

## Research Article

### The Impact of Processing on Antioxidant Activity of Faba Bean (*Vicia faba* L.)

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

**Abstract:** Antioxidant activity of 70% acetone extracts of raw and processed seeds of faba bean (*Vicia faba* L.) was evaluated by various *in vitro* antioxidant assays, including total antioxidant, free radical scavenging, reducing power, metal ion chelating,  $\beta$ -carotene/linoleic acid bleaching and antihemolytic activities. The total phenolics and tannin contents were higher in the extract of seeds processed by autoclaving with 1% ash solution (3.6 and 1.9/100 g extract, respectively). In general, all the extracts of processed seeds exhibited higher activity in various antioxidant systems, when compared to raw seeds but significant differences were noticed between processing methods. The extract of seeds autoclaved with 1% sugar solution showed higher DPPH radical scavenging activity (IC<sub>50</sub>7.4 mg/mL). Interestingly, the extract of dry heated seeds registered higher inhibition of hemolysis (76.8%) compared to standards butylated hydroxyl anisole (BHA) (61.8%) and  $\alpha$ -tocopherol (52.6%) at the concentration of 500  $\mu$ g/mL.

**Keywords:** Antioxidant activity, antihemolytic, legume, total phenolics

## INTRODUCTION

Leguminous seeds are an important source of nutrient compounds such as protein, starch, dietary fiber and minerals (Leterme, 2002; Rizkalla *et al.*, 2002), particularly in third-world countries. Incorporation of leguminous seeds into the human diet in developing countries can offer protective effects against chronic diseases (Anderson and Major, 2002). Legumes contain a number of bioactive substances including phenolics that can diminish protein digestibility and mineral bioavailability (Chung *et al.*, 1998; Sendberg, 2002). On the other hand, phenolic compounds such as flavonoids, phenolic acids, lignans and tannins have antioxidant properties and these are very important from nutritional and technological points of view. Various evidences suggest that oxidative stress is closely associated with a diverse assortment of diseases such as cancer and cardiovascular disease. The relationship between the intake of food rich in antioxidant components and the alleviation of illnesses caused by oxidative damage has become an important research topic in the food and medicine arena.

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.

However, information with respect to the antioxidant and free radical scavenging activity of raw as well as processed seeds of faba bean is scanty. In this context, we attempted to evaluate the antioxidant potential of the 70% acetone extracts of raw and processed seeds of this beans by employing different chemical and *in vitro* antioxidant assays.

## MATERIALS AND METHODS

**Chemicals:** ABTS<sup>+</sup>, DPPH<sup>•</sup>, TPTZ, Trolox, 3-(2-pyridyl)-5, 6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nitro blue tetrazolium (NBT) and linoleic acid were purchased from Sigma (Nanjing, China). Potassium persulfate, Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), ferrous chloride, hydrogen peroxide, hydroxylamine hydrochloride, riboflavin, Trichloroacetic Acid (TCA), Tween 40 and ferric chloride were obtained from Sigma (Nanjing, China). All other reagents used were of analytical grade.

**Seed samples and processing:** Faba beans (Qidou 2, cultivated in Jiangsu Province and harvested in 2012) were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. The mean

**Corresponding Author:** Yu-Wei Luo, College of Horticulture, Jinling Institute of Technology, Zhongyangmen, Xiaozhuang Village 130#, 210038, Nanjing, Jiangsu Province, P.R. China, Tel.:+86-25-8539-3314; Fax:+86-25-8539-3314

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moisture, protein, carbohydrate, fat, ash content were 12.6, 21.2, 55.4, 0.4, 3.2% respectively. The seeds (100 g) were dry heated in a hot air oven at 150°C for 30 min and the beans were allowed to cool at room temperature. For the pressure-cooking treatment, the seeds (100 g) were pre-soaked in distilled water in the ratio of 1:10 (seed: water, w/v) for 12 h at room temperature (25°C). After decanting the water, the soaked seeds were subjected to autoclaving (seed: water, 1:5, w/v) for 20 min at 120°C. For other treatments, water soaked seeds were autoclaved with sodium bicarbonate solution (0.1%)/1% ash solution/1% sugar separately. Excess water was drained off and the seeds were rinsed in distilled water and dried at 55°C. The raw and processed seed samples were ground to fine powder (particle size of about 0.25 mm) and stored in separate screw cap bottles at -20°C before analysis.

