



# EFFECT OF DIFFERENT PROCESSING METHODS ON POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF BROAD BEANS (*VICIA FABEA*)

Pinki Saini, Priyanka Singh, Shreyasi Dubey, and Ayushi Srivastava

Centre of Food Technology, University of Allahabad, Allahabad, India.

## ABSTRACT

**Objective:** The effects of processing on total phenolic components and antioxidant activity in commonly consumed broad bean was investigated.

**Methods:** The raw and processed samples were extracted with 70% methanol and analysed for antioxidant components and antioxidant activity.

**Results:** Processing of legumes caused decrease in total phenolic content when compared to the raw samples. However, the dry heating caused remarkable increase in tannin contents ( $6.98 \pm 0.53$  g TAE/100 g extract). The flavanoid and  $\beta$  carotene content was significantly reduced on processing of samples. Raw sample of *D. lablab* was found to possess the highest DPPH ( $73.5 \pm 2.5\%$ ), Reducing power ( $4.9 \pm 0.68$  mg ascorbic acid/gm) and Iron chelating capacity than other samples.

**Conclusion:** Maximum retention of antioxidant activity was observed in dry heated samples. Higher correlation was found between phenolic content and chelating capacity ( $r^2=0.945$ ) but a poor correlation with DPPH. Moreover, the content of tannins gave good correlation ( $r^2=0.745-0.913$ ) with Iron chelating and DPPH assays.

**Key Words:** Processing, Antioxidant, Broad beans, Total phenolics, Correlation

## INTRODUCTION

Legumes belong to the family Leguminosae, one of the most important families in Dicotyledons, including around 700 genera and 20,000 species. Legumes are the second most important source of food and fodder, green manures and forages. In comparison of cereal grains, legumes are good source of proteins, dietary fibers, low glycemic indexes, low levels of fat (2-5%) and high amounts of carbohydrates (55-60%) (Xu *et al.*, 2007). Recently demand for plant based proteins has increased and hence there are more studies on functional proteins from legumes such as chickpea, lentil, cowpea, lupins, pea and broad beans as alternative to soybean. The epidemiological evidence indicates that the consumption of dietary antioxidant such as legume seed proteins provided protective effects for several chronic diseases like cardiovascular diseases, cancer, obesity diabetes and hypercholesterolemia.

Broad beans also known as the field bean or fava bean is a species of bean native to North Africa and extensively cultivated in south and south west Asia. In India, it is an important legume used as a pulse and vegetable for human consumption and forage. Broad beans (*Vicia faba*) are a potential source of protein, dried seeds of bean contain 20–28% crude protein and the amino acids are moderately well balanced with especially high lysine content. They are rich in L-dopa, a substance used medically in the treatment of Parkinson's disease. L-dopa is also a natriuretic agent, which might help in controlling hypertension. An antifungal protein Dolichin, has also been purified from the seeds of the field bean. Dolichin inhibited Human Immuno-deficiency Virus (HIV) reverse transcriptase, D and E-glucosidases which are glycohydrolases implicated in HIV infection. It had very low ribonuclease and cell-free translation-inhibitory activities (Ye *et al.*, 2000). Further, the dietary protein concentrates of broad beans showed potential hypocholesterolemic effect (Chau *et al.*, 1998).

### Corresponding Author:

Pinki Saini, Centre of Food Technology, University of Allahabad, Allahabad, India.  
E-mail: pspinki55@gmail.com

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The antioxidant activities and phenolic compounds in fresh legumes have been reported earlier in several communications (Amarowicz *et al.*, 2003; Xu *et al.*, 2007). However the legumes require sufficient processing before consumption. The effects of processing methods on phenolics and antioxidant activities have not been systematically studied. In addition, very little information is available in the literature regarding the changes in antioxidant activity of the processed beans. The aim of the present study is to investigate the effects of processing methods (boiling, drying and pressure cooking) on the phenolic contents and antioxidant activities of broad beans.

## MATERIAL AND METHODS

### Sample collection and Processing

Broad beans were procured from local market of Allahabad city. They were washed, dried and stored under refrigeration. The fresh Broad beans (100g) were boiled using beans: water ratio of 1:5 (w/v) until they became tender. Pressure cooking of samples (100g) was done in a pressure cooker for 20 min with water ratio of 1:3 (w/v). Water was decanted, boiled and pressure cooked samples were dried at 50°C until constant weight reached. Another 100g of sample was dried at 160°C for 15 min in a microwave oven.

