Original Research Article

Extraction Of Phenolic And Flavonoid From Seeds Of Black Soybean (*Glycine max* L.)

ABSTRACT

Aims: The study was conducted on black soybean seeds (Glycine max. L) to optima the conditions of some factors affecting the extracted yield of polyphenol compounds.
Study design: The factors include the types of solvent used (methanol, ethanol and acetone); solvent concentrations (40, 50, 60, 70, 80 and 90 % v/v); ratio of sample and solvent (1:4,1:6, 1:8, 1:10) and cycle of extractions (2, 3, 4); extract times (2, 3, 4h) and temperature (30, 40, 50, 60^{o}C). The efficiency of polyphenol extract was shown by the content of total phenolic (TPC) and flavonoid (TFC).
Place and Duration of Study: Department of Biotechnology and Food technology, Thai Nguyen University of Agriculture and Forestry, Viet Nam, from March 2021 to March 2022.
Methodology: The black soybean seeds were pulverized by a mill and defatted using the Soxhlet system with hexane for 10 hrs as described by Weidner et al. (2012). TPC was determined by the Folin-Ciocalteu method of SusuGiang et al (2013). TFC was determined by colorimetric method as described by Ozsoy et al (2008).
Results: The results showed that the extraction efficiency was high when using 70% acetone solvent; the appropriate ratio of soybean and solvent was 1:10 with 3 cycles of extraction. The extraction efficiency can be improved when extracting samples at temperature at 60^{o}C for 4 hours, TPC and TFC content reached about 5.02±0.04 mgGAE/g and 2.74±0.03 mgQE/g.
Conclusion:70% acetone is the most suitable solvent to extract polyphenols from black soybean. The ratio of sample in solvent is 1:10 (w/v), three times of extraction in 4 hours for each extraction cycle at 60°C for the highest extraction efficiency of TPC and TFC, reaching 7.02 ±0.04 mgGAE/g and 2.74±0.03 mgQE/g, respectively.

Keywords: Extraction, solvent, black soybean seeds, polyphenol, phenolic, flavonoid

1. INTRODUCTION

Black soybean seeds are one of the traditional foods widely used in Asia countries such as Taiwan, Korea, and Japan to promote health due to its ability to prevent cancer, cardiovascular disease, osteoporosis and menopausal symptoms [1]. The significant health benefits are the polyphenols with high biological activity, including antioxidant, anti-proliferative, and cholesterol-lowering effects as well as binding to estrogen receptors. Polyphenol compounds are known as antioxidants in the body [2].
The extraction of polyphenols from black soybean seeds is less studied. Various solvent systems have been used to extract polyphenols from plant materials [3]. Both extraction efficiency and extraction activity are highly dependent on the solvent used [4]. The antioxidant capacity of polyphenol compounds was strongly affected by the polarity of the solvent used for extract. Therefore, the choice of solvents is very important for plant material samples. The extraction solvent system is usually selected according to the purpose, the polarity of the target components, the polarity of the unwanted components, the total cost, safety and environmental concerns. Acetone, ethanol and methanol solvents have been widely used to extract polyphenol components from plant materials, especially herbs and medicinal plants [5]. The extract efficiency depends not only on the solvent but also on the extraction method. In addition, there is no single extraction method applicable to all food

samples because of the complexity of polyphenol compounds and its interactions with other bioactive compounds in food samples. Several factors may contribute to the influence of extract rate and quality of extracted bioactive polyphenol compounds, including extract method, solvent type, solvent concentration, and time two-phase contact, extract temperature, ratio of raw materials and solvents along with particle size of materials [3], [5], [6].

According to Silva *et al.* (2007) [8], extraction methods for polyphenol compounds are different for each plant material and an ideal phenol extraction method for a particular feedstock must be tailored and optimized. Therefore, it is necessary to evaluate the effectiveness of different types of solvents, solvent concentration, sample to solvent ratio as well as number of extract cycles, temperature and extraction time on the ability to separate polyphenol compounds and their antioxidant activity in black soybean.

2. MATERIAL AND METHODS

2.1. Seed materials

Black soybean seed with code GBVN006665 provided by Plant Resource Center (https://prc.org.vn).