**Solvent extraction:** Raw/processed ground seed samples (20 g) were extracted by stirring with 100 mL of 70% acetone at 25°C for 24 h and filtered through filter paper. The residues were re-extracted with an additional 50 mL of 70% acetone, as described above, for 3 h. The solvent of the combined extract was evaporated under reduced pressure, in a rotary vacuum-evaporator at 40°C and the remaining water was removed by lyophilization. The freeze-dried extract thus obtained was used directly for total phenolics and tannin estimation and also for the assessment of antioxidant capacity. One-hundred mg of the sample extract was generally dissolved in 100 mL of the solvent and generally used in different assays.

**Determination of total phenolic and tannin contents:** The total phenolic content of faba bean seed extracts was determined by Folin-Ciocalteu method. Using the same extract the tannins were estimated after treatment with Polyvinyl Polypyrrolidone (PVPP). The amount of total phenolics and tannins were calculated as the tannic Acid Equivalents (GAE) (Siddhuraju and Becker, 2003).

**Ferric Reducing/Antioxidant Power (FRAP) assay:** The antioxidant capacity of seed extracts was estimated according to the method described previously by Pulido *et al.* (2000) absorbance of the reaction mixture was read at 593 nm. The values are expressed as mmolFe (II)/mg extract.

**Antioxidant activity by the ABTS<sup>+</sup> assay:** Radical scavenging activity of faba bean seed extracts was assessed spectrophotometrically by ABTS<sup>+</sup> cation decolorization assay and the absorbances were taken at 734 nm (Re *et al.*, 1999). The unit of Total Antioxidant Activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as  $\mu\text{mol/g}$  extracts.

**Metal chelating activity:** The chelating of ferrous ions

by faba bean seed extracts was estimated by the method described by Dinis *et al.* (1994). Absorbance of the solution was measured spectrophotometrically at 562 nm. The results were expressed as mg EDTA equivalent/g extract.

**Phosphomolybdenum (AEAC) assay:** The antioxidant activity of seed extracts was evaluated by the green phosphomolybdenum complex formation according to the previously described method of Prieto *et al.* (1999). The absorbance of the mixture was measured at 695 nm. The results reported are mean values expressed as g of ascorbic acid equivalents/100 g extract.

**Assay of superoxide radical scavenging activity:** Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (1971). The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The absorbance was measured at 590 nm.

**Assay of Nitric Oxide (NO) scavenging activity:** The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. The absorbance of the chromophore formed was read at 546 nm (Sreejayan and Rao, 1997).

**Hydroxyl radical scavenging activity:** The scavenging activity of faba bean seed extracts on hydroxyl radical activity was measured according to the previously described method (Klein *et al.*, 1991). The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity.

**Free radical scavenging activity on DPPH:** The DPPH radical scavenging activity of the different extracts of faba bean seed was measured according to the method of Blios (1958). IC<sub>50</sub> values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

**$\beta$ -Carotene/linoleic acid antioxidant activity:** The  $\beta$ -carotene/linoleic acid antioxidant activity of the antioxidant (seed extracts, BHA, or  $\alpha$ -tocopherol) solution was measured according to the previously described method (Taga *et al.*, 1984) at 470 nm. And the antioxidant activity of the bark extracts and standard was evaluated as % of antioxidant activity.

**Antihemolytic activity:** Antihemolytic activity of the extracts was assessed as described by Naim *et al.* (1976). The extent of hemolysis was determined by measuring the absorbance at 540 nm and % inhibition of hemolysis was calculated.

