

Research Article

The Impact of Different Extraction Techniques on Antioxidant Activity of Faba Bean (*Vicia faba* L.)

Jing Li, Yu-Wei Luo, Qian Wang, Xiao-Xiao Jin and Zhen-Ping Hao
Jinling Institute of Technology, 210038, Nanjing, P.R. China

Abstract: The effects of three extraction techniques (shaking, soaking and stirring) and two solvents (80% methanol and 80% ethanol) on the antioxidant attributes of extracts from seeds of faba bean have been investigated. The yield of faba bean extracts varied between 5.45 and 7.69 g/100 g of dry matter. Faba bean extracts contained a considerable amount of phenolics (1.35-1.65 g GAE/100 g) and flavonoids (2.01-2.31 g CE/100 g). An appreciable level of reducing power (1.24-2.14) at 10 mg/mL extract concentration, inhibition of linoleic acid peroxidation (84.6-90.2%) and DPPH radical scavenging activity (IC₅₀ value 17.4-45.1 µg/mL) were also documented. Overall, the efficacy of an extraction system in isolating potent antioxidant components from faba bean seeds followed the order: shaking, 80% methanol>shaking, 80% ethanol>stirring, 80% methanol>stirring, 80% ethanol>soaking, 80% ethanol>soaking, 80% methanol. The yield and antioxidant activity of the faba bean extracts varied significantly (p<0.05) as function of extraction techniques and solvents employed.

Keywords: DPPH radical, extraction techniques, faba bean antioxidants, flavonoids, linoleic acid oxidation, total phenolic contents

INTRODUCTION

Legumes are important sources of both macro and micro nutrients and also play important role in the traditional human diet of many regions throughout the world. In addition to their nutritive value, some of them have health benefits and therapeutic properties (Geil and Anderson, 1994). They have been shown to have low glycemic indexes (Foster-Powell and Miller, 1995), hypocholesterolaemic effects (Anderson *et al.*, 1999), breast cancer prevention (Adebamowo *et al.*, 2005) and health benefits with respect to cardiovascular disease (Kushi *et al.*, 1999). A decreasing incidence of such diseases have been observed in epidemiological studies, upon consumption of foods with high content of phenolics, such as fruits, vegetables, grains and legumes (Kris-Etherton *et al.*, 2002; Miller *et al.*, 2000).

The faba bean is one of the oldest crops that ranks sixth in production among the different legumes grown in the world. Faba beans are a good source of energy, proteins, vitamins, minerals and dietary fiber. They are relatively inexpensive compared to meat foods and as they have a high carbohydrate content (50-65%). Among that 50-65% carbohydrates only about 40% are starch and sugars and other parts are dietary fibers. Therefore, faba beans are beneficial for human nutrition perspectives because they are good source of energy,

protein and dietary fiber. In China, plant foods provide at least 50% of the dietary energy and nutrients and faba bean is one of the most important legumes.

A recent epidemiological study showed that among several common fruits and vegetables, only the consumption of bean and lentil is related to lower incidence of breast cancer (Adebamowo *et al.*, 2005). Recently it has been reported in several experimental studies that legumes exhibit significant antioxidant activity (Heimler *et al.*, 2005; Madhujith and Shahidi, 2005; Xu and Chang, 2007a). These reports suggest that legumes may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion.

Antioxidant extracts from a plant material can be isolated using different extraction processes. The yields and antioxidant properties of the resultant extracts noticeably vary depending upon the efficacy and dynamism of the extraction methods (Cheung *et al.*, 2003; Peschel *et al.*, 2006). It is generally considered that pure or aqueous mixtures of methanol or ethanol are more efficient towards recovering phenolic antioxidant components from plant-based materials (Abdille *et al.*, 2005; Li *et al.*, 2006; Rehman, 2006; Sultana *et al.*, 2007). Similarly, different extraction devices, e.g., rotary shaker, multi-wrist shaker, sonicator, soxhlet and supercritical fluid-extractor, etc., are in practice for extraction of antioxidant components from plant matrices (Luthria *et al.*, 2007).

In view of the high nutritive value and functional food potential of faba bean seed, it is important to ascertain an effective method for the isolation of antioxidants from this valuable legume crop. So far, few studies have been reported in the literature on the extraction of faba bean antioxidants involving the use of different techniques and solvents leading towards exploring an optimum extraction method. The main objective of this study was to evaluate and quantify the effects of different techniques, namely, shaking, stirring and soaking as well as two solvents (80% methanol and 80% ethanol) on the recovery of potent antioxidant components from faba bean seeds, so as to devise an optimized extraction protocol.

MATERIALS AND METHODS

Material: Faba beans (*Vicia faba* L.) were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. The chemicals and reagents, namely, Folin-Ciocalteu reagent, 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical, linoleic acid, β -carotene and Butylated Hydroxy Toluene (BHT) were obtained from Sigma (Nanjing, China). Other reagents used were of analytical grade.

