytochemicals (Obendorf et al., 2008). Therefore, soybeans can be utilized in a variety of uses, mainly as food and feed, both direct consumption and processed into various foods. Soybean has been also an important economic crop in Thailand. Since 1975, the Chiang mai60, Thai soybean cultivar, was bred and developed by the Chiang mai Field Crop Research Center, Thailand. This cultivar has been popular and widespread in northern Thailand because of high productivity, resistance to diseases (rust and mildew disease, etc.) and acclimatization to geographic change.

Apart from utilizing as protein foods, Chiang mai60 was reported as a rich source of raffinose family oligosaccharides (RFOs) (Wongputtisin et al., 2015). These oligosaccharides are accepted as an effective prebiotic in functional food products, contributing to the balance of intestinal microflora. In addition, isoflavones are the group of polyphenolic phytochemicals that are commonly found as large quantity in soybean seed. Natural isoflavones can be classified in to 4 types, i.e. aglycones, glucosides, acetylglucosides and malonylglucosides (Wang et al., 2013). The main functionality of isoflavones is accepted as an antioxidant, resulting of reduce the risk and treatment of several diseases such as antitumor, antimenopausal (female) osteoporosis and anti-aging properties, improvement of learning and memory skills of menopausal women, prevention and treatment of heart disease and diabetes, and so on (Wang et al., 2013; Lante et al., 2018). From the benefits of soy isoflavones mentioned above, several isoflavones-based food products have been nowadays developed and commercialized to functional food market.

Our research group has been interested in development of functional food supplement from Chiang mai 60 soybean. However, antioxidant activity and isoflavone content of Chiang mai60 have not been yet investigated. Therefore, the optimization for antioxidant extraction process and quantification of isoflavones content in seed of soybean cultivar Chiang mai60 were aimed in this study for further application in functional food development.

2. MATERIALS AND METHODS

2.1 Raw material

Soybean seed, cultivar Chiang mai60, was kindly obtained from Chiang mai Field Crop Research Center, Chiang mai, Thailand. The seed was grind into fine powder by electric grinder and then dried at 55ºC for 12 h soybean powder was kept under -20ºC.

2.2 Optimization for process of antioxidant extraction

Three factors (variables), including ratio of water to powder, extraction temperature and extraction time, were optimized for the maximum antioxidant extraction from soybean powder by using statistical experimental design strategy. The central composite design (CCD) method was applied, resulting of established 20 experimental treatments. The range and level of each setting variables are shown in Table 1 and experiments were established as in Table 2. The experiments were carried out and the mixtures were centrifuged at 14,000 rpm, 4ºC for 10 min. Supernatants were kept at -80°C during waiting for antioxidant activity determination by ABTS inhibition assay. The obtained data were subjected to regression and graphical analysis using Design Expert® software.

Table 1. The range and level of each setting variables in the central composite design

Variables	Parameter	Range and levels		
		-1	0	1
A	Ratio of water to powder (ml : g)	10.00	20.00	30.00
B	Temperature (ºC)	30.00	45.00	60.00
C	Time (h)	2.00	4.00	6.00

Table 2. The established treatments according to the central composite design experiment

Treatments	Variables		
	Ratio of water to soybean powder (ml : g)	Temperature (°C)	Time (h)
1	10.00	30.00	2.00
2	30.00	30.00	2.00
3	10.00	60.00	2.00
4	30.00	60.00	2.00
5	10.00	30.00	6.00
6	30.00	30.00	6.00
7	10.00	60.00	6.00
8	30.00	60.00	6.00
9	3.18	45.00	4.00
10	36.82	45.00	4.00
11	20.00	19.80	4.00
12	20.00	70.20	4.00
13	20.00	45.00	0.64
14	20.00	45.00	7.36
15	20.00	45.00	4.00
16	20.00	45.00	4.00
17	20.00	45.00	4.00
18	20.00	45.00	4.00
19	20.00	45.00	4.00
20	20.00	45.00	4.00

2.3 ABTS inhibition activity assay

Antioxidant activity of the extracts were determined by measurement of free radical scavenging activity using ABTS inhibition assay. Briefly, ABTS cation radical ($ABTS^{\bullet+}$) solution was diluted with DI water to obtain an absorbance of 0.700 at 734 nm. 10 µl of extract was added to 990 µl of $ABTS^{\bullet+}$ solution, mixed well and recorded the decreasing of A_{734} every 1 min until stable. Percent inhibition was calculated using the formula,

$$\frac{A_{734}\text{ at 0 min} - A_{734}\text{ at 1 min}}{A_{734}\text{ at 0 min}} \times 100$$