**Statistical analysis:** Data were analysed with SPSS 13.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by One-Way Analysis of Variance (ANOVA). Duncan's multiple range tests was used to separate means. Significance was accepted at probability  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Recovery percent, total phenolics and tannin content of extracts:** The data on the extract yield, total phenolics and tannins of acetone extracts of raw and processed seeds of faba bean are shown in Table 1. The extract of autoclaved seeds gave the maximum yield (20.3%), whereas, the extract of raw seeds registered the minimum yield (7.5%). All the processing methods generally increased the total phenolics and tannin contents than raw seeds. The extracts of the seeds autoclaved with 1% ash solution or 1% sugar registered higher level of total phenolics (3.6 and 3.1 g GAE/100 g extract, respectively) and tannins (1.9 g GAE/100 g extract) than other processing methods. This might be due to higher extractability, facilitated by the heat processing, in the extracting solvent than raw seeds. Interestingly, the increased contents of total phenolics and tannins in the processed seeds as recorded in the present study are contrary to the observations of Xu and Chang (2008) and Siddhuraju (2006).

**FRAP assay:** The FRAP assay measures the ability of antioxidants to reduce the TPTZ-Fe(III) complex to the intensely blue colored TPTZ-Fe(II) complex in acidic

medium. Antioxidant potential of the various extracts of raw and processed seeds was estimated from their ferric reducing ability and the results are expressed as concentration of substances having ferric-TPTZ reducing ability equivalent to that of 1 mmol concentration of Fe (II). The data on the ferric reducing potential of acetone extracts of raw and processed Faba bean seeds are presented in Table 2. All the processing methods, with the exception of dry heat method, significantly ( $p < 0.05$ ) increased the FRAP activity when compared with the raw seeds. However, autoclaving with 1% ash solution or 0.1% sodium bicarbonate resulted in the highest FRAP activity (135.6 and 112.4 mmol Fe (II)/mg extract, respectively). These results suggest that the seed extracts have the ability to donate electrons and thus can scavenge free radicals. However, this activity was lesser when compared to other legume seeds.

**ABTS radical scavenging activity:** ABTS assay is based on the inhibition of the absorbance of the radical action ABTS, which has a characteristic long wavelength absorption spectrum (Sanchez-Moreno, 2002). Total Antioxidant Activity (TAA) of differentially processed seed samples of Faba bean are presented in Table 2. As for FRAP activity, the extract of seeds processed by autoclaving with 1% ash solution showed higher TAA (2301.6  $\mu\text{mol/g}$  extract) followed by the extract of autoclaved seeds (2089.3  $\mu\text{mol/g}$  extract). The ABTS radical scavenging ability of the extracts decreased in the order of  $T5 > T3 > T6 > T4 > T2 > T1$ . The difference in the TAA

Table 1: Extract yield percentage, total phenolics and tannin content of acetone extracts of raw and processed faba bean seeds<sup>1,2</sup>

Sample	Extract yield (%)	Total phenolics (g GAE/100 g extract)	Tannin (g GAE/100 g extract)
T1	7.50	1.6 $\pm$ 0.1	1.2 $\pm$ 0.1
T2	9.60	1.7 $\pm$ 0.1	0.9 $\pm$ 0.1
T3	20.3	2.4 $\pm$ 0.2	1.3 $\pm$ 0.1
T4	8.90	2.3 $\pm$ 0.1	1.1 $\pm$ 0.1
T5	12.7	3.6 $\pm$ 0.2	1.9 $\pm$ 0.2
T6	15.8	3.1 $\pm$ 0.2	1.9 $\pm$ 0.2

<sup>1</sup>: T1: Raw; T2: Dry heated; T3: Soaking followed by autoclaving; T4: Soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: Soaking followed by autoclaving with 1% ash solution; T6: soaking followed by autoclaving with 1% sugar; <sup>2</sup>: Values are mean $\pm$ SD (n = 3); Total phenolics and tannin activity are expressed as Gallic Acid Equivalent (GAE)

