
Effects of extraction process on phenolic content and antioxidant activity of soybean

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Abstract: In this research, a set of experiments was carried out for identifying the optimum conditions of independent variables affecting phenolic content extraction efficiency and antioxidant activity of soybean seeds (*Glycine max L.*). They included the use of different organic solvents (methanol, ethanol and acetone); concentrations of solvent (40, 50, 60, 70, 80, and 90 v/v, %); the soybean-to-solvent ratio (1:4, 1:6; 1:8 and 1:10) and the number extraction cycles (2, 3 and 4); the extraction time (2, 3 and 4 hours) and the temperature (30, 40, 50 and 60°C). The extraction abilities of phenolics manifested in forms of total phenolic and total flavonoid contents (TPC and TFC) as well as the antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical scavenging (DPPH) were used as assessment indicators. Generally, high extraction yield was obtained using aqueous acetone 70% as solvent; the most suitable soybean-to-solvent ratio was 1:6 for 3 cycles of extraction. The extraction yield could further be increased using a prolonged time of 3 hours at the temperature of 40°C.

Keywords: Soybeans, Polyphenols, Flavonoids, Extraction, Antioxidant Activity, DPPH Radical Scavenging

1. Introduction

Soybean (*Glycine max L. Merrill*) is used extensively for food formulation and it is considered a desirable ingredient for health promotion because of its ability on prevention of cancer, cardiovascular diseases, osteoporosis and menopause symptoms (Adlercreutz and Mazur, 1997; Omoni and Aluko, 2005; Bolanho and Beléia, 2011). These health benefits have been ascribed substantially to the bioactivity of soy polyphenols, including their antioxidant, anti-proliferative, and hypocholesterolemic effects as well as binding to oestrogen receptors (Isanga and Zhang, 2008). The phenolic compounds are known as active antioxidant and as such they are able to scavenge free radicals – important due the deleterious effect of such reactive species, since they are able to oxide bio molecules which may damage cells and cause tissue alterations (Madhavi *et al.*, 1995; Bolanho and Beléia, 2011).

Extraction process is widely known as a separation process of a crude extract of phytochemicals from the raw materials (Chew *et al.*, 2011). Different solvent systems have been used for extraction of polyphenols from plant materials (Chavan *et*

al., 2001). Both extraction yield and extraction activity are strongly dependent on the solvent (Tan *et al.*, 2013). Antioxidative potential of phenolic compounds is strongly affected by the polarity of solvent used in extraction. Hence, the selection of extraction solvents is critical for the complex plant samples. An extraction solvent system is generally selected according to the purpose of extraction, polarity of the interested components, polarity of undesirable components, overall cost, safety and environmental concern (Wang *et al.*, 2008). Aqueous mixtures of acetone, ethanol and methanol have been widely used for extracting phenolic components from botanical materials, especially herbs and medicinal plants (Tabart *et al.*, 2007; Wang *et al.*, 2008). Extraction yield is not only dependent on the solvent but also on the method of extraction (Goli *et al.*, 2004). The extraction method must enable complete extraction of the compounds of interest and must avoid their chemical modification (Zuo *et al.*, 2002). In addition, there is no single universal extraction method applicable for all food matrices because of the complexity of phenolic compounds and its

interaction with other bioactive compounds presented in the food matrices. There are few factors would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds, including method of extraction, solvent type, solvent concentration, contact time, extraction temperature, solid to solvent ratio and particle size (Chew *et al.*, 2011; Jin Dai *et al.*, 2010; Pinelo *et al.*, 2005).

According to Silva *et al.* (2007), the extraction method of phenolic compounds differs from plant to plant and an ideal extraction method for a particular phenolic source has to be individually designed and optimized. The objective of this study was to evaluate the efficacy of solvent types, solvent concentrations, the soybean-to-solvent ratios as well as the number extraction cycles, the extraction temperature and time for the extraction of phenolic contents and antioxidant activity of soybean.

2. Materials and Methods

Preparation of samples: Soybeans (*Glycine max* L., MTĐ 760 variety) were supplied from Department of Agricultural Genetic, College of Agricultural and Applied Biology, Cantho University. The cleaned soybeans were ground (Giao *et al.*, 2009), defatted in a Soxhlet extractor with hexane for 10h (Weidner *et al.*, 2012) and then were stored at 5°C after removal of hexane.

Experimental design for the extraction of TPC and TFC from soybean: A mass of 0.5g of defatted soybean powder was extracted twice with 5ml solvent with shaking and the two extracts were combined for determination the TPC, TFC and antioxidant potential.