The fresh, boiled, pressure cooked and dried bean samples were finely powdered using a Willy Mill of 60 mesh size. All the powdered samples were stored separately in a screw capped bottles at a room temperature until further analysis

**Proximate analysis:** All the samples were analysed for proximate composition using AOAC (2005) methods. All the chemicals used were of analytical grade obtained from Merck or Sigma.

**Preparation of solvent extract:** Raw and processed bean powder (100 g) was extracted with 500 ml of 70% methanol (w/v) using a shaker, the sample was shaken occasionally for 24 h. The extracts were centrifuged at 5,000 rpm for 20 min and the supernatants obtained were concentrated with a rotary vacuum evaporator (RV-10, IKA) at 45° C. The resultant extracts were stored in amber vials at 4°C until assayed. The extract recovery percentage of raw, boiled, dry heated and pressure cooked samples of Broad beans were found to be 1.66%, 1.25%, 1.50% and 1.12%, respectively.

**Estimation of Total Phenolic Content (TPC):** Total phenolic content was determined by adopting Folin-Ciocalteu method (Velioglu *et al.*, 1998; Ying *et al.*, 2013). Basically, 0.2 ml of extracts was added with 1.5 ml of Folin-Ciocalteu reagent and mixture was allowed to stand at room temperature for 5 minutes. Then 1.5 ml of sodium carbonate solution

(6%) was added into the mixture. Absorbance was measured using spectrophotometer at 725 nm after incubating the sample to stand for 1½ hours at room temperature. Results were expressed as gallic acid equivalent in mg/100 g dry weight (DW).

**Estimation of Total Tannin Content (TC):** Tannin content was determined by the method of Ranganna, 2005. Powdered sample (0.5 g) was boiled with water (75ml) for 30 minutes and centrifuged at 2000 rpm for 20 minutes and the supernatant was collected. Folin Denis reagent and sodium carbonate was added to the sample extract, solution was diluted to 100ml with water and absorbance is taken at 700 nm after 30 minutes.

**Estimation of Total Flavonoid Content:** A colorimetric assay (Kim *et al.*, 2003) with some modification was used to quantify total flavonoid content. Briefly, 25 microliter of diluted sample was added to 125 microliter of double distilled H<sub>2</sub>O. Subsequently, 7.5 microliter of 5% NaNO<sub>2</sub> was added to the mixture and was allowed to stand for 5 minute thereafter 15 microliter of 10% AlCl<sub>3</sub> was added. The mixture was incubated at ambient temperature (25°C) for an additional 5 minute. Following that and 50 microliter of 1 M NaOH was then added to the mixture. The mixture was immediately diluted by addition of 27.5 microliter of ddH<sub>2</sub>O and the absorbance of the mixture was measured at 510 nm against a blank prepared with ddH<sub>2</sub>O using microplate reader (synergy HT, BioTek instrument, USA).

**Estimation of Beta carotene:** Beta carotene was analyzed by column separation method (Rangana 2005). The absorbance was measured using spectrometer at 452nm. Petroleum ether and acetone mixture was used as blank.

**DPPH free radical scavenging assay:** The free radical scavenging activity of the field bean extracts was measured by measuring the decrease in absorbance of ethanolic DPPH solution at 517 nm in the presence of the extract (Krings and Berger, 2001; Koolen *et al.*, 2013). The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. In cases where the absorbance decreased too much before the 30 minutes period the sample was appropriately diluted. The antioxidant activity was expressed as:-

**Estimation of Reducing power:** The reducing power of the extracts was determined by using potassium ferricyanide-ferric chloride method (Oyaizu, 1986). Different dilutions of extracts amounting to 1 ml were added to 2.5 ml 0.2 M phosphate buffer (pH=6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 minutes, after which 2.5 ml trichloroacetic acid (10%) was added. 2.5 ml of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% ferric chloride. The absorbance at 700 nm was

measured after allowing the solution to stand for 30 minutes.

**Chelating Capacity on Fe<sup>2+</sup>:** Fe<sup>2+</sup> chelating capacity was measured by 2, 2'-bipyridyl competition assay (Yamaguchi *et al.*, 2000). The reaction mixture contained 0.25 ml of 1 mM FeSO<sub>4</sub> solution, 0.25 ml of sample extract, 1 ml of 0.2 M Tris-HCl buffer (pH 7.4), 1 ml of 2,2'- bipyridyl solution (0.1% in 0.2 M HCl), 0.4 ml of 10% hydroxylamine-HCl, and 2.5 ml of ethanol. The final volume was made up to 5 ml with distilled water. The absorbance at 522 nm was determined and used to evaluate Fe<sup>2+</sup> chelating activity using ethylenediamine tetra acetate (EDTA) as a standard. The results were expressed as mg EDTA equivalent/ g of seed extracts.

