Effects of processing conditions on the stability of polyphenolic contents and antioxidant capacity of *Dolichos lablab* L.

Vellingiri Maheshu ·
Deivamarudhachalam Teepica Priyadarsini ·
Jagathala Mahalingam Sasikumar

Revised: 5 April 2011 / Accepted: 19 April 2011 / Published online: 8 May 2011
© Association of Food Scientists & Technologists (India) 2011

Abstract The effects of raw, dry heated and pressure cooked samples on total phenolic components and antioxidant activity in commonly consumed field bean, *Dolichos lablab* L. was investigated. The raw and processed samples were extracted with 70% methanol. Processing of legumes caused decreases in total phenolic content when compared to the raw samples. However, the dry heating caused remarkable increase in tannin contents (1.809 ± 0.25 g GAE/100 g extract). Dry heated samples of *D. lablab* was found to possess the highest DPPH (IC_{50} , 2.53 ± 0.17 μ g/ml), TEAC (4649.8 ± 38.4 μ mol/g DM), $OH^{\cdot}$ radical (IC_{50} , 42.2 ± 0.67 μ g/ml) scavenging activities, inhibition of linoleic acid and ferric reducing capacity than other samples. The raw samples displayed the highest antihemolytic activity ($59.6 \pm 1.53\%$) and chelating capacity (74.2 ± 1.37 mg EDTA/g). Dry heat processing exhibited several advantages in retaining the antioxidant components and activities. The higher correlation was found the phenolic content with chelating ($r^2=0.933$) and antihemolytic ($r^2=0.839$) activities, but a poor correlation with other assays. Moreover, the content of tannins gave good correlation ($r^2=0.644-0.997$) with all antioxidant assays. The low correlation values between total phenols and the antioxidative activity suggest that the major antioxidant compounds in studied seeds might be tannins.

Keywords Antioxidant activity · *Dolichos lablab* L. · Field bean · Phenolic content · Processing methods

Introduction

Legumes are of great world economic importance. They are used for human food, animal feed and other commercial applications. Thus, legumes play a paramount role in food nutrition, especially in developing countries (Duranti 2006). Legumes contain different bioactive compounds that may have beneficial effect, if consumed on a regular basis, against metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Tharanathan and Mahadevamma 2003; Pastor-Cavada et al. 2009). Polyphenols from dry beans may possibly act as antioxidants, hindering the formation of free radicals that eventually leads to the deterioration of biological molecules (Boateng et al. 2008). Thermal processing decreases the content of polyphenols and tannins of legumes and modifies their oxidation state. These modifications can decrease or increase the antioxidant capacity. In general, thermal treatment decreases the antioxidant activity of beans; however, products of the Maillard reactions can also be generated; these products have been identified as excellent antioxidants, particularly as scavengers of free radicals (Kaur and Kapoor 2002; Granito et al. 2008).

Dolichos lablab L., commonly referred to as field bean or hyacinth bean, is a legume widespread throughout the tropics. In India, it is an important legume used as a pulse and vegetable for human consumption and forage. Dried seeds of lablab bean contain 20–28% crude protein and the amino acids are moderately well balanced with especially high lysine content. Lablab bean leaves are also rich in protein and among legumes it is considered to be one of the best sources of iron (The Wealth of

V. Maheshu · D. T. Priyadarsini · J. M. Sasikumar (✉)
Department of Biotechnology, Karpagam University,
Coimbatore 641 021 Tamil Nadu, India
e-mail: jmsashikumar@yahoo.co.in

India 1992). The potential breast cancer fighting flavonoid known as kievitone was also found in hyacinth bean (Obboh 2006). Nonetheless, an antifungal protein Dolichin, has been purified from the seeds of the field bean *D. lablab*. Dolichin inhibited Human Immuno-deficiency Virus (HIV) reverse transcriptase and 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 *D. lablab* seeds showed potential hypocholesterolemic effect (Chau et al. 1998).

Antioxidant activities and phenolic compounds in raw legumes have been reported in several earlier communications (Amarowicz et al. 2003; Xu et al. 2007). Legumes must be processed before consumption. However, effects of processing methods on health promoting phenolics and antioxidant activities have not been systematically studied. In addition, very little information is available in the literature regarding the change of antioxidant components and antioxidant activity of the processed legumes. The aim of the present study is to investigate the effects of processing methods (dry heating and pressure cooking) on the phenolic contents and antioxidant activities of *D. lablab* seeds.

Materials and methods

Chemicals Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH[•]), potassium persulfate, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetra acetic acid (EDTA) disodium salt, 2,2'-bipyridyl and hydroxylamine hydrochloride were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Seed samples and processing The dry seeds of *D. lablab* black variety were purchased from the market located at Mettupalayam, Tamilnadu, India during October 2006. Raw seeds (100 g) were dry heated at 160°C for 15 min in a microwave oven. Another 100 g of the raw seeds were cooked in a pressure cooker for 20 min, used a seed: water ratio of 1:3 (w/v). Water was decanted and the cooked seeds were dried at 50°C until a constant weight was reached. The raw, dry heated and pressure cooked seeds 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.