Preparation of faba bean antioxidant extracts: The hull-free faba bean seeds were ground into a fine powder using a commercial coffee grinder. The material that passed through the 80-mesh sieve was used for extraction of antioxidant components. The extraction of faba bean antioxidant components was performed by three extraction techniques, i.e., shaking, soaking and stirring, using as well as two solvents (80% methanol and 80% ethanol). In orbital shaking, 20 g of faba bean ground seed material was taken in a 500-mL Erlenmeyer flask and extracted individually with 200 mL of 80% methanol (methanol: water, 80:20 v/v) and 80% ethanol (ethanol: water, 80:20 v/v) for 8 h at room temperature using an orbital shaker. In the soaking method, the material (20 g) was soaked separately in 200 mL of 80% methanol and 80% ethanol in a 500-mL Erlenmeyer flask for 8 h. Extraction using stirring was carried out by mixing the ground material independently with 200 mL of 80% methanol and 80% ethanol in a 500-mL Erlenmeyer flask followed by magnetic stirring on a stirring plate using polyvinylidene fluoride coated 50 mm magnetic bar. After complete extraction in each case, the solid residue was separated from the extract by filtering through Whatman No. 1 filter paper. The residues were re-extracted twice using fresh solvent and the three extracts were pooled. The excess solvent was then removed by distilling off under reduced pressure at 45°C, using a rotary vacuum evaporator. The crude concentrated extracts obtained were stored at -4°C until used for further analyses.

Determination of Total Phenolic Contents (TPC):

The total phenolic contents of faba beans (*Vicia faba* L.) were determined according to Xu and Chang (2007b) with slight modifications. After adding Folin-Ciocalteu reagent and sodium carbonate to aliquots of samples, the mixtures were set in a 40°C water bath for 20 min. The absorbance was measured at 740 nm using a spectrophotometer (Unico, Shanghai, China) and total phenolic contents were expressed as milligrams of ferulic acid equivalents per grams of defatted sample.

Determination of Total Flavonoid Contents (TFC):

The total flavonoids content of samples was determined by Xu and Chang (2007b) with slight modifications. Briefly, properly diluted samples (1 mL) and NaNO₂ (0.3 mL, 5%) were mixed. AlCl₃ (10%, 0.3 mL) was added at 5 min and 1 M NaOH (2 mL) was added after one additional minute. The absorbance readings of samples were taken at 510 nm. Total flavonoid contents were expressed as milligrams of (+) -Catechin Equivalents (CE) per gram of sample.

DPPH radical scavenging assay: The antioxidant activity was determined by DPPH assay according to Llorach *et al.* (2008) with some modifications. Aliquot of 200 μ L sample mixed with 3.8 mL DPPH solution (200 mM in methanol) was incubated in dark at room temperature for 60 min, then its absorbance at 517 nm was measured by a spectrophotometer. Scavenging ability of the sample to DPPH radical was determined according to the following equation.

Antioxidant Activity (AA) was expressed as percentage inhibition of DPPH radical by using below equation:

$$AA = 100 - [100 \times (A_{\text{sample}}/A_{\text{control}})]$$

where,

A_{sample} = The absorbance of the sample at $t = 60$ min

A_{control} = The absorbance of control

Antioxidant activity in linoleic acid system: The inhibition of β -carotene bleaching in a coupled oxidation with linoleic acid is a well-known methodology used for evaluating the antioxidant activity. This spectrophotometric technique monitors the bleaching of the carotenoid caused by its interaction with peroxy radicals produced during linoleic acid oxidation (Yamauchi *et al.*, 1993). The bleaching of β -carotene could be inhibited by antioxidants, which are capable of reducing the rate of chain reaction initiated during lipid peroxidation and transforming the reactive end product to a more stable form. In the present study, the extracts of raw and differentially processed seeds of Faba bean exhibited moderate levels of inhibition of β -

carotene bleaching by using β -carotene/linoleic acid emulsion system when compared with the standards BHA and α -tocopherol. All the processing methods generally enhanced peroxidation inhibition activity with the exception of autoclaved seeds. The autoclaved seeds with 0.1% sodium bicarbonate registered the highest β -carotene bleaching activity (35.7% at 200 $\mu\text{g/mL}$). The antioxidant effect of the seed extracts may be of biological relevance as it may prevent oxidation of lipid components within cell membranes.