## RESULTS

**Nutritional composition of broad beans:** Raw broad beans had crude protein (2.69%) and crude fat (1.02%), ash (2.1%) and crude fibre (1.82%). Raw broad beans had a high protein, crude fibre and calcium content as compared to processed field bean. The phosphorous was highest in boiled broad beans whereas iron content was highest in pressure cooked bean samples.

**Antioxidant components:** Plant phenolics are free radical scavengers and act as antioxidants. The content of polyphenols in broad beans is depicted in Table 2. Total phenolics ranged from 3.33 to 5.65 mg GAE/g extract. The raw broad beans had highest phenolic content followed by dry heated and boiled samples. Tannin content varied from 5.13 to 6.16 mg TAE/g. Raw field bean samples had highest tannin content followed by dry heated and boiled samples. Flavonoid content was in the range of 0.65 to 1.54 mg/100ml (Table 2). Highest flavonoid content was found in raw broad beans which reduced on processing. Maximum reduction was observed in pressure cooked broad beans. The  $\beta$  carotene content varied from 0.31 to 4.41  $\mu$ g/100g. The highest  $\beta$  carotene content was found in raw field bean samples followed by pressure cooked samples (0.6  $\mu$ g/100g).

Linear correlation coefficient between composition and antioxidant capacity of broad beans has been discussed in Table 4. High correlation coefficient was found between TPC and Iron chelating capacity ( $r^2=0.945$ ) and FRAP ( $r^2=0.678$ ), but a poor correlation with DPPH ( $r^2=0.248$ ). Similarly tannin content showed high correlation coefficient with iron chelating capacity ( $r^2=0.913$ ) and DPPH ( $r^2=0.745$ ). A high correlation coefficient was observed between Total Flavanoids and DPPH ( $r^2=0.945$ ) and Iron chelating capacity ( $r^2=0.784$ ).  $\beta$  carotene showed a poor correlation with antioxidant assays ( $r^2=0.086-0.652$ ).

**Antioxidant activity of broad beans:** DPPH was used to

determine the free radical scavenging activity of the methanol extracts of raw and processed field bean samples (Table 3). The raw broad beans showed highest DPPH content (73.5%) followed by boiled and dry heated samples. The pressure cooked broad beans had lowest DPPH content (22.8%), Ascorbic acid and BHT were positive controls and exhibited DPPH content as 79.5% and 83.4% respectively.

**Ferric ion reducing capacity:** The reducing properties are related with the presence of reductones, which exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada *et al.*, 1992). Ferric ion reducing capacity of samples and standards are found to be in following order (Table 3): ascorbic acid (5.1 $\pm$ 0.29 mg AA/gm) > raw (4.9 $\pm$ 0.68 mg AA/gm) > BHT (4.3 $\pm$  0.54 mg AA/gm) > pressure cooked (3.5 $\pm$ 0.34 mg AA/gm) > dry heated (2.6 $\pm$ 0.12 mg AA/gm) > boiled (1.2 $\pm$ 0.23 mg AA/gm). The dry heated samples had higher reducing power than pressure cooked and boiled samples.

**Fe chelating:** In this study, the chelating ability of the raw and processed seed sample extracts of *D. lablab* towards ferrous ions were examined (Table 3). All the samples examined showed Fe<sup>2+</sup> ion chelating effect and the activity was expressed as mg EDTA equivalent. The raw samples showed a chelating capacity of 70.2 $\pm$ 1.05 mg EDTA/g of extract followed by dry heating (67.4 $\pm$ 1.12 mg EDTA/g). The boiled broad beans showed minimum Fe capacity (55.2 $\pm$ 1.3 EDTA/g).

## DISCUSSION

### Nutritional composition:

The proximate analysis of beans is given Table 1. Raw beans showed a higher nutritional value as compared to processed beans. The decrease in the ash content of processed vegetables could be as a result of processing during which some of the inorganic salt in the vegetables might have leached off (Yaciuk and Sofose 1981). The protein content also showed reduction may be due to the fact that during boiling cellular protein are denatured and the chlorophyll which is bound to protein may be released, such free chlorophyll are highly unstable and are readily converted to pheophytin which is olive green to brown in colour (Komolafe and Obayanju, 2003).

