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Soybean and Fermented Soybean Extract Antioxidant Activities

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***Abstract*—**Today, people are more interested in the foods beneficial on their health. However, there are still lacks of accurate knowledge in the field of biological properties, functional properties, including the application of legume in foods. This study focused on antioxidant activity of soybean (SB) and fermented soybean (FSB) crude extracts evaluating to have more information in fortification SB and FSB crude extracts in food products and/or dietary supplement. SB and FSB crude extracts were prepared by infusion with water and ethanol. The antioxidant activity of crude extracts was studied with DPPH and ABTS assay including commercial standard. From both DPPH and ABTS assay, the antioxidant activity of SB and FSB water crude extract showed higher antioxidant activity than ethanol crude extract, and FSB crude extract showed higher antioxidant activity than SB crude extract. In DPPH assay, BHT and vitamin C showed IC_{50} values at 0.241, 0.039 mg/ml, in ABTS assay. In addition, Trolox showed IC_{50} at 0.058 mg/ml respectively. FSB water crude extract showed high antioxidant activity. Finally, the functional properties study of both water and ethanol crude extracts should be done for beneficial in application of these extracts in food products and dietary supplement in the near future.

***Keywords*—**Antioxidant activity, Fermented soybean (FSB) crude extracts, soybean (SB) crude extracts.

I. Introduction

SOYBEAN is a scientific name *Glycine max(L.) Merrill,* in the family is *Leguminosae*. The plant is popular with consumed because of the high nutritional value such as vitamin A, B, C and minerals that the body needs high volume.[1]. A medical report on the benefits of eating soybean in the prevention and treatment of various diseases such as prevent obesity [2] cancer [3-4] osteoporosis [5] cardiovascular disease [6-8] and renal obstruction [9]. Soybean is also a source of isoflavones (e.g. genistein, daidzein), they are known to possess the protective effect against oxidative damage related with cancer and atherosclerosis [10].

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Thua nao is a traditional Thai fermented soybean product which has been produced and consumed for several decades. This product is popular especially for people in the Northern part of Thailand [11]. Conventional production of thua nao is as following: soybeans are washed, soaked overnight, cooked by boiling for about 3-4 h, gently smashed and wrapped inside banana leaves. The fermentation generally proceeds for 2-3 days at ambient temperature [12]. Similar fermented soybean products have been described in several countries, ie *kinema* in *India*, *schuidouchi* in *China*, and *natto* in *Japan*. The best-characterized fermented soybean product is probably *natto* – the Japanese styled fermented soybeans [12]. Bacillus subtilis, the main microorganism found in fermented soybean [13]. The ability of B. subtilis active growth between pH 5.5 – 8.5 and to produce several enzymes (ie proteases) and other useful biological compounds seems a likely reason for its superiority in the soybean fermentation [12].

Most antioxidant nutrients are polyphenolic compounds, acting as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers. Soybean and different soybean products are known to contain phenolic compounds. Concentration of these compounds in soybean is reported to increase after fermentation [13].The main source of the biologic activities of fermented soybean foods is isoflavones that reported exhibiting estrogenic, antioxidative, antiosteoporotic, and anticarcinogenic activities by several researchers. Heating or fermentation process affects total isoflavone content and its composition in soy products. It is known that glycoside isoflavones in soybeans are hydrolyzed by β-glucosidase produced by microorganisms, thereby increasing aglycone isoflavones during fermentation [14]. During natto fermentation, nattokinase, a clot-dissolving agent that has been used in the treatment of cardiovascular disease, is produced. This property is one reason why natto has been enticed attention from both medical researchers and health experts alike as a healthy food source [15].

This study was aimed at evaluating the antioxidant activity of soybean (SB) and fermented soybean (FSB) crude extracts for getting more information related to how to fortified or apply SB and FSB crude extracts in food products and dietary supplement.

II. Material and Method

A. Sample Preparation Soybean

Fresh soybean (Glycine max L. Merill) samples of Chiang Mai 60 variety were purchased from Loei Field Crop Research Center (Loei Province, Thailand), dried and grounded to powder, sieve by mesh size 2 mm and kept in vacuum package at 4° C until used.