Table 2: FRAP, ABTS scavenging, metal chelating and phosphomolybdenum reducing activities exhibited by acetone extracts of raw and processed faba bean seeds<sup>1,5</sup>

Sample	FRAP <sup>2</sup> (mmol Fe (II)/mg extract)	TEAC <sup>3</sup> ( $\mu\text{mol/g}$ extract)	Metal chelating (mg EDTA/g extract)	AEAC <sup>4</sup> (g AA/100 g extract)
T1	86.3 $\pm$ 3.8 <sup>d</sup>	1463.3 $\pm$ 62.1 <sup>c</sup>	8.4 $\pm$ 0.6 <sup>b</sup>	4.7 $\pm$ 0.2 <sup>c</sup>
T2	86.7 $\pm$ 3.6 <sup>d</sup>	1562.4 $\pm$ 72.6 <sup>d</sup>	4.2 $\pm$ 0.4 <sup>c</sup>	7.5 $\pm$ 0.3 <sup>a</sup>
T3	96.4 $\pm$ 4.1 <sup>c</sup>	2089.3 $\pm$ 87.2 <sup>b</sup>	4.1 $\pm$ 0.5 <sup>c</sup>	3.2 $\pm$ 0.4 <sup>d</sup>
T4	112.4 $\pm$ 3.2 <sup>b</sup>	1854.1 $\pm$ 72.4 <sup>c</sup>	4.8 $\pm$ 0.3 <sup>c</sup>	5.6 $\pm$ 0.3 <sup>b</sup>
T5	135.6 $\pm$ 2.7 <sup>a</sup>	2301.6 $\pm$ 96.5 <sup>a</sup>	10.2 $\pm$ 0.7 <sup>a</sup>	3.8 $\pm$ 0.2 <sup>d</sup>
T6	107.5 $\pm$ 2.1 <sup>b</sup>	1926.3 $\pm$ 88.4 <sup>c</sup>	2.6 $\pm$ 0.2 <sup>d</sup>	3.6 $\pm$ 0.3 <sup>d</sup>

<sup>1</sup>: T1: raw; T2: dry heated; T3: soaking followed by autoclaving; T4: soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: soaking followed by autoclaving with 1% ash solution; T6: soaking followed by autoclaving with 1% sugar; <sup>2</sup>: Concentration of substance having ferric-TPTZ reducing ability in mmolFe(II) equivalent; <sup>3</sup>: Trolox equivalent antioxidant capacity ( $\mu\text{mol}$  equivalent Trolox performed by using ABTS<sup>•+</sup> radical cation); <sup>4</sup>: Ascorbic acid equivalent antioxidant capacity (g equivalent of ascorbic acid/100 g extract) through the formation of phosphomolybdenum complex; <sup>5</sup>: Values are mean $\pm$ SD (n = 3); Total phenolics and tannin activity are expressed as Gallic Acid Equivalent (GAE)

Table 3: Superoxide, nitric oxide and hydroxyl radical scavenging activity exhibited by acetone extracts of raw and processed faba bean seeds<sup>1,2,3</sup>

Sample	Radical scavenging activity		
	Superoxide (1 mg)	Nitric oxide (1 mg)	Hydroxyl (500 µg)
T1	35.4±2.3	51.6±2.5	22.8±1.5
T2	45.7±2.6	64.7±2.4	28.7±1.8
T3	62.5±2.5	72.3±1.8	65.6±2.1
T4	60.8±3.1	73.8±3.1	18.7±1.6
T5	15.9±1.8	76.5±2.6	64.6±1.7
T6	59.6±3.5	76.8±2.7	67.5±2.3
α-tocopherol	33.8±2.7	35.4±1.4	40.3±1.3

<sup>1</sup>: T1: raw; T2: dry heated; T3: soaking followed by autoclaving; T4: soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: soaking followed by autoclaving with 1% ash solution; T6: soaking followed by autoclaving with 1% sugar; α-tocopherol, mass of standard in the reaction mixture was 100 µg; <sup>2</sup>: Mass of extract used in the reaction mixture; <sup>3</sup>: Values are mean±SD (n = 3); Mean values followed by different superscripts in a column are significantly different (p<0.05)

values could be attributed to the level of total phenolic content in the seed extracts.