Determination of reducing power: The determination of reducing potential of FE was made following the method of Yen and Duh (1994). Briefly, variable extract concentrations (2.5-10.0 mg/mL of distilled water) were mixed with 5.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 5.0 mL of 1.0% aqueous solution of potassium ferricyanide. The mixture produced was incubated at 50°C for 20 min in a water bath followed by the addition of 5 mL of 10% trichloroacetic acid. The mixture was then centrifuged (980 g) for 10 min at 5°C using a refrigerated centrifuge machine (CHM-17, Kokusan Denki, Tokyo, Japan). The upper layer of the centrifuged solution (5.0 mL) was recovered and further diluted with 5.0 mL of distilled water and 1.0 mL of ferric chloride (0.1%). Finally, the absorbance of the resulting solution was recorded at 700 nm using a spectrophotometer.

Statistical analyses: Data were analyzed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. The data were analyzed by one-way Analysis of Variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at a probability $p < 0.05$.

RESULTS AND DISCUSSION

The extract yields of faba bean seed-derived antioxidant components ranged from 5.45 to 7.69% (Table 1). The maximum yield (7.69%) was obtained with 80% ethanol using a stirring technique, while the minimum (5.45%) with 80% methanol was via a soaking method. The stirring technique, involving 80% ethanol as solvent, was found to be the most effective

mean for extraction of antioxidant components from faba bean seeds. In agreement with the present findings, Abdille *et al.* (2005) and Peschel *et al.* (2006) reported that methanol and ethanol (in pure form or as aqueous mixtures) have had superior efficacy towards extraction of antioxidant compounds from plant-based materials. As is revealed by some previous studies, the yield of plant antioxidant fractions is mainly affected and enhanced by the use of proper extraction procedure as well as the ability of extraction solvent to dissolve indigenous components (Siddhuraju and Becker, 2003; Sultana *et al.*, 2007).

Total Phenolic Contents (TPC) of Faba bean Extracts (FE) varied between 1.35 and 1.65 GAE/g/100 g dry matter (Table 1). TPC of 80% methanolic extract, produced by a shaking method, was found to be the highest (1.65 g/100 g), while the lowest (1.35 g/100 g) was for 80% methanolic extract produced by a stirring method. The present results showed significant ($p < 0.05$) variations for the phenolic contents of FE as function of extraction procedures and solvents employed. Sun and Ho (2005) also found that antioxidant activity and phenolics concentrations of buckwheat extracts were influenced by the extraction system.

Total Flavonoid Contents (TFC) of faba bean extracts ranged from 1.76 to 2.31 CEg/100 g of dry matter (Table 1). TFC in 80% methanolic extract of a soaking method was found to be the highest (2.31 g/100 g), whereas the least (1.76 g/100 g) in 80% ethanolic extract of soaking method. These results revealed significant ($p < 0.05$) variations for TFC of faba bean extracts in relation to the extraction methods and solvents used. According to Oomah *et al.* (2005) faba bean contained considerable amounts of flavonoids, which might be correlated to the high anthocyanins contents.

The data depicting the ability of FE to scavenge DPPH free radical is given in Table 2. DPPH, a stable, organic free radical having deep violet/purple color, shows absorption maxima at 515-528 nm. Upon receiving proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and becomes yellow. It is generally accepted that as the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds increases, DPPH radical

Table 1: Percent yield, total phenolic and total flavonoid contents of faba bean extracts as affected by extraction technique/solvent

Extraction technology	Shaking		Soaking		Stirring	
	80% methanol	80% ethanol	80% methanol	80% ethanol	80% methanol	80% ethanol
Mung bean extracts						
Percent yield	7.42±0.13aA	7.41±0.16aA	5.45±0.22bB	6.27±0.23bB	7.18±0.31aA	7.69±0.28aA
TPC GAE (g/100 g)	1.65±0.06aA	1.63±0.04aA	1.41±0.07bB	1.46±0.03bB	1.35±0.04bB	1.42±0.02bB
TFC CE (g/100 g)	1.87±0.05bB	2.26±0.07aA	2.31±0.04aA	1.76±0.02bB	2.14±0.06aA	2.01±0.05aA

Values are means±S.D. of three faba bean samples for each treatment, analyzed individually in triplicate; Small letters in superscript indicate significant differences ($p < 0.05$) of means between the extraction solvents; Capital letters in superscript indicate significant differences ($p < 0.05$) of means among the extraction techniques; GAE: Gallic acid equivalent; CE: Catechin equivalent

Table 2: Inhibition of linoleic acid peroxidation and DPPH free radical scavenging activity of faba bean extracts as affected by extracting technique/solvent

Extraction technology	Shaking		Soaking		Stirring		BHT
	80% methanol	80% ethanol	80% methanol	80% ethanol	80% methanol	80% ethanol	
Mung bean extracts							
DPPH IC ₅₀ (µg/mL)	18.6±0.43dD	17.4±0.38dD	26.1±0.52cC	19.3±0.44dD	39.3±0.37bB	45.1±1.12aA	10.7±1.23cE
Percent inhibition of linoleic acid	86.7±3.12aB	86.4±1.64aB	90.2±2.69aA	84.7±3.11aB	88.6±2.69aA	84.6±2.65aB	85.3±3.85aB