2.4 Isoflavones determination by HPLC

Samples were extracted by 80% methanol (1:1), mixed well and stand overnight at -20ºC. The precipitate was removed by centrifugation under 10ºC. Soybean isoflavones were analyzed from clear supernatant according to (Lante et al., 2018). The HPLC Ultratechsphere C18 analytical column (size 4.6x250 mm) was used with controlled temperature at 35°C and 10 µl sample injection. The mobile phase was 0.25% (v/v) trifluoroacetic acid (TFA) in water (solvent A) and acetonitrile (ACN) (solvent B). A linear HPLC gradient was used as follow, 15% of solvent B for 6 min, then increased gradually to 30% over 4 min, to 40% over 2 min, to 50% over 1.50 min and 50% over 1.50 min. The duration of the analysis was 15 min at a solvent flow rate of 1.3 ml/min. Standard of aglycones isoflavones (daidzein, genistein, glycitein) and glycosides forms (daidzin, genistin, glycitin) were obtained from a commercial source Wako Pure Chemical Industries, Ltd., (Osaka, Japan).

3. RESULTS AND DISCUSSIO

The antioxidant activity, in term of ABTS inhibition activity, of the established 20 treatments according to CCD experiment were shown in Table 3 with different values. Among these responses, treatment number 9 and 10 exhibited the highest and lowest antioxidant activities, respectively. The analysis of variance (ANOVA) was carried out for the determination of significant factors and to predict the antioxidant activity as a function of these three factors. The analyzing data were shown in Table 4-5. It was found that simulated model was significant at $p=0.0004$ but not significantly fit to the quadratic

model according to lack of fit ($p<0.0001$). The estimated coefficient of three factors and their interaction were also analyzed. We found only the ratio between water and powder was highly significant ($p<0.001$) to antioxidant extraction with negative effect. Extraction time was another negative factor to antioxidant extraction from Chiang mai60 powder but not significant. The interactions between time and other two factors were negative but slightly influenced to antioxidant activity ($P>0.05$). Subsequently, the quadratic model for prediction of antioxidant extraction from the powder of Chiang mai60 seed was simulated by the software as follow:

$$Y = 23.04 - 16.02A + 0.40B - 0.54C + 0.055AB - 0.33AC - 0.62BC + 8.02A^2 - 1.23B^2 - 1.05C^2$$

Where
Y = ABTS inhibition activity (%), A= code value of ratio of water to soybean powder
B = code value of extraction temperature, C = code value of extraction time

The response surface graphs of this model were also plotted (Fig 1). The results confirmed the optimal level of extraction time and temperature were around 3-4 h and 45-50°C, while that of ratio of water and powder should be low. Less volume of water, for example around 3:1 as applied in this experiment, was effectively and enough for antioxidant extraction from Chiang mai60 seed. To enhance yield of antioxidant extraction from soybean seed, other strategies can be assisted in the process, for example ultrasonic (Lai et al., 2013) and UV radiation (Lante et al., 2018)

The highest antioxidant activity of treatment 9 correlated to its total isoflavones content which was the highest content among those treatments, meanwhile that of treatment 10 was in the group with low content of total isoflavones as shown in Table 4. Genistin and genistein were the major isoflavones found in seed of Chiang mai60, furthermore, glucosides isoflavones naturally accumulate in soybean seed higher than aglycones isoflavones (Baú and Ida, 2015). But it was noticed that proportions of total aglycones in most of the treatment which exposed to 45°C were higher than those of total glucosides. It was possibly to explain by the activity of endogenous β-glucosidase in soybean seed. The optimal conditions for soybean β-glucosidase were at 45°C and pH 4.5-5.0 (Matsuura and Obata, 1993; Chiou et al., 2010). Glycosidic bonding on glucoside molecules might be hydrolyzed resulting of free aglycones released. Aglycones exhibit greater antioxidant activity than that from glucosides since their smaller molecular size (Baú and Ida, 2015). Interestingly, aglycones content of extract from treatment 9 was also the highest amount. This might be another reason to explain the great antioxidant activity of this treatments.

Table 3. The actual and predicted antioxidant activity results optimization for process of antioxidant extraction by the central composite design

Treatments	Ratio of water to soybean powder (ml : g)	Temperature (°C)	Time (h)	ABTS scavenging activity (%)	
				Actual	Predicted
1	10.00	30.00	2.00	37.37	30.52
2	30.00	30.00	2.00	16.41	38.34
3	10.00	60.00	2.00	41.48	18.02
4	30.00	60.00	2.00	17.08	13.74
5	10.00	30.00	6.00	38.93	35.29
6	30.00	30.00	6.00	12.98	15.75
7	10.00	60.00	6.00	36.90	23.38
8	30.00	60.00	6.00	14.83	14.25
9	3.18	45.00	4.00	85.55	22.88
10	36.82	45.00	4.00	10.96	45.26
11	20.00	19.80	4.00	21.84	12.94
12	20.00	70.20	4.00	22.33	14.03
13	20.00	45.00	0.64	22.20	18.42
14	20.00	45.00	7.36	22.98	16.20
15	20.00	45.00	4.00	23.58	15.74
16	20.00	45.00	4.00	21.98	15.06
17	20.00	45.00	4.00	23.06	20.31
18	20.00	45.00	4.00	23.56	19.23
19	20.00	45.00	4.00	21.76	20.40
20	20.00	45.00	4.00	23.41	17.41