### Antioxidant components:

The polyphenolic and tannin content of broad beans is depicted in Table 2. The results are in accordance with Pascharicha *et al.* (2014) who also reported total phenolic content of 22.415 GAE equivalents ( $\mu$ g GAE/mg sample) in faba seeds. Siddhuraju (2007) reported that processed samples had lower concentration of phenolic fractions possibly due to the poor extractability by the formation of insoluble tannin- protein

and tannin-carbohydrate complexes. The Reduction of phenolic content in broad beans may be due to lixiviation (Siddhuraju and Becker 2003) and the phenols may also bound to other compounds and form insoluble complexes (Fernandez *et al.*, 2003). Similar decrease in phenolics content of broad beans has also been reported by Maheshu *et al.* (2013). The results are also in accordance with Barroga *et al.* (1985) who found that boiling and cooking reduced the amount of phenolics in legumes by 75%. However this might be caused in part by diffusion of phenolics from the seed coat to cooking water (Rocha-Guzman *et al.* 2007).

The total flavonoid content (Table 2) of the raw and processed samples was estimated by the aluminium chloride method. It has been recognized that flavonoids show antioxidant activity. The total flavonoid content in the dried *faba* seeds was estimated to be 7.814 in  $\mu\text{g}$  of Catechin equivalents (CE) / mg (Milo, 2004). The  $\beta$ -carotene content of beans was found to decrease on processing. The high sensitivity of  $\beta$ -carotene to light and heat is well recognized and its loss is therefore expected during heat-processing. Some workers have reported losses of  $\beta$ -carotene from vegetables, including spinach, amaranth and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching and pressure cooking (Yadav and Sehgal, 1995 and Yadav and Sehgal, 1997).

The linear correlation between composition and antioxidant capacity of broad beans (Table 4) show that antioxidant activity is not alone dependent on total phenolics. Also the synergistic equation between antioxidants in mixture makes them dependent on concentration as well as on structure and interaction among them (Djeridane *et al.*, 2006). The antiradical and antioxidant activities of beans depend on the amount and composition of the antioxidants they contain. The research conducted by Oomah *et al.* (2005) with Canadian bean cultivars revealed differences between the cultivars in antioxidant and antiradical activities.

### Antioxidant activity of Broad beans

DPPH content was highest in raw samples followed by boiled and dry heated samples (Table 3). Saini and Singh (2015) have reported that ethanolic extracts of raw spices and herbs show higher DPPH content as compared to other extracts. The antiradical scavenging activity of untreated and treated seed extracts are related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability (Brand-Williams *et al.* 1995). According to Tsai and She (2006) a change in phenolic compounds after heating might be contributed to a decrease in DPPH-scavenging ability.

Ferric ion reducing capacity was found to be higher in dry heated samples as compared to pressure cooked and boiled samples. Higher antioxidant activity of dry heated broad beans might be due to the formation of products from Mail-

lard reaction. Tsai and She (2006) concluded that there was a change in the phenolic compounds after heating which resulted in increase in reducing power. The decrease in reducing power of pressure cooked samples correlates with the low level of phenolic contents since, during cooking, a part of phenolics diffuse from the seed coat to cooking water (Rocha-Guzman *et al.*, 2007).

The results of the study show that Fe chelating activity was higher in raw and dry heated samples. Similar results have also been reported in the raw and processed legumes of *Macrotyloma uniflorum* and *D. lablab* methanol and acetone extracts (Siddhuraju *et al.*, 2008). The extract of peanut seed testa (Yen *et al.*, 2005) and faba bean (Carbonaro *et al.*, 1996) also showed a significant  $\text{Fe}^{2+}$  chelating effect.

## CONCLUSION

The raw and dry heated samples of broad beans showed higher antioxidant activity than the pressure cooked and boiled samples. The results indicated that not only the phenolic constituent from raw samples but also the phenolics and Maillard products of processed samples are found to be potent antioxidant suppliers. Therefore, consumers may obtain optimal health benefits along with nutrient assimilation without any negative implications. As dry beans contain compounds other than phenolics that may have significant antioxidant potential, it will be useful to investigate their potential and maximize their use in food industry.

