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RESEARCH ON EXTRACTION OF ISOFLAVONES FROM SOYBEAN GERM

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ABSTRACT

Isoflavones in soybean including genistein, daidzein, glycitein and their acetyl and malonyl derivatives have week estrogenic activity, therefore, they are called phytoestrogen. There are many papers publishing the research results about isoflavones from soybean seed and soybean sprout. Isoflavones are found to balance estrogenic hormone and support woman to prevent menopause symptoms. They have other precious biological activities such as anti - cancer, anti – oxidant and anti - osteomalacia. On the market, we find many functional foods of isoflavones from soybean seed and soybean sprout. Soybean germ is the most abundant isoflavones in different part of soybean seed, and the isoflavones content in soybean germ higher than that in the whole soybean seed about 5 times. In this paper, the total isoflavones were extracted from soybean germ. The five factors that influence the isoflavones extraction process including solvent concentration, extraction temperature, extraction time, extraction pH and the material/solvent ratio were optimized. The content of isoflavones in soybean germ material is from 1.8 to 2 percent. After extraction with optimized conditions, we obtain the total crude isoflavones extract that contains 3.8% of isoflavones.

Keywords: extraction, purification, analysis, isoflavones, phytoestrogen, soybean germ (soygerm).

1. INTRODUCTION

Soybean is a valuable crop which is abundant in Vietnam. Soybean provides an ideal source of vegetable protein containing essential amino acids with well-balanced ratio and high biological absorption. Moreover, soybean is a predominant source of isoflavones, the main class of phytoestrogen. In addition to having biological activities of flavonoids such as antioxidant activity, atherosclerosis prevention and increased permeability of the cell, these compounds have weak estrogenic activity. They can regulate female hormone and prevent breast cancer, prostate cancer, osteoporosis, cardiovascular diseases and menopausal symptoms in women [1, 2, 3].

There are four kinds of isoflavones in soybean: aglycone isoflavones (genistein, daidzein and glycitein), glycoside isoflavones (genistin, daidzin and glycitin), acetyl glycoside

isoflavones (acetyl genistin, acetyl daidzin and acetyl glycitin), and malonyl glycoside isoflavones (malonyl genistin, malonyl daidzin and malonyl glycitin) [4, 5]. Soybean germ is only 2 % of the whole soybean seed including seed cost, cotyledons and soy germ. However, the isoflavones content in soy germ is five to six times higher than that in whole soybean seed [6].

For the extraction of isoflavones from soybean, various techniques have been developed. Extraction based on Soxhlet method is widely used, but needs a long time of operation and large amount of solvent at high temperature. Pressurized solvent extraction [7], ultrasonic assisted extraction [8], and microwave assisted extraction [9] can also extract isoflavones from soybean, but need intensive energy and cost, and are difficult to be applied on a large scale. Ethanol extraction of isoflavones from soybean flour at alkaline condition was also studied [5].

In this study, we used maceration method to extract the total crude isoflavones from defatted soybean germ flour, with ethanol as the extraction solvent. Five influential factors during extraction process such as the ethanol concentration, extraction temperature, extraction time, pH and material/solvent ratio were optimized to obtain higher extraction content of total crude isoflavones.

2. MATERIALS AND METHODS

2.1. Soygerm flour

Soygerm flour was prepared by VINANUSOY and stored at 20–25 ^{o}C.

Soygerm flour with the size of 0.1 mm, yellow, and moisture content of 5.57 % containing 75–80 % of embryos and 20–25 % of cotyledon fragmentations.

2.2. Chemicals

The standard isoflavones (genistein, daidzein and glycitein; genistin, daidzin and glycitin; acetyl genistin, acetyl daidzin and acetyl glycitin; malonyl genistin, malonyl daidzin and malonyl glycitin) were purchased from Wako (Japan) and stored at -45 ^{o}C. Ultrapure water, HPLC grade acetonitrile and other analytical grade chemicals were from Merck; hexane (95 %), ethanol (96 %), and NaOH were from Duc Giang Chemical Company (Vietnam).

2.3. Preparation of defatted soygerm flour

The fatty acids were extracted from soygerm flour to obtain defatted soygerm flour by using the solvent hexane 95 %, extraction temperature: 40 ^{o}C, extraction time: 7 hours, the material/solvent ratio: 1/5 [10] and shaking speed: 180 rpm. Then, the extract was centrifuged at 6000 rpm in 15 minutes to separate the solvent with fatty acids; the remainder was dried to obtain the defatted soygerm flour.