Studying effects of solvent types on TPC, TFC and antioxidant activity was carried out with three types of solvents as methanol, acetone and ethanol (70%, v/v). Studying effects of solvent concentrations on TPC, TFC and antioxidant activity was carried out with the concentration of solvent as 40, 50, 60, 70, 80 and 90 (% v/v). To study the effects of soybean-to-solvent ratio (1:4, 1:6, 1:8 and 1:10) and the number extraction cycles (2, 3 and 4) on TPC, TFC and antioxidant activity was designed in form two factor experiment. Similarly, the effects of extraction time (2, 3 and 4 hours) and temperature (30, 40, 50 and 60°C) on TPC, TFC and antioxidant activity was investigated by two factor design.

Determination of the TPC in the extracts: The TPC was estimated by Folin-Ciocalteu method (Susu Jiang *et al.*, 2013). The total phenolic content of samples was expressed as milligrams gallic acid equivalents per gram of dry matter (mg GAE/g).

Total flavonoid content (TFC) The TFC was determined by the colorimetric method as described by Ozsoy *et al.* (2008). The results were expressed as milligrams of quercetin equivalents (QE) per g of dry matter sample (mg QE/g).

Antioxidant activity: Antioxidant activity of the phytochemicals extracted from soybean was assessed by measuring their radical scavenging activity that was measured by the bleaching of the purple-coloured methanol

solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent and was described by Anshu *et al.* (2011).

Statistical analysis: All the experimental results were analysed using Portable Statgraphics Centurion software (Version 15.2.11.0). Every assay was done in triplicates. Analysis of variance (ANOVA) with LSD test was used to determine the significant differences ($p < 0.05$) between means.

3. Results and Discussions

3.1. Effects of Types of Solvent on the Contents of TPC, TFC and Antioxidant Activity

Figures 1 show the TPC and TFC results of soybean extracted from three types of solvents as acetone, methanol and ethanol. Most efficient extractor of polyphenols (TPC and TFC) among the examined solvents seemed to be acetone solvent. The result is suitable to their ability to deactivate stable DPPH radicals has been shown in Figure 2. The strongest properties to DPPH radicals showed acetone extracts (% scavenging radicals at the level of 76.4). Many studies showed that acetone is the best solvent for proanthocyanidins and tannins extraction (Chirinos *et al.*, 2007; Tabart *et al.*, 2007). These facts are in accordance with polarity of the solvent used for the extraction and solubility of phenolic compounds in them. It is interesting to note that the polarity of acetone, ethanol and methanol is 0.355, 0.654 and 0.762, respectively (Tan *et al.*, 2013). Aqueous acetone is a good solvent for polar antioxidants and more useful for extracting.

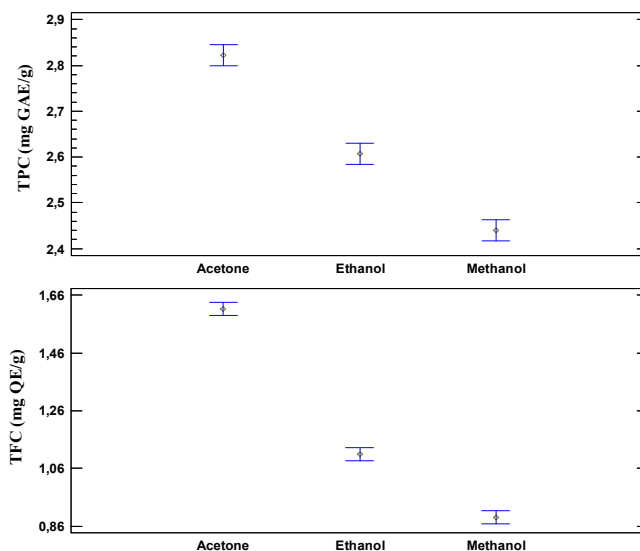


Figure 1. TPC and TFC contents from soybean extracted by various solvents (means and 95% LSD intervals).

Polyphenols from protein matrices, since they appear to degrade the polyphenol-protein complexes (Chirinos *et al.*, 2007; Al-Farsi and Lee, 2008). In fact, the use of aqueous acetone has several advantages to the use of aqueous ethanol

and aqueous methanol, for example higher extraction efficiency, suggesting the use of aqueous acetone as extraction solvent for the following stages in this study. However, there is still a need to check if using different water percentages in acetone (% v/v) is possible to increase the extraction efficiency of TPC from soybean in the experiment.