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**Table 1: Proximate composition of raw and processed Broad beans**

Sample	Moisture %	Protein %	Fat %	Crude Fibre %	Ash %	CHO %	Ca (mg/100g)	P (mg/100g)	Fe (mg/100g)
Raw	64.5±0.28 <sup>c</sup>	2.7±0.26 <sup>bc</sup>	1.02±0.04 <sup>b</sup>	6.82±0.07 <sup>a</sup>	2.1±0.03 <sup>a</sup>	29.7±0.15 <sup>a</sup>	223.12±2.64 <sup>d</sup>	185.33±3.05 <sup>c</sup>	7.7±0.05 <sup>a</sup>
Boiled	8.6±0.35 <sup>b</sup>	1.5±0.31 <sup>a</sup>	0.08±0.02 <sup>a</sup>	6.22±0.09 <sup>d</sup>	3.5±0.17 <sup>b</sup>	87.68±0.38 <sup>b</sup>	122.67±2.51 <sup>b</sup>	248.33±2.4 <sup>d</sup>	7.0±0.18 <sup>a</sup>
Dry Heated	5.7±0.43 <sup>a</sup>	2.0±0.2 <sup>c</sup>	0.06±0.13 <sup>a</sup>	3.24±0.15 <sup>c</sup>	2.0±0.04 <sup>a</sup>	91.23±0.71 <sup>c</sup>	74.06±1.21 <sup>a</sup>	162.67±2.52 <sup>b</sup>	9.5±0.6 <sup>b</sup>
Pressure Cooked	7.18±0.79 <sup>a</sup>	2.2±0.4 <sup>b</sup>	1.09±0.14 <sup>b</sup>	2.41±0.09 <sup>b</sup>	3.2±0.08 <sup>b</sup>	86.5±0.51 <sup>b</sup>	135.33±2.51 <sup>c</sup>	101.34±1.53 <sup>a</sup>	10.9±0.3 <sup>c</sup>

<sup>a</sup>Each value represents the mean and standard deviation from three lots;

Means with different superscripts for each sample are significantly different (p<0.05).

**Table 2: Antioxidant components of raw and processed Broad beans**

Sample	Total Phenolics (mg GAE/g)	Tanin content (mg TAE/g)	Total flavonoid (mg/100ml)	β carotene (μg/100g)
Raw	5.65±0.85 <sup>a</sup>	6.16±0.28 <sup>ab</sup>	1.54±0.19 <sup>a</sup>	4.41±0.44 <sup>c</sup>
Boiled	3.48±0.72 <sup>c</sup>	5.56±0.97 <sup>ab</sup>	1.44±0.22 <sup>b</sup>	0.35±0.01 <sup>b</sup>
Dry Heated	4.62±0.38 <sup>b</sup>	6.98±0.53 <sup>a</sup>	1.31±0.03 <sup>b</sup>	0.316 ±0.11 <sup>a</sup>
Pressure Cooked	3.33±0.41 <sup>d</sup>	4.84±0.61 <sup>b</sup>	0.65±0.02 <sup>a</sup>	0.609±0.014

<sup>a</sup>Each value represents the mean and standard deviation from three lots;

Means with different superscripts for each sample are significantly different (p<0.05).

**Table 3: DPPH, FRAP and Fe chelating activity of Broad beans raw and processed samples**

Sample	DPPH%	FRAP (mg Ascorbic Acid/gm)	Fe chelating (mg EDTA/g)
Raw	73.5±2.5 <sup>d</sup>	4.9±0.68 <sup>c</sup>	70.2±1.05
Boiled	67.2±1.74 <sup>c</sup>	1.2±0.23 <sup>a</sup>	55.2±1.31
Dry Heated	53.5±1.13 <sup>b</sup>	3.5±0.12 <sup>a</sup>	67.4±1.12
Pressure cooked	22.8±1.78 <sup>a</sup>	2.6±0.22	60.5±0.98
Ascorbic acid	79.5±1.18	3.8±0.29	-
BHT	83.4±1.63	4.3±1.28	-

<sup>a</sup>Each value represents the mean and standard deviation from three lots;

Means with different superscripts for each sample are significantly different (p<0.05).

**Table 4: Linear correlation coefficients between composition and antioxidant capacity of Broad beans.**

	DPPH	FRAP	Fe chelating
TPC	0.248	0.678	0.945
Tannin content	0.745	0.264	0.913
Total Flavonoids	0.954	0.126	0.784
β carotene	0.316	0.086	0.652

TPC: Total Phenolic Content