Table 4. Analysis of variance (ANOVA) for the model regression

Source	SS	DF	MS	F-value	Significant value (p-value)
Model	4544.58	9	504.95	11.33	0.0004
Residual	445.77	10	44.58		
Lack of fit	442.44	5	88.49	132.89	<0.0001
Pure error	3.33	5	0.67		
Total	4990.35	19			
R^2= 0.9107					

SS = sum of squares, DF = degrees of freedom, MS = mean square

Table 5. Coefficient estimates by the regression model

Independent variables (parameter)	Coefficient	Standard error	Significant value (p-value)
Intercept	23.04	2.72	
A-ratio	-16.02	1.81	<0.0001
B-temp	0.40	1.81	0.8304
C-time	-0.54	1.81	0.7707
AB	0.055	2.36	0.9819
AC	-0.33	2.36	0.8908
BC	-0.62	2.36	0.7982

*Statistically significant at 95% of confidence level.

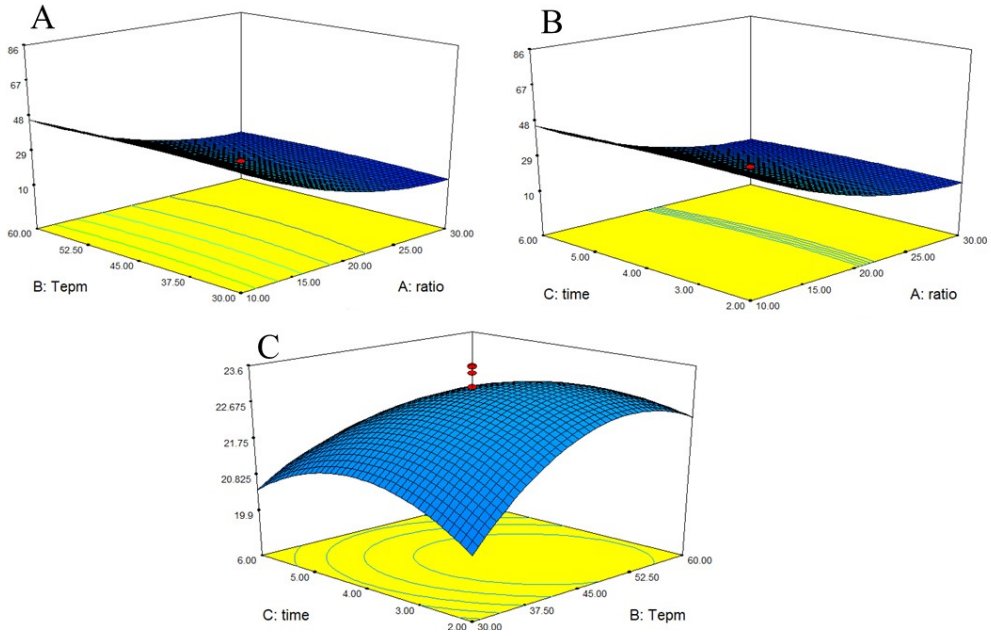

Figure 1. The response surface graphs exhibiting the antioxidant activity of the extract obtained as the function of three factors (A: ratio of water to soybean powder, B: extraction temperature and C: extraction time)