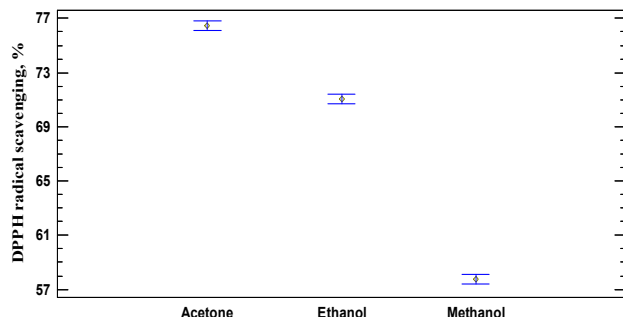


Figure 2. Antioxidant activities from soybean extracted by various solvents (means and 95% LSD intervals).

3.2. Effects of Acetone Concentration on the TPC, TFC Contents and Antioxidant Activity

The effects of acetone concentration in the extraction solvent on the content of polyphenols and flavonoids in soybean extracts were shown in Figure 3. The correspondently soybean antioxidant activities was expressed on Figure 4. The acetone concentration had a significant effect ($p < 0.05$) on the extraction efficiency of both TPC and TFC from soybean.

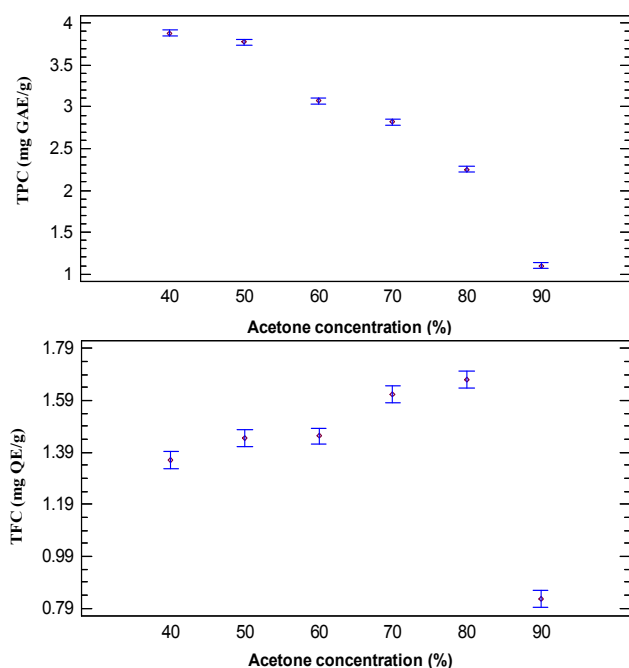


Figure 3. TPC and TFC contents from soybean extracted by various concentrations of acetone (means and 95% LSD intervals)

As can be seen from Figure 3, the TPC content increases as decreasing the acetone concentration. However, the TFC content follows a curve shape and TFC of soybean extracts

reached a maximum at 70-80% acetone (v/v) followed by a significant ($p < 0.05$) decrease with further increase in the concentration of the acetone in the extraction medium. The change in TFC content was same to the change in antioxidant activity (Figure 4) that reached to maximum value at 70% acetone. The different rule in the change of TPC might be due to the more water in aqueous acetone solution is the more water soluble proteins extracted. The reaction of these soluble proteins and Folin reagent resulted in the change of absorbance reading. Form all observations, a moderately polar solvent of 70% acetone (v/v) was chosen for the next step of study.

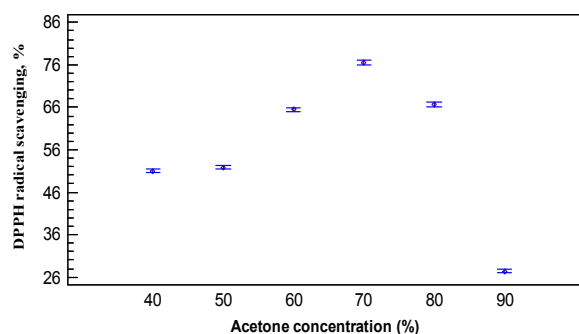


Figure 4. Antioxidant activities from soybean extracted by various concentrations of acetone (means and 95% LSD intervals).