Fermented soybean

Initially, soybeans were washed and soaked in tap water for 12 h at ambient temperature (~25°C). After decanting the water, soaked soybeans were placed in a plastic bag and autoclaved at 121°C for 15 min. After cooling, the steamed soybeans (500 g) were inoculated with the test organism by evenly spraying 1 mL spore suspension of B. subtilis SB-MYP-1. After a thorough mixing, fermentation process was allowed to proceed at 37°C for 72 h. Fermented soybeans were freeze-dried and grounded to powder, sieve by mesh size 2 mm and kept in vacuum package at 4° C until used.

B. Preparation of Water and Ethanol Extract of Soybean and Fermented Soybean

The ground powder of the samples was extracted using the method previously [16]. Two grams of freeze-dried samples were dissolved in 10 ml of water or ethanol in a 50 mL screw-cap tube, vortexes for 1 min, sonicated in sonicator for 15 min, and centrifuged at 3200 rpm for 30 min. The sample remains were subjected to repeated extractions with 10 mL of water or ethanol. Supernatants from two extractions were then combined, concentrated with an evaporator and dried by nitrogen gas flow at room temperature for ethanol extract. For water extract were dried by freeze dryer. The samples were kept at -20° C until used [19-21].

C. DPPH Free Radical Scavenging Assay

The scavenging activity of the stable 1,1-diphenyl-2-picrylhy-drazyl (DPPH) free radical was determined by the method described by [17], [22]. Briefly, 62.5 µM solution of DPPH in methanol was prepared. Aliquots (0.1 mL) of gallic standard/sample/blank transferred into test tube. After addition of 1.90 mL DPPH solution, mix solution and let stand for 15 min and read absorbance at 515 nm. The BHT and Ascorbic acid in MeOH solution were used as positive controls. Inhibition of free radical DPPH was calculated according to the formula:

$$\%\ Antioxidant\ Activit = \left[\frac{(Abd-(As-Abs)}{Abd}\right] \times 100 \qquad (1)$$

where Abd is the absorbance of MeOH Blank, As is the absorbance of sample and Abs is the absorbance of sample Blank. All samples were analyzed in triplicate.

D. ABTS$^+$ Radical-Scavenging Assay

ABTS radical-scavenging activity of extracts was determined according to [18]. The ABTS$^+$ cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h.

Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 1 ml, the reaction mixture comprised 950 µl of ABTS$^+$ solution and 50 µl of the sample extracts at various concentrations and distilled water for control. The reaction mixture was homogenized and its absorbance was recorded at 734 nm after at least 6 min. Similarly, the mixture of standard group reaction was obtained by mixing 950 µl of ABTS$^+$ solution and 50 µl of Trolox. The inhibition percentage of ABTS radical was calculated using the following formula:

$$ABTS\ scavenging\ effect\ = \left[\frac{(A_0-A_1)}{A_0}\right] \times 100 \qquad (2)$$

where A_0 is the absorbance of the control at 6 min, and A_1 is the absorbance of the samples at 6 min. All samples were analyzed in triplicate.

III. Result and Discussion

A. DPPH Free Radical Scavenging Assay

The IC50 of water and ethanol soybean extracts are shown in Table I.

TABLE I
DPPH Scavenging Activity of Soybean and Fermented Soybean Crude Extracts using Water and Ethanol Extraction Solvents

Samples	**IC_{50} (mg / ml)**	
	Water	Ethanol
	(mg extract/ ml)	(mg extract/ ml)
Soybean	21.091 ± 0.558	41.294 ± 3.184
Fermented Soybean	14.277 ± 0.532	29.468 ± 1.215
BHT	0.241 ± 0.332	0.241 ± 0.332
Ascorbic acid	0.039 ± 0.032	0.039 ± 0.032

Note: The values are mean ± SD.

DPPH radical scavenging activity of SB and FSB water and ethanol crude extracts reveal antioxidant potency based on IC_{50} values when compare with BHT and ascorbic acid as shown in Table I. At lower value of IC_{50} indicates a higher antioxidant activity. In fermented soybean, antioxidant activity of water crude extract by DPPH assay showed higher antioxidant activity than ethanol crude extract at IC_{50} values 14.277 mg crude extract/ml and 29.468 mg crude extract/ml, respectively. Whereas soybean, water crude extract showed higher antioxidant activity than ethanol crude extract at IC_{50} values 21.091 mg crude extract/ml and 41.294 mg crude extract/ml, respectively. The antioxidant activity of fermented soybean crude extract showed higher antioxidant activity than soybean crude extract.