2.2. Prepare materials

The black soybean seeds were pulverized by a mill and defatted using the Soxhlet system with hexane for 10 h as described by Weidner *et al*. (2012) [9] and stored at 5 °C after hexane removed. 0.5g of defatted soybean meal was extracted in a solvent according to the ratio as arranged in the experiment, the extraction was used to determine the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity.

2.3. Experiment set-up

Study on the effect of solvent on TPC, TFC and antioxidant activity was carried out using three solvents such as methanol, acetone and ethanol (70%, v/v). The study of the effect of solvent concentration on TPC, TFC and antioxidant activity was performed with concentrations of 40, 50, 60, 70, 80 and 90 respectively (%, v/v). The experiment evaluated the effect of the sample in solvent ratio (1:4, 1:6, 1:8 and 1:10) and the number of extract times (2, 3 and 4 times) on the extract efficiency of TPC and TFC. Similarly, the experiment also assessed the effects of extract time (2 h, 3 h and 4 h) and temperature (30, 40, 50 and 60^{o}C) on TPC and TFC content.

2.4. Determination of the total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu method of SusuGiang*et al*. (2013) [10]. The total phenolic content of the sample was shown as mg gallic acid equivalents per gram of dry matter (mg GAE/g).

2.5. Determination of total flavonoid content (TFC)

TFC was determined by colorimetric method as described by Ozsoy*et al*. (2008) [11]. Results are expressed in mg of quercetin equivalent (QE) per g of dry matter analyzed (mg QE/g).

2.6. Statistical analysis of the data

Experimental results were analyzed using Statgraphics Centurion software. Each experiment was repeated three times. Analysis of variance (ANOVA) with LSD test was used to determine the significant difference ($p<0.05$) between means.

3. RESULTS AND DISCUSSION

3.1. Effect of solvents on extracting polyphenols from black soybean

Total phenolic and flavonoid content (TPC and TFC) extracted from three solvents, acetone, methanol and 70% ethanol are presented in Table 1. Among the surveyed solvents, acetone solvent gave the most effective phenolic and flavonoid extract yield, TPC and TFC content reached 4.96 mg GAE/g and 2.97 mg QE/g, respectively. Next is methanol (TPC=4.67 mg

GAE/g, TFC=2.65 mg QE/g), ethanol (TPC=4.29 mg GAE/g, TFC=2.46 mg QE/g). In the case of powder samples extracted with water, the TPC and TFC contents were very low, reaching only 1.53 mg GAE/g and 0.92 mg QE/g (Table 1, Fig. 2A). This result proves that the addition of solvent increases the extract efficiency of polyphenol compounds in black soybean. Acetone was selected as the solvent to conduct further experiments.

Table 1. Effect of extraction solvents on yield of TPC and TFC

Solvents	Total phenolic content (TPC, mgGAE/g)	Total Flavonoid (TFC, mgQE/g)
Water	1.53±0.03[a]	0.92±0.02[a]
Methanol	4.67±0.05[c]	2.65±0.02[c]
Acetone	4.96±0.03[d]	2.97±0.01[d]
Ethanol 70%	4.29±0.04[b]	2.46±0.01[b]

3.2. Effect of acetone concentration on ability to extract polyphenols from black soybean

The results of the study in Table 2 show that the concentration of acetone has a significant effect on the ability to extract polyphenols from black soybean. When increasing the acetone concentration from 40 to 90%, the TPC content decreased from 4.96 to 3.01 mg GAE/g (Table 2). In contrast, the TFC content varied with acetone concentration and TFC reached the maximum (2.77 and 2.76 mgQE/g) with 60 and 70% acetone concentrations, respectively. The TFC content then gradually decreased at high acetone concentrations >80% or low <50% (Table 2, Fig.2B).

Table 2. Effect of acetone concentration on the ability to extract TPC and TFC from black soybean

Concentration acetone (%)	Total phenolic content (TPC, mgGAE/g)	Total Flavonoid (TFC, mgQE/g)
40	4.94±0.01[d]	1.92±0.02
50	4.96±0.02[d]	2.65±0.03
60	4.78±0.04[c]	2.77±0.02
70	3.91±0.02[b]	2.76±0.04
80	3.07±0.03[a]	2.64±0.02
90	3.01±0.02[a]	2.12±0.02