**Metal chelating activity:** Presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton-type reactions, resulting in the generation Of Hydroxyl radicals (OH<sup>•</sup>). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH<sup>•</sup> generation and inhibition of peroxidation processes of biological molecules (Chew *et al.*, 2009). The extent of chelation of ferrous ions by the extracts of raw and differentially processed seed samples of faba bean are listed in Table 2. When compared with the raw seeds (8.4 mg EDTA/g extract), all the processed seeds generally decreased the chelating activity except the seeds processed by autoclaving with 1% ash solution (10.2 mg EDTA/g extract). Among the processed seed extracts, the seeds autoclaved with 1% sugar registered the least metal chelating activity (2.6 mg EDTA/g extract). Therefore, it can be said that the extracts have mild metal chelating activity in the processed seeds. It was reported that the chelating agents, which form σ-bonds with metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gülçin *et al.*, 2004).

**Phosphomolybdenum assay:** This assay has been routinely used to evaluate the antioxidant capacity of extracts. Also, it is a quantitative one, since the antioxidant activity is expressed as ascorbic acid equivalent (g/100 g plant extract). This method depends on the reduction of Mo (VI) to Mo(V) by the antioxidant agents and the subsequent formation of a green-colored phosphomolybdenum V complex with a maximal absorption at 695 nm. Phosphomolybdenum reduction by the raw and processed seed samples of faba bean is presented in Table 2. The extract of dry heated seeds showed higher phosphomolybdenum reduction (7.5 g AA/100 g extract) followed by seeds autoclaved with 0.1% sodium bicarbonate (5.6 g AA/100 g extract). All the other heat treatment processes showed lesser activity. Variations in

antioxidant activity of the extracts may be attributed to the differential processing methods. Hydrogen/electron transfer from antioxidants to DPPH radical and Mo (VI) complex occur in the DPPH<sup>•</sup> and phosphomolybdenum assays, respectively. Loo *et al.* (2008) reported transfers occur at different redox potentials in the 2 assays and also depend on the structure of the antioxidants.

**Superoxide radical scavenging activity:** As a precursor of the more reactive oxygen species, superoxide radical is known to be very harmful to cellular components, contributing to tissue damage and various diseases (Halliwell and Gutteridge, 1999). The percentage inhibition of superoxide by the raw and processed seed samples of faba bean is presented in Table 3. Generally, all the extracts were found to be efficient scavengers of the superoxide radical generated in riboflavin-NBT-light system in vitro. The decrease in the absorbance at 590 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The autoclaved seeds exhibited the highest percentage of superoxide inhibition (62.5% at 1 mg/mL) when compared to raw seeds, whereas the seeds autoclaved with 1% ash solution (15.9% at 1 mg/mL) showed the least activity. These results reveal that the tested extracts have a noticeable effect on scavenging superoxide radical when compared with the standard α-tocopherol. Their scavenging efficiency depends on their concentration as well as the numbers and location of hydroxyl groups (Benavente-Garia *et al.*, 1997).

**NO scavenging activity:** NO plays an important role in the various pathophysiological processes. In the present study, the extracts of raw and processed seeds of faba bean were evaluated for their scavenging effects on NO derived from sodium nitroprusside (Table 3). All the processing methods significantly (p<0.05) enhanced NO scavenging potential of the test seeds. However, the wet heat processings appeared to be more effective than the dry heat processing. The scavenging effects of the extracts on NO is decreasing in the order of T6>T5>T4>T3>T2>T1. The nitric oxide radical (NO<sup>•</sup>), which is produced in vivo by a variety of cell types, is

an important bio-regulatory molecule with a number of physiological functions. However, under oxidative stress this Reactive Nitrogen Species (RNS) reacts with other reactive species to produce more toxic RNS and Reactive Oxygen Species (ROS) (Valko *et al.*, 2007). Therefore, the scavenging effect of the said plant extracts was assessed against this radical. It can be seen from the data that all the extracts are capable of scavenging NO<sup>•</sup>.