2.4. Extraction of total crude isoflavones from defatted soygerm flour

Extraction of total crude isoflavones from 3 g defatted soybean flours was performed in a triangle flask 100 ml equipped with a Shaking incubator at 180 rpm. The basic extraction conditions were the following [5, 11]: extraction time 60 min, extraction temperature 40 ^{o}C, ethanol concentration 50 %, material/solvent ratio 1/10 and extraction pH 7.0. The extraction time (60–150 min), extraction temperature (30–45 ^{o}C), ethanol concentration (40–70 %),

material/solvent ratio (1/5–1/20) and extraction pH (5.0–11.0) were optimized for the isoflavones extraction. The optimal conditions were described in Table 1. After extraction, the extract liquid was separated from insoluble fractions by filtration; the solvent was evaporated from extract liquid by using Rotavapor IKA RV-10 (Heidolph, Germany) at 40 ^{o}C, then the total crude isoflavones extract was freeze dried to constant mass. The content of extracted total crude isoflavones compared to defatted soybean flour material was defined as follows:

Content of extracted total crude isoflavones (mg/g) = Total crude isoflavones obtained (mg)/defatted soygerm flour material (g).

Table 1. Optimal conditions for isoflavones extraction from defatted soygerm flour.

Extraction time (min)	Extraction temperature (^{o}C)	Ethanol concentration (%)	Material/solvent ratio (g/ml)	Extraction pH
60	30	40	1/5	5
90	35	50	1/10	7
120	40	60	1/15	9
150	45	70	1/20	11

2.5. HPLC analysis

Isoflavones were analyzed by Alliance HPLC system (Waters, US) equipped with C18 column (5 µm × 4.6 mm × 150 mm), detector PDA 2996. The HPLC conditions were set at 35 ^{o}C of column temperature, 260 nm of detective wavelength, acetonitrile/water as mobile phase and 1.0 ml/min of flow rate.

2.6. Statistical analysis

All measurements were carried out in triplicate and analyzed statistically by analysis of variance ANOVA. Significant difference was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extration to obtain defatted soygerm flour

The fatty acids were extracted from soygerm flour by hexane 95 %. The extraction conditions was described in the content 2.3 above. Defatted soygerm flour was obtained with humidity 5.64 %.

The content of extracted lipid in soygerm was 10.16 %, less than that in the hole soybeen (20 %). Our extracted result was silmilar with the result published by Sun-Lin Kim *et al.* which reported that the lipid content in soygerm was 10.1 % [12].

3.2. Extraction of total crude isoflavones from defatted soygerm flour

Based on the basic extraction conditions described in the content 2.4, we evaluated the influence of each of the five factors (extraction time, the material/solvent ratio, extraction pH, extraction temperature and ethanol concentration) one by one on the content of extracted total

crude isoflavones (this is called classic optimization method). The amount of isoflavones obtained at different extraction conditions (Table 1) were investigated.

3.2.1. Influence of extraction time on the total crude isoflavones obtained

The total crude isoflavones were extracted from defatted soygerm flour by ethanol 50 %, material/solvent ratio 1/10, extraction pH 7.0, extraction temperature 40 ^{o}C; extraction times were 60, 90, 120 and 150 min. The effect of extraction time on extraction amount of total crude isoflavones was shown in Fig. 1**.**

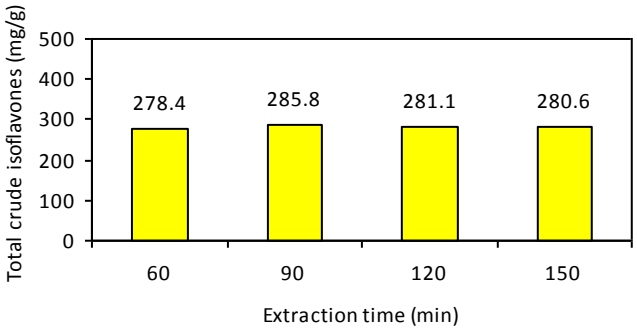

Figure 1. Total crude isoflavones obtained at different extraction times.

As can be seen in Fig. 1, the total crude isoflavones was mainly obtained in 60 min early of extraction time, increased to the maximum at 90 min, but decreased slowly more and more at 120 and 150 min of extraction time. In the other extraction conditions, Sang-MonBae *et al.* obtained maximum amount of isoflavones from defatted soygerm at 120 min, and Mengfan Wang *et al.* obtained a maximum amount of isoflavones from soybeen at 60 min; the amount of obtained isofavones decreased when the extraction time continued to increase [5, 11]. Based on this extraction result, we have selected extraction time for 90 min, and the total crude isoflavones obtained at this time were 285.8 mg/g defatted soygerm flour. This value of time factor was applied for continuing research.