Table 6. The results of soybean extract contained isoflavones by HPLC

Trt	Isoflavones content (mg/g)								
	Glucosides			Aglycones			Total glucosides	Total aglycones	Total Isoflavones
	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein			
1	0.04	0.02	0.11	0.02	0.01	0.01	0.16	0.07	0.23
2	0.06	0.02	0.16	0.04	0.01	0.10	0.23	0.15	0.38
3	0.09	0.03	0.17	0.02	0.01	0.05	0.29	0.08	0.37
4	0.09	0.02	0.21	0.03	0.00	0.09	0.32	0.12	0.44
5	0.02	0.01	0.07	0.03	0.01	0.06	0.10	0.09	0.19
6	0.04	0.01	0.10	0.04	0.01	0.11	0.15	0.15	0.30
7	0.09	0.03	0.15	0.02	0.01	0.06	0.26	0.09	0.35
8	0.09	0.02	0.20	0.03	0.01	0.11	0.32	0.14	0.46
9	0.05	0.02	0.17	0.07	0.02	0.20	0.24	0.30	0.54
10	0.01	0.00	0.07	0.04	0.00	0.14	0.08	0.18	0.27
11	0.05	0.02	0.12	0.02	0.00	0.08	0.18	0.10	0.28
12	0.10	0.03	0.19	0.02	0.00	0.09	0.32	0.12	0.44
13	0.05	0.02	0.14	0.03	0.01	0.09	0.20	0.13	0.33
14	0.02	0.00	0.06	0.04	0.01	0.12	0.08	0.16	0.24
15	0.02	0.00	0.09	0.04	0.01	0.10	0.11	0.15	0.26
16	0.00	0.00	0.08	0.04	0.01	0.09	0.08	0.13	0.21
17	0.02	0.01	0.09	0.04	0.01	0.10	0.12	0.15	0.27
18	0.02	0.00	0.08	0.04	0.01	0.09	0.10	0.14	0.24
19	0.02	0.00	0.07	0.04	0.00	0.10	0.10	0.14	0.23
20	0.02	0.00	0.07	0.04	0.00	0.10	0.10	0.14	0.23

4. CONCLUSION

It could be concluded that the optimal processes for antioxidant extraction from seed of soybean cultivar Chiang mai60 could be carried out at 45-50°C, 3-4 h and low ratio between water and soybean powder. Under these conditions, the highest isoflavones content with the highest antioxidant activity were subsequently obtained. It might be indicated that seed of soybean cv. Chiang mai60 was one of good source of potential antioxidants to be utilized as ingredient for functional food development.

ACKNOWLEDGMENT

The authors would like to thank the financial support from the Research and Researchers for Industries (RRI) scholarship, The Thailand Science Research and Innovation, Thailand, and the facility support from the Program in Biotechnology, Faculty of Science, Maejo University.

REFERENCES

Bau, T.R and E.I. Ida, 2015. Soymilk processing with higher isoflavone aglycones content. *Food Chemistry,* 183: 161-168.

Chiou, T. Y., Y. H. Lin, N. W S. and M. H. Lee, 2010. β-Glucosidase isolated from soybean Okara shows specificity toward glucosyl isoflavones. *Journal of Agricultural and Food Chemistry,* 58(15): 8872-8878.

Choe, E. and D.B. Min. 2009. Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety*, 8(4): 345-58.

Dajantra, K. and H. Rongkom. 2017. Effects of drying temperature on the isoflavone content and antioxidant capacity of fermented soybean (Thua Nao). *KKU Science Journal*, 45(1): 138-150.

Lai, J., C. Xin, Y. Zhao, B. Feng, C. He, Y. Dong, Y. Fang. and S. Wei. 2013. Optimization of ultrasonic assisted extraction of antioxidants from black soybean (*Glycine max* var) sprouts using response surface methodology. *Molecules*, 18: 1101-1110.

Lante, A., G. Barion, S. Zannoni, M. R. Pastore, F. Tinello, C.D. Cortivo, T. Vamerali. and G. Mosca. 2018. An ecofriendly procedure to extract isoflavones from soybean seeds. *Journal of Cleaner Production*, 170: 1102-1110.

Matsuura, M. and A. Obata. 1993. β-Glucosidases from soybeans hydrolyze daidzin and genistin. *Journal of Food Science,* 58(1): 144–147.

Obendorf, R.L., A.D. Zimmerman, P.A. Ortiz, A.G. Taylor. and S.R. Schnebly. 2008. Imbibitional chilling sensitivity and soluble carbohydrate composition of low raffinose, low stachyose soybean seed. *Crop Science*, 48-6.

Rostagno, M.A., A. Villares, E. Guilamon, A. L. Garcia, and J.A. Martinez, 2009. Sample preparation for the analysis of isoflavones from soybeans and soy foods. *Journal of Chromatography A,* 1216: 2-29.

Sangla, L. 2015. Research and development on soybean production technology for nutritional enhancement. *Department of Agriculture,* 1-92.

Tipkanon, S., P. Chompreeda, V. Haruthaithanasan, T. Suwonsichon, W. Prinyawiwatkul. and Z. Xu. 2010. Optimizing time and temperature of enzymatic conversion of isoflavone glucosides to aglycones in soy germ flour. *Journal of Agricultural and Food Chemistry*, 58: 11340–11345.

Wang, Q., X. Ge, X. Tian, J. Zhang. and P. Zhang. 2013. Soy isoflavone: The multipurpose phytochemical (Review). *Biomedical Reports,* 1: 697-701.

Wongputtisin, P., R. Ramaraj, Y. Unpaprom, R. Kawaree and N. Pongtrakul. 2015. Raffinose family oligosaccharides in seed of *Glycine max* cv. Chiang mai 60 and potential source of prebiotic substances. *International Journal of Food Science and Technology,* 5: 1750-1756.