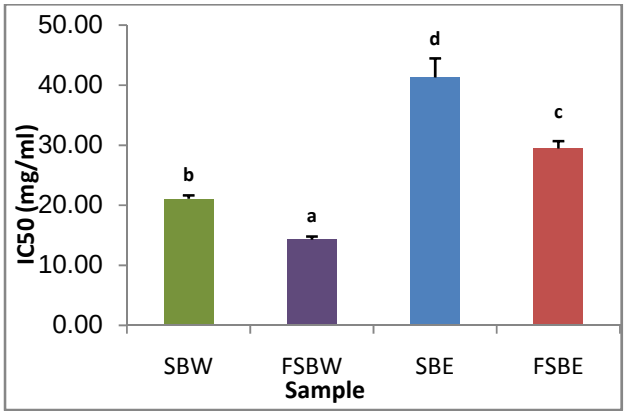

Fig. 1 DPPH scavenging activity (mg crude extract / ml) of soybean and fermented soybean crude extracts (SBW: water soybean crude extract, FSBW: water fermented soybean crude extract, SBE: ethanol soybean crude extract and FSBE: ethanol fermented soybean crude extract). Measurements were carried out in triplicate. Means and standard deviation are represented, and different letters above the bars indicate significantly different (P < 0.05) means

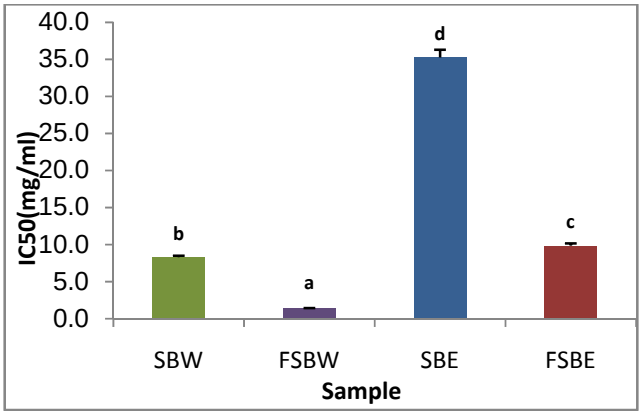

Fig. 2 ABTS$^+$ radical-scavenging (mg crude extract /ml) of soybean and fermented soybean crude extracts (SBW:water soybean crude extract , FSBW: water fermented soybean crude extract ,SBE: ethanol soybean crude extract and FSBE: ethanol fermented soybean crude extract). Measurements were carried out in triplicate. Means and standard errors are represented, and different letters above the bars indicate significantly different (P < 0.05) means

B. ABTS$^+$ Radical-Scavenging Assay

In Table II, ABTS$^+$ radical-scavenging of water and ethanol crude extracts of soybean and fermented soybean reveal antioxidant potency considering based on the IC_{50} values in comparison with trolox. At low value of IC_{50} indicates a higher scavenging activity. In fermented soybean, ABTS$^+$ radical scavenging activity of water extract had higher scavenging activity than ethanol extract. Both water and ethanol fermented soybean crude extracts also showed IC_{50} values at 1.400 mg crude extract/ml and 9.859 mg crude extract/ml by ABTS assay respectively. Though soybean, water crude extract showed higher antioxidant activity than ethanol crude extract at IC_{50} values 8.306 mg crude extract/ml and 35.264 mg crude extract/ml, respectively. The ABTS$^+$ radical scavenging activity of fermented soybean crude extract showed higher antioxidant activity than soybean crude extract.

TABLE II
ABTS$^+$ RADICAL-SCAVENGING OF SOYBEAN AND FERMENTED SOYBEAN CRUDE EXTRACTS

Samples	IC_{50} (mg / ml)	
	Water	Ethanol
	(mg extract/ ml)	(mg extract/ ml)
Soybean	8.306 ± 0.207	35.264 ± 1.044
Fermented Soybean	1.400 ± 0.035	9.859 ± 0.309
TROLOX	0.058 ± 0.008	0.058 ± 0.008

Note. The values are mean ± SD.

IV. CONCLUSION

Fermented soybean crude extract showed higher antioxidant potency than soybean crude extract. Furthermore, extraction with water give higher antioxidant activity crude extracts than ethanol extraction in both DPPH free radical scavenging assay and ABTS$^+$ radical-scavenging assay. In the conclusion, the SB and FSB crude extracts could be applied or fortified as food ingredient or dietary supplement with functional properties research.

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