3.2.2. Influence of extraction temperature on the total crude isoflavones obtained

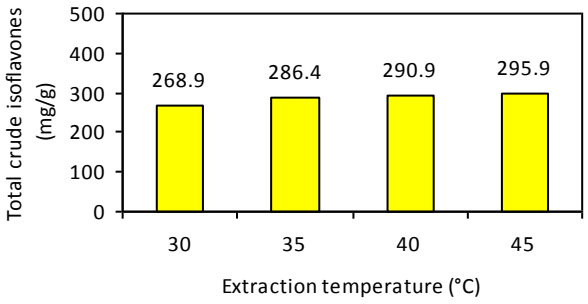

Figure 2. Total crude isoflavones obtained at different extraction temperatures.

To evaluate the influence of extraction temperature, we extract total crude isoflavones from defatted soygerm flour by ethanol 50 %, extraction times 90 min, material/solvent ratio 1/10, extraction pH 7.0; extraction temperature were 30, 35, 40 and 45 ^{o}C. The effect of extraction temperature on total crude isoflavones obtained was presented in Fig. 2**.**

The result in Fig. 2 indicated that total crude isoflavones obtained increased significantly when the extraction temperature increased from 30 to 35 ^{o}C, then increased more slowly and insignificantly when extraction temperature continued to increase from 35 to 40 and to 45 ^{o}C. Therefore, extraction temperature 40 ^{o}C was chosen for next research.

3.2.3. Influence of the ethanol concentration on the total crude isoflavones obtained

We continued to extract total crude isoflavones from defatted soygerm flour with the following conditions: extraction times 90 min, extraction temperature 40 ^{o}C, material/solvent ratio 1/10, extraction pH 7.0; and ethanol concentrations were 40, 50, 60 and 70 %. The effect of ethanol concentration on total crude isoflavones obtained was described in Fig. 3**.**

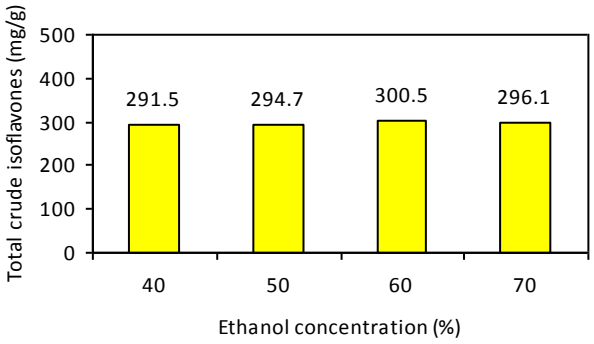

Figure 3. Total crude isoflavones obtained at different ethanol concentrations.

The result in Fig. 3 showed that total crude isoflavones obtained maximum at ethanol concentration 60 %. Therefore, ethanol concentration 60 % was chosen as reasonable value for this factor. In the research of Mengfan Wang *et al*., ethanol concentration 65 % was chosen to extract isoflavones from soybeen [5].

3.2.4. Influence of the material/solvent ratio on the total crude isoflavones obtained

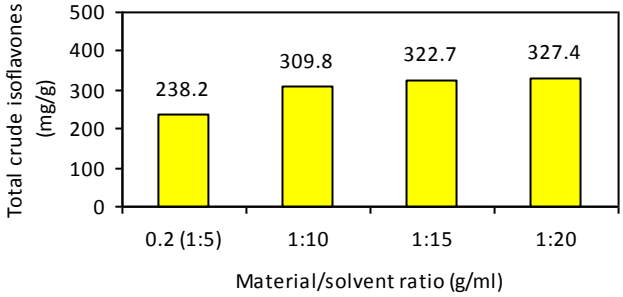

Figure 4. Total crude isoflavones obtained at different material/solvent ratio.

The total crude isoflavones were extracted from defatted soygerm flour by ethanol concentration 60 %, extraction times 90 min, extraction temperature 40 ^{o}C, extraction pH 7.0; material/solvent ratio were 1/5, 1/10, 1/15 and 1/20. The effect of material/solvent ratio on extraction amount of total crude isoflavones was presented in Fig. 4.

The result in Fig. 4 showed that the extracted total crude isoflavones increased when the material/solvent ratio increased from 1/5 to 1/20. However, the total crude isoflavones increased fast when the material/solvent ratio increased from 1/5 to 1/10; and increased slowly when this ratio increased from 1/10 to 1/15 and then to 1/20. We can choose material/solvent ratio 1/15 as reasonable value for this factor. But because the difference of total crude isoflavones obtained at material/solvent ratio 1/10 and 1/15 was insignificant; and as we aim to apply on a larger production scale, we have selected material/solvent for 1/10 to save the solvent and decrease the production cost.