3.3. Effects of the Soybean-to-Solvent Ratio and the Number Extraction Cycles on the TPC and TFC Contents

The effects of soybean-to-solvent ratio and number extraction cycles on total phenolic content (TPC) and total flavonoid content (TFC) are shown in can be observed that both the number extraction cycles and the soybean-to-solvent ratio effect significantly on TPC and TFC contents. Soybean-to-solvent ratio of 1:6 (w/v) showed high amount of TPC and TFC. A further increase in soybean-to-solvent ratio from 1:6 to 1:10 did not significantly ($p > 0.05$) increase both values of TPC and TFC. According Tan *et al.* (2011), a high solid to solvent ratio was found to be favorable in extraction of phenolic compounds. These results were consistent with mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent. A high solid-to-solvent ratio could promote an increasing concentration gradient, resulting in an increase of diffusion rate that allows greater extraction of solids by solvent (Cacace and Mazza, 2003; Al-Farsi and Chang, 2007). In addition, the chance of bio-active components coming into contact with extracting solvent expanded with increase amount of extraction solvent, leading to higher leaching out rates (Zhang *et al.*, 2007). However, active component yields will not continue to increase once equilibrium is reached (Herodež *et al.*, 2003).

Multiple-step extraction is an important method to improve the extraction yield of polyphenols (Chen *et al.*, 2013). Three cycles extraction showed higher yield of both TPC and TFC than that of using two cycles of extraction.

However, four cycles of extraction could not improve the yield of both TPC and TFC (Fig. 5).

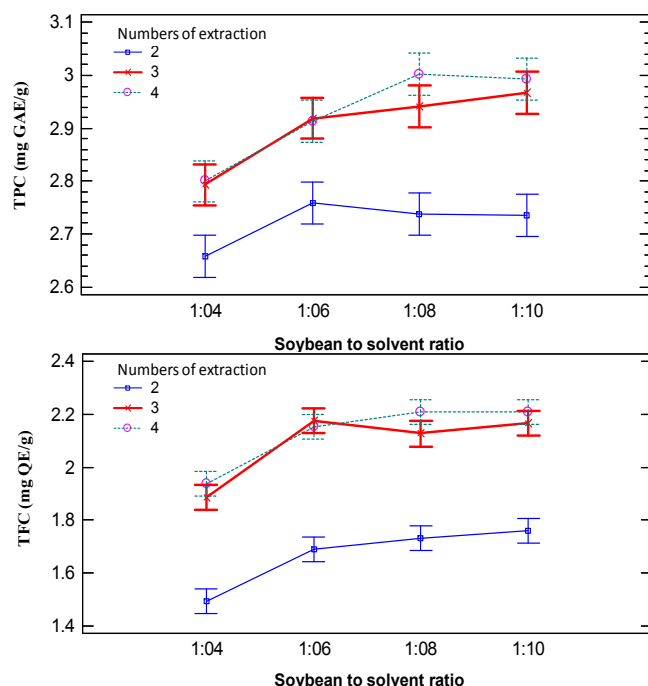


Figure 5. Effects of soybean-to-solvent ratio and number extraction cycles on TPC and TFC of soybean (means and 95% LSD intervals)

From obtained results, the soybean-to-solvent ratio of 1:6 (w/v) and three cycles extraction are optimum conditions for extraction TPC and TFC from soybean.

3.4. Effects of the Extraction time and Temperature on the Contents of TPC, TFC and Antioxidant Activity

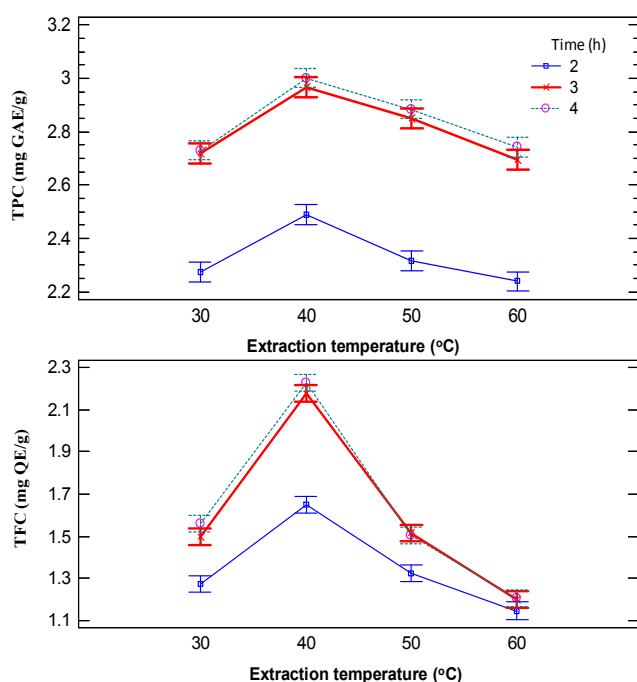


Figure 6. Effects of extraction time and temperature on TPC and TFC contents of soybean (means and 95% LSD intervals)