**Hydroxyl radical scavenging activity:** The Hydroxyl radical (OH<sup>•</sup>) in the cells can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. As such, removal of OH<sup>•</sup> is very important for the protection of living systems (Yang *et al.*, 2008). Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radical formed undergoes oxidation reaction with Dimethyl Sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent. The hydroxyl radical scavenging activity of differentially processed seeds of Faba bean is presented in Table 3. Autoclaving of seeds with 0.1% sodium bicarbonate significantly ( $p < 0.05$ ) decreased hydroxyl radical scavenging activity. However, the other wet heat processing methods markedly enhanced the hydroxyl scavenging activity. Compared to raw seeds (22.8% at 500  $\mu\text{g/mL}$ ), the dry heating process increased the hydroxyl radical scavenging activity only marginally (28.7% at 500  $\mu\text{g/mL}$ ). Yen and Hsieh (1995) attributed the hydroxyl radical scavenging potential to the combined effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen.

**DPPH radical scavenging activity:** DPPH radical has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts. It determines the ability of pure substances or crude extracts for trapping this unpaired-electron species by donating hydrogen atoms or electrons and producing in consequence the radical disappearance and the formation of less reactive species derived from the antioxidant (Brand-Williams *et al.*, 1995). DPPH free radical scavenging capacities of the extracts from differentially processed seeds of Faba bean are presented in Fig. 1. All the extracts demonstrated DPPH scavenging activity in a concentration dependent manner. Further, the various heat processing methods improved the radical scavenging potential when compared with the raw seeds, the only exemption being autoclaved seeds with 1% ash solution. Faba bean seeds

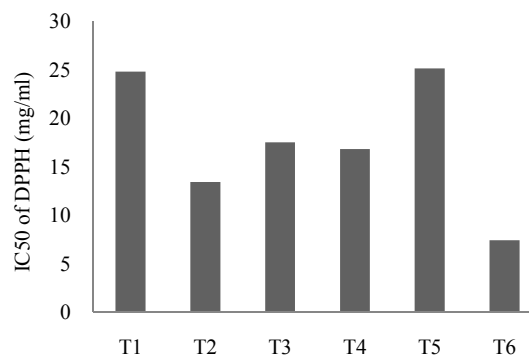


Fig. 1: DPPH scavenging activity exhibited by acetone extracts of raw and processed faba bean seeds; T1: raw; T2: dry heated; T3: soaking followed by autoclaving; T4: soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: soaking followed by autoclaving with 1% ash solution; T6: soaking followed by autoclaving with 1% sugar; Values are mean $\pm$ SD (n = 3); Mean values followed by different superscripts in a column are significantly different ( $p < 0.05$ )

processed by autoclaving with 1% sugar were a more efficient method to scavenge DPPH (IC<sub>50</sub> 7.4 mg/mL) compared to other methods. Thus, the heat processing methods have ability in preventing the initiation and propagation of free radical-mediated chain reaction which contributes to the oxidative stress.

**Antioxidant activity in the  $\beta$ -carotene bleaching assay:** The inhibition of  $\beta$ -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  $\beta$ -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  $\beta$ -carotene bleaching by using  $\beta$ -carotene/linoleic acid emulsion system when compared with the standards BHA and  $\alpha$ -tocopherol (Fig. 2). 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  $\beta$ -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.