3.3. Effect of the ratio of sample to solvent and number of extraction times on yield of polyphenols from black soybean

The sample to solvent ratio significantly affects the extraction efficiency of TPC and TFC from black soybean. The results showed that the sample:solvent ratio of 1:10 (w/v) gave the highest TPC and TFC content, reaching 5.02 mgGAE/g and 2.74 mgQE/g, respectively. TPC and TFC content decreased with increasing or decreasing the sample:solvent ratio. TPC and TFC content was only 4.11-4.97 mgGAE/g and 2.14-2.44 mgQE/g when extracted with sample:solvent ratio from 1:4 to 1:8 (w/v)) or greater than 1:10 (Table 3, Fig. 1). In addition, the number of extraction also affects the TPC and TFC content. At the same sample:solventratio, the number of extraction times will increase the TPC and TFC content, and the three times of extraction gave the highest efficiency (Table 3). This result showed that the sample:solvent ratio was 1:10 for the highest yeild of TPC and TFC after 3 times of extraction.

Table 3. Effect of sample/solvent ratio and extraction times on yeild of TPC and TFC

Sample:solvent ratio	Number of extraction	Total phenolic content (TPC, mgGAE/g)	Total Flavonoid (TFC, mgQE/g)
1:4	1	1.25±0.04	1.12±0.02
	2	3.06±0.02	2.01±0.03
	3	5.16±0.05	3.14±0.02
	4	4.11±0.05	2.14±0.02
1:6	1	1.31±0.03	1.22±0.03
	2	3.84±0.02	2.26±0.02
	3	4.92±0.04	2.87±0.02
	4	4.12±0.04	2.27±0.02
1:8	1	1.65±0.03	1.42±0.02
	2	4.03±0.02	2.13±0.01
	3	5.22±0.04	3.24±0.03
	4	4.62±0.04	2.54±0.03
1:10	1	1.95±0.02	1.95±0.02
	2	4.73±0.03	2.51±0.04
	3	7.92±0.04	3.74±0.03
	4	7.02±0.04	2.14±0.03
1:14	1	1.92±0.02	1.87±0.02
	2	4.35±0.04	2.61±0.04
	3	5.59±0.03	3.24±0.03
	4	4.89±0.03	2.84±0.03
1:16	1	1.90±0.02	1.76±0.02
	2	4.43±0.03	2.14±0.02
	3	5.97±0.03	3.72±0.04
	4	4.32±0.04	2.14±0.03

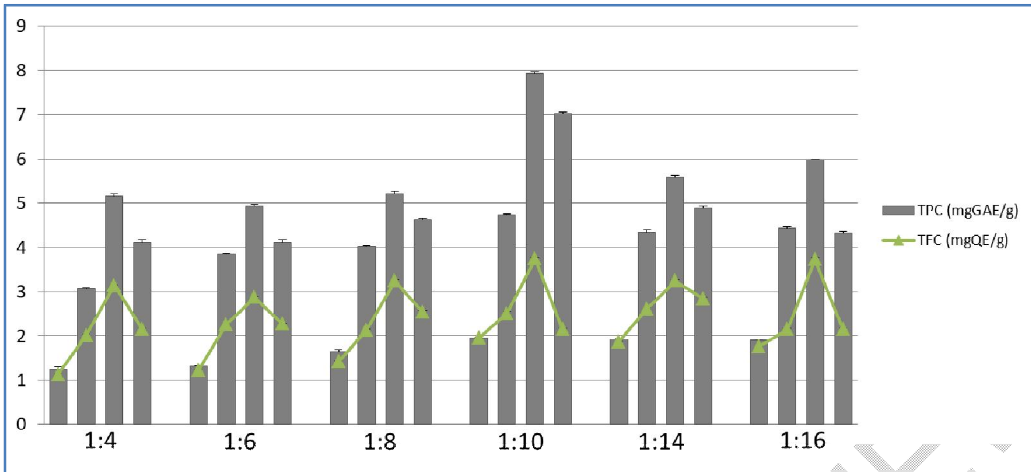

Figure 1.Effect of sample and solvent ratio and extraction times on yeild of TPC and TFC from black soybean seeds

3.4. Effect of extraction time and temperature on yield of TPC and TFC

Extraction time had a significant impact on the extraction efficiency of TPC and TFC. The results showed that TPC and TFC contents changed with increasing extraction time from 2h to 7h. When extracting samples from 2 h to 4 h, the concentration of TPC and TFC gradually increased from 3.46 to 4.87 mgGAE/g for TPC and 1.63 to 2.78 mgQE/g for TFC (Table 4, Fig. 2C). However, the content of TPC and TFC decreased gradually when increasing the extraction time from 5h to 7h.