3.2.5. Influence of the extraction pH on the total crude isoflavones obtained

We continue to extract total crude isoflavones from defatted soygerm flour by ethanol concentration 60 %, extraction times 90 min, material/solvent ratio 1/10, extraction temperature 40 ^{o}C; extraction pH were 5.0, 7.0, 9.0 and 11.0. The effect of extraction pH on total crude isoflavones obtained was shown in Fig. 5.

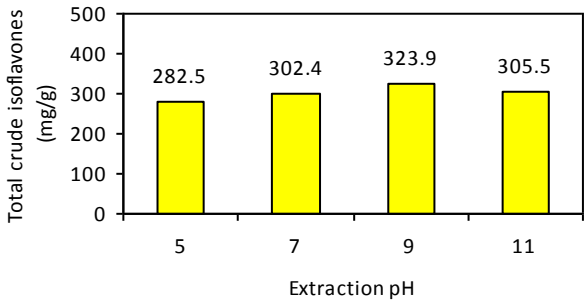

Figure 5. Total crude isoflavones obtained at different extraction pH.

We found in Fig. 5 that total crude isoflavones obtained maximum (323.9 mg/g defatted soygerm flour) at pH 9.0, therefore, we chose the value pH 9.0 for this factor. This could be explained that at alkaline pH 9.0, the solubility of isoflavones in aqueous ethanol solution reached the highest value, which could result in the highest extraction of total crude isoflavones. Moreover, in the alkaline condition, ester form of soygerm isoflavones (acetyl glycoside isoflavones and malonyl glycoside isoflavones) could be converted into their glycoside form which dissolved better in aqueous ethanol solution than in ester form [5].

According to the above results, reasonable extraction conditions obtaining total crude isoflavones were chosen as follows: extraction time 90 min, extraction temperature 40 ^{o}C, ethanol concentration 60 %, material/solvent ratio 1/10 and extraction pH 9.0. In our other research [13], we optimated isoflavones extraction process by applying the Response Surface Methodology and the optimal results of studying factors were similar to the optimal results in this research.

3.3. Quantitative analysis of isoflavones in the total crude isoflavones

Realized extraction with the above conditions, the total crude isoflavones obtained were freeze dried (humidity was 2.7 %). The isoflavones in soygerm flour material and in total crude

isoflavones were quantitatively analyzed by HPLC method. The results of HPLC analysis was shown in Table 2.

Table 2. Amount of 12 compounds (3 isoflavones and their derivatives) in soy germ material and in total crude isofavones extract from defatted sogerm flour.

Isoflavones and their derivatives	Amount of isoflavones in soy germ material (mg/100 g soy germ)	Amount of isoflavones in total crude isoflavones (mg/100 g total crude isoflavones)
Daidzin	454.10	822.99
Glycitin	272.87	550.34
Genistin	144.09	208.12
Malonyldaidzin	325.10	501.13
Malonylglycitin	166.81	279.07
Malonylgenistin	81.52	165.52
Acetyldaidzin	310.70	624.28
Acetylglycitin	145.82	333.84
Acetylgenistin	105.44	179.27
Daidzein	29.99	70.18
Glycitein	32.21	79.09
Genistein	7.29	13.33
Total	2075.9	3827.16

As can be seen in Table 2: the isoflavones content in soy germ material was 2.08 % (2075.9 mg/100 g soy germ); and the isoflavones content in total crude isofavones extract from defatted sogerm flour was 3.83 % (or 3827.16 mg/100 g total crude isoflavones). Therefore, after the extraction of isoflavones from defatted soy germ flour with selected reasonable conditions, we obtained total crude isoflavones with isoflavones content which was 1.84 times higher than that in soygerm flour material. The result in Table 2 also indicated that regarding the total crude isoflavones: in the three of free isoflavones, the content of glycitein was the highest (79.09 mg/100 g), followed by daidzein (70.18 mg/100 g), and genistein (13.33 mg/100 g); however, the content of glycoside, malonyl and acetyl of daidzein were the highest, followed by that of glycitein, and that of genistein.

4. CONCLUSIONS

By using the classic optimal method to optimize extraction process of isoflavones from defatted soy germ flour, reasonable extraction conditions to obtain total crude isoflavones were determined as follows: extraction time 90 min, extraction temperature 40 ^{o}C, ethanol concentration 60 %, material/solvent ratio 1/10 and extraction pH 9.0. After extraction of isoflavones from defatted soy germ flour with the above reasonable conditions, we obtained that

isoflavones content in total crude isoflavones (3.83 %) was 1.84 times higher than that in soygerm flour material (2.08 %).

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