**Antihemolytic activity:** Erythrocytes, which are the most abundant cells in the human body and possess

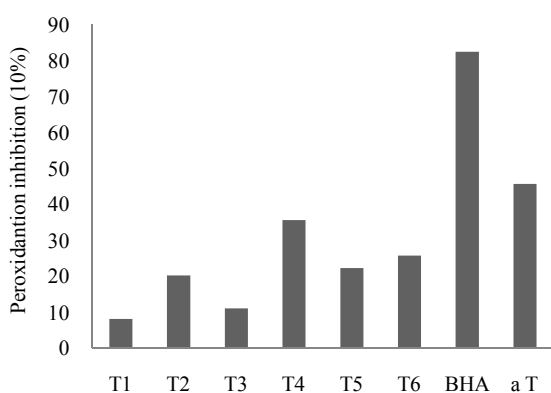


Fig. 2:  $\beta$ -Carotene/linoleic acid peroxidation inhibition activity exhibited by acetone extracts of raw and processed faba bean seeds; T1: raw; T2: dry heated; T3: soaking followed by autoclaving; T4: soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: soaking followed by autoclaving with 1% ash solution; T6, soaking followed by autoclaving with 1% sugar BHA, butylatedhydroxyanisole;  $\alpha$ T,  $\alpha$ -tocopherol. Values are mean $\pm$ SD (n = 3); Bars having different letters are significantly different (p<0.05)

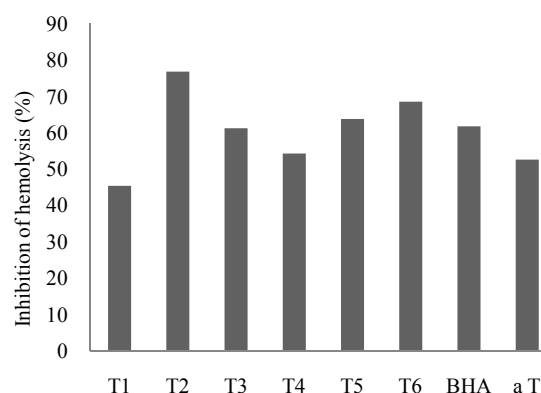


Fig. 3: Antihemolytic activity exhibited by acetone extracts of raw and processed faba bean seeds; T1: raw; T2: dry heated; T3: soaking followed by autoclaving; T4: soaking followed by autoclaving with 0.1% sodium bicarbonate; T5: soaking followed by autoclaving with 1% ash solution; T6: soaking followed by autoclaving with 1% sugarBHA, butylatedhydroxyanisole;  $\alpha$ T,  $\alpha$ -tocopherol. Values are mean $\pm$ SD (n = 3); Bars having different letters are significantly different (p<0.05)

desirable physiological and morphological characteristics, are exploited extensively in drug delivery (Hamidi and Tajerzadeh, 2003). In this study, we used a biological test based on free radical-induced erythrocyte lysis in cow blood. This assay is useful for screening studies on various molecules and their metabolites, especially on the one hand molecule having an oxidizing or antioxidizing activity and on the other hand molecule having a long-term action (Djeridane *et al.*, 2006). Figure 3 shows the antihemolytic activity of raw and processed seed extracts of Faba bean and standards at the concentration of 500  $\mu$ g/ mL. The results clearly indicate that the extracts of raw and processed seeds were highly potent in inhibition of erythrocytes hemolysis. Among them, extract of the dry heated seeds (76.8%) registered the highest capability to inhibit the hemolysis by H<sub>2</sub>O<sub>2</sub> induced membrane damage in cow blood erythrocytes when compared to standards BHA and  $\alpha$ -tocopherol. The extracts and standards on inhibition of hemolysis are decreasing in the order of T2>T6>T5>BHA>T3> $\alpha$ T>T4>T1. Chaudhuri *et al.* (2007) noted that binding of flavonoids to the red blood cell membranes significantly inhibits lipid peroxidation and at the same time enhances their integrity against lysis.

The results from in vitro experiments demonstrate that the raw and processed seeds of Faba bean have significant antioxidant activities. They are natural and economic sources of dietary antioxidant, which may prevent diseases caused by free radical. The difference in the antioxidant activity of the processed seed extracts

may be ascribed to the changes in the chemistry and in the level of total phenolic contents as influenced by the processing methods. Further studies are needed on the nutritional and antinutritional properties of the above said processed samples which may result in increased utilization of faba bean seeds in human and animal diet.

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