Table 4. Effect of extraction time on yield of TPC and TFC

Extraction time	Total phenolic content (TPC, mgGAE/g)	Total Flavonoid (TFC, mgQE/g)
2h	3.46±0.01^{a}	1.63±0.02^{a}
3h	4.03±0.02^{c}	1.89±0.05^{b}
4h	4.87±0.04^{f}	2.73±0.03^{e}
5h	4.73±0.03^{e}	2.78±0.04^{e}
6h	4.21±0.02^{d}	2.65±0.02^{d}
7h	3.83±0.03^{b}	2.40±0.04^{c}

The extraction temperature of about 60^{o}C after 4 hours gave the highest concentration of TPC and TFC, reaching 3.97 mgGAE/g and 2.14 mgQE/g, respectively. At temperatures higher or lower than 60oC both reduce TPC and TFC content (Table 5, Fig. 2D). From the research results, the extract efficiency of TPC and TFC was highest after 4 hours at 60oC.

Table 5. Effect of extraction temperature on yield of TPC and TFC

Temp (^{o}C)	Total phenolic content (TPC, mgGAE/g)	Total Flavonoid (TFC, mgQE/g)
20	1.42±0.02	0.89±0.03
30	1.55±0.03	0.93±0.02
40	2.03±0.04	1.21±0.03
50	2.96±0.02	1.53±0.02
60	3.97±0.04	2.14±0.04
70	3.63±0.03	2.02±0.02
80	3.41±0.02	2.10±0.01

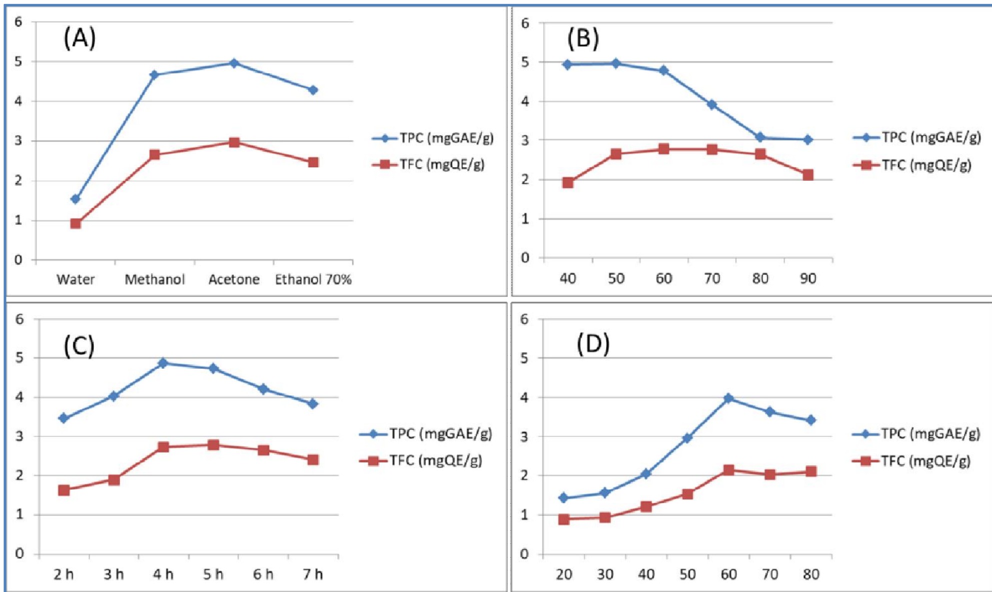

Figure 2. Effect of extraction parameters on yield of TPC and TFC extracted from black soybean seeds including solvent types (A), concentration of Acetone (B), extraction time (C) and extraction temperature (D).