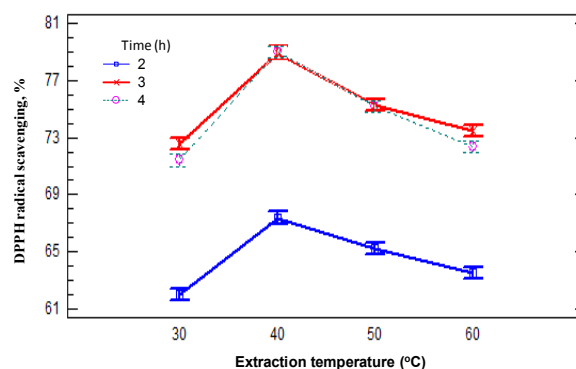


Figure 7. Effects of extraction time and temperature on antioxidant activity of soybean (means and 95% LSD intervals)

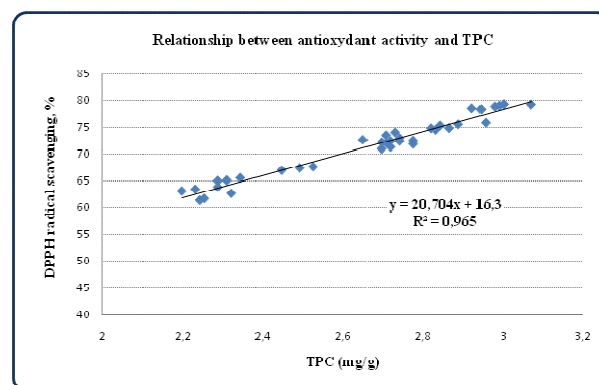


Figure 8. Correlation between DPPH free radical scavenging and TPC content

The selection of appropriate extraction time and temperature was the final step in experiments. The changes in TPC and TFC contents by extraction time and temperature were described in Figure 6. The effect of these factors on antioxidant activity was shown in Figure 7. The TPC and TFC contents increased significantly with increasing temperature reaching a peak at 40°C, and then decreased significantly.

According Spigno *et al.* (2007), extraction temperature impacts the solubility, mass-transfer rate, and stability of phenolic compounds. The results confirmed the fact that, below a certain limit, higher temperatures improve the efficiency of extraction due to enhancement of the diffusion rate and the solubility of analytes in solvents (Ju and Howard, 2003). Beyond the limit, high extraction temperatures decrease TPC and TFC contents. It has been observed in the study that with the prolongation of the extraction time from 2 to 3 hours the output of polyphenols and flavonoids in solvent increased significantly. But there is no significant difference in TPC and TFC contents with the prolongation of extraction time up to 4 hours. These phenomena could be well explained by the Fick's second law of diffusion, predicting that a final equilibrium between the solute concentrations in the solid matrix and in the solvent might be reached after a certain time (Silva *et al.*, 2007). Effects of time and temperature on DPPH free-radical scavenging potential showed a similar trend of TPC and TFC. Interesting result was the fine linear correlation between DPPH free

radical scavenging and TPC content with $R^2 = 0.97$ (Figure 8), confirming that polyphenols are likely to contribute to the radical scavenging activity of these plant extracts (Miliauskas *et al.*, 2004). Similar results have also been reported for different plants by various studies (Katalinic' *et al.*, 2004; Maksimovic' *et al.*, 2005; Miliauskas *et al.*, 2004; Yu *et al.*, 2005 and Turkmen *et al.*, 2006).

4. Conclusions

Acetone was found to be the best solvent for the soybean extraction. Subsequently, the optimum conditions for maximum TPC, TFC and antioxidant activity were found to be acetone concentration 70% (v/v), soybean-to-solvent ratio of 1:6 (w/v), three cycles extraction, 3 hours for each cycle extraction at the temperature 40°C. Operating with these conditions, the TPC and TFC contents were 2.97 ± 0.041 mgGAE/g (db) and 2.18 ± 0.057 mgQE/g (db), respectively. The DPPH free radical scavenging reaches $78.96 \pm 0.34\%$ and has a good correlation to the total polyphenol content.

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