Values are means±S.D. of three faba bean samples for each treatment, analyzed individually in triplicate; Small alphabets in superscript indicate significant differences (p<0.05) of means between the extraction solvents; Capital alphabets in superscript indicate significant differences (p<0.05) of means among the extraction techniques; BHT: Butylatedhydroxy toluene

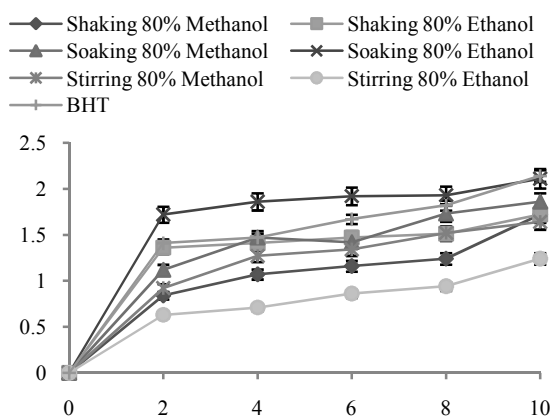


Fig. 1: Reducing power of faba bean seed extracts as affected by extracting technique

scavenging capacity and, thus, antioxidant activity of the plant materials also increases. The tested FE were found to be strongly effective in reducing the stable DPPH radical (purple-colored) to yellow-colored DPPH product. The scavenging capacity of FE (in terms of IC₅₀) was noted to be increased in a concentration dependent manner. The lowest activity (IC₅₀ 45.1 µg/mL) was observed for 80% ethanol extract obtained by the stirring method whereas the highest activity (IC₅₀ 17.4 µg/mL) was observed for 80% ethanol extract by the shaking method (Table 2). The lower the value of IC₅₀, the higher will be the ability of the extract to act as a free radical scavenger.

Antioxidant activity of FE was also examined by assessing their efficiency towards inhibition of linoleic acid (C18:2) peroxidation. In this test, linoleic acid acts as a substrate and forms peroxides on oxidation, which oxidizes ferrous (Fe²⁺) to Fe³⁺ (ferric) ions, the latter forms a complex with thiocyanate. The absorption intensity of the resulting colored complex is measured at 500 nm (Sultana *et al.*, 2007). It is evident from the data presented in Table 2 that 80% methanol extract of faba bean, produced by a stirring method, exhibited the highest inhibition of linoleic acid oxidation (88.6%), reflecting superior antioxidant activity. On the other hand, 80% ethanol extract, prepared by a soaking method, offered the lowest inhibition (84.7%). The ability of FE to inhibit linoleic acid oxidation might be ascribed to the presence of appreciable amounts of

phenolics and flavonoids, which are well accepted to act as potent antioxidants of legumes.

In a previous study, Anwar *et al.* (2007) reported that extracts from different mung bean cultivars effectively inhibited the linoleic acid oxidation, ranging from 49.8 to 89.2% as against 89.4% for BHT. Measurement of reducing potential can also be used to express the antioxidant activity of plant extracts. Ferric ions are reduced to ferrous ions with change in color from yellow to bluish green. The intensity of the color depends on the reducing potential of the antioxidant compounds present in the extract. The greater the intensity of the color of the reaction mixture, the higher will be the absorption, corresponding to higher antioxidant activity of extracts. The extent of reducing potential can be directly correlated to the amount of phenolic compounds (Siddhuraju *et al.*, 2002). The tested FE, having concentrations between 2-10 mg/mL of reaction solvent, exhibited a strong reducing potential. As is evident from Fig. 1, reducing power of the tested extracts increased in a concentration dependent manner. The reducing power for the extract (typical concentration of 10 mg/mL) of a soaking (with 80% ethanol) method was found to be the maximum (2.11), whereas the minimum (1.64) was for the extracts by a stirring technique (80% ethanol) (Fig. 1).

CONCLUSION

The results of this study revealed that both the extraction techniques and extraction solvents significantly affected the yield and antioxidant properties of faba bean extracts. Generally speaking, of the extraction solvents used, 80% methanol was found to be more promising for extraction of faba bean antioxidant components. Regarding the effects of extraction techniques on the recovery of antioxidant components from faba bean, with few exceptions, the results mainly revealed the superior efficacy of the shaking process, followed by stirring, while least amounts of antioxidants were recovered by the soaking technique. It could be concluded that an optimum extraction protocol/system might be selected for isolation of potent faba bean antioxidant components. A detailed study is further recommended to fully characterize and quantify the individual phenolic acids and flavonoids components of faba bean seeds using state-of-the-art chromatographic approaches coupled

with some *in vivo* antioxidant activity trials of the whole extracts produced.

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