Acetone is a commonly used as solvent for polar antioxidants and is highly effective when extracting polyphenols from protein complexes [12]. In fact, the use of acetone solvents has many advantages over the use of ethanol and methanol solvents and gives a higher extraction yield. However, the research results showed that the extraction efficiency of TPC and TFC depends on other factors such as acetone concentration, solvent ratio, time, temperature and number of extraction times. Based on the above analysis results, acetone 70% (v/v), sample:solvent ratio of 1:10 (w/v) gave the highest TPC and TFC content. These results are consistent with the mass transfer principle that the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent. A high solvent ratio can promote an increasing concentration gradient, leading to increased diffusion rates allowing better solvent extraction [13]. However, the amount of bioactive ingredients will not continue to increase once equilibrium has been reached [14].
Multiple extractions are an important method to improve the content of polyphenols (Chen et al., 2013). Three extraction times resulted in higher TPC and TFC content. However, four extraction cycles could not improve the yield of both TPC and TFC components. According to Spigno et al (2007) [15], extraction temperature affects the solubility, mass transfer rate and stability of polyphenol compounds. The results confirm the fact that below a certain limit, high temperature enhances extraction efficiency by enhancing the degree of diffusion and solubility of the analyte in solvents [16]. Beyond that certain limit, high extraction temperature will reduce TPC and TFC. Observation of the study results showed that extending the extraction time from 2 to 5 hours TPC and TFC in the solvent increased significantly. However, there was no significant difference in both TPC and TFC when extending extract time up to 5 h. This result can be explained by Fick's second law of diffusion when predicting the final equilibrium between the solute concentrations in the solid matrix in the solvent can be reached after a given time determined [8].

4. CONCLUSION

70% acetone is the most suitable solvent to extract polyphenols from black soybean. The ratio of sample in solvent is 1:10 (w/v), three times of extraction in 4 hours for each extraction cycle at 60°C for the highest extraction efficiency of TPC and TFC, reaching 7.02 ±0.04 mgGAE/g and 2.74±0.03 mgQE/g, respectively

REFERENCES

1 BolanhoBC, Adelaide DPB, Bioactive compounds and antioxidant potential of soy products. AlimNutrAraraquara. 2011; 22 (4): 539-546.

2 IsangaJ and Zhang G, Soybean bioactive components and their implications to health - a review. Food Reviews International. 2008;(24): 252–276.

3 Chew KK, Ng SY, Thoo YY, Khoo MZ, Aida WM and Ho CW, Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphonstamineus extracts. International Food Research Journal. 2011;(18): 1427-1435.

4 Tan MC, Tan CP and Ho CW, Effects of extraction solvent system, time and temperature on total phenolic content of henna (Lawsoniainermis) stems. International Food Research Journal. 2013; 20(6): 3117-3123.

5 Wang J, Sun BG, Cao Y, TianY and Li XH, Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chemistry. 2012; 106: 804-810.

6 Dai J and Russell JM, Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules. 2010; 15: 7313-7352.

7 Pinelo M, Rubilar M, Jerez M, SineiroJ and Nuñez MJ, Effect of solvent, temperature, solvent to solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. Journal of Agricultural and Food Chemistry. 2005; 53: 2111-2117.

8 Silva EM, Rogez H and Larondelle Y, Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. Separation and Purification Technology. 2007; 55: 381-387.

9 Weidner S, Powałka A, KaramaćM and Amarowic R, Extractios of phenolic compounds from seeds of three wild grapevines—Comparison of their antioxidant activities and the content of phenolic compounds. Int. J. Mol. Sci. 2012; 13: 3444–3457.

10 Jiang S, CaiW and Xu B, Food Quality Improvement of Soy Milk Made from Short-Time Germinated Soybeans. Foods. 2013;2:198-212.

11 Ozsoy N, CanA, Yanardag R and Akev N, Antioxidant activity of Smilax excelsa L. leaf extracts. Food Chemistry. 2008; 110: 571-583.

12 Al-Farsi MA and Chang YL, Optimization of phenolics and dietary fiber extract from date seeds. Food Chemistry. 2007; 108: 977-985.

13 CacaceJE and Mazza G, Mass transfer process during extraction of phenolic compounds from milled berries. Journal of Food Engineering. 2003; 59: 379–389.

14 Herodež ŠS, Hadolin M, ŠkergetM and Knez Ž, Solvent extract study of antioxidants from *Melissa officinalis* L. leaves.Food Chemistry. 2003; 80: 275-282.

15 Spigno G, TramelliL and De Faveri DM, Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering. 2007; 81:200 – 208.

16 Ju ZY, Howard LR,Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. J Agric Food Chem. 2003; 51(18):5207–5213.