Solvent extraction Raw and processed seed powder (100 g) was extracted with 500 ml of 70% methanol (w/v) and 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 (Buchi type, Flawil, Switzerland) at 45°C. The resultant extracts were stored in amber vials at 4°C until assayed. The extract recovery percentage of raw, dry heated and pressure cooked samples of *D. lablab* were found to be 1.75%, 1.40% and 1.15%, respectively.

Estimation of total phenolic content (TPC) The total phenolic content of raw and processed *D. lablab* seed samples were determined according to the method described by Makkar et al. (1997). Aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. To this, Folin–Ciocalteu reagent (0.5 ml) and 20% sodium carbonate solution (2.5 ml) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of TPC was calculated as gallic acid equivalents from a calibration curve.

Estimation of tannin content (TC) The tannin content of the methanol extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a test tube and to this 1 ml distilled water and then 1 ml of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4°C for 15 min. The sample was centrifuged (5,000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of non-tannin phenolics on a dry matter basis (Siddhuraju et al. 2008). From the above results, the tannin content of the sample as tannic acid equivalents were calculated as follows: Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

DPPH radical-scavenging assay (DPPH-RSA) The antioxidant activity of *D. lablab* bean seed extracts and standards was measured in terms of hydrogen donating or radical-scavenging ability, using the DPPH method (Sanchez-Moreno et al. 1998). A methanol solution (0.1 ml) of the sample extracts at various concentrations was added to 3.9 ml (0.025 g/L) of DPPH[•] solution. The solution was incubated at room temperature for 60 min and decrease in absorbance at 515 nm was determined at the end of incubation period with a Spectrophotometer. The remaining

concentration of DPPH[•] in the reaction medium was calculated from a calibration curve obtained with DPPH[•] at 515 nm.

$$\text{DPPH}(\%) = [(\text{Abs. 515 nm})_{\text{sample}} / (\text{Abs. 515 nm})_{\text{control}}] \times 100.$$

The percentage of remaining DPPH[•] against the sample/standard concentration was plotted to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH[•] by 50% (IC₅₀).

Determination of trolox equivalent antioxidant capacity (TEAC) This method was based on the reduction of the ABTS radical cations (ABTS^{•+}) according to the procedure described by Re et al. (1999). ABTS^{•+} radical cation was produced by reacting 7 mM aqueous ABTS with 2.45 mM (final concentration) potassium persulfate and keeping the mixture in the dark at room temperature for 16 h. The blue green solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The stock solution of seed extracts in ethanol were diluted such that, after introduction of a 10 μ l aliquot of each dilution into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1.0 ml of diluted ABTS^{•+} solution to 10 μ l of antioxidant compounds or trolox standards (final concentration 0–15 μ M) in ethanol was taken at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as mmol/kg seed extracts on dry matter basis.

Hydroxyl radical scavenging activity (HRSA) The scavenging activity of the extracts of raw and processed *D. lablab* seed samples on hydroxyl radical were measured according to the method of Klein et al. (1991). Various concentrations of extracts were added to 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5% w/v). To this, Nash reagent (3 ml) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured at 412 nm in a spectrophotometer. The hydroxyl radical

scavenging activity (%) was calculated by the following formula:

$$\% \text{HRSA} = 1 - (\text{difference in absorbance of sample} / \text{difference in absorbance of blank}) \times 100.$$

Antioxidant activity in linoleic acid emulsion system The antioxidant activity in linoleic acid emulsion system was determined using the ferric thiocyanate (FTC) method (Kikuzaki and Nakatani 1993). A sample extract having a concentration of 0.25 mg was taken. To this 0.5 ml of linoleic acid (2.51%) in ethanol (99.5%), 1 ml of phosphate buffer (0.05 M, pH 7), and 0.5 ml of distilled water was placed in a screw capped tube and then incubated in dark oven at 40°C. Every 6 h, 0.1 ml aliquots of this solution were taken and 9.7 ml of ethanol (75%) and 0.1 ml of ammonium thiocyanate (30%) were added. Precisely 3 min after the addition of 0.1 ml of ferrous chloride (0.02 M) in hydrochloric acid (3.5%) to the reaction mixture, the absorbance was measured at 500 nm until the absorbance of the control reached the maximum. The antioxidant activity (AA) was calculated as percentage of inhibition relative to the control using the following equation:

$$\text{AA}(\%) = 1 - (\text{absorbance of sample at 54 h} / \text{absorbance control at 54 h}) \times 100.$$

Ferric ion reducing capacity (Fe^{3+} to Fe^{2+}) Ferric ion reducing capacity was measured out according to the method of Oyaizu (1986). Briefly, different concentrations of extracts were dissolved in 1 ml of phosphate buffer were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (1%), and the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 ml of trichloro acetic acid (10%) was added to the mixture, which was centrifuged at 3,000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl_3 (0.1%), and the absorbance was measured at 700 nm.

Antihemolytic activity Antihemolytic activity of the extracts was assessed as described by Naim et al. (1976). The erythrocytes from cow blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. Extract (50 μ g/ml) of saline buffer was added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. After incubation of 5 min at room temperature, 0.5 ml of H_2O_2 solution in saline buffer was added to induce the oxidative

degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 240 min. After incubation, the reaction mixture was centrifuged at 1,500 rpm for 10 min and extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

Chelating capacity on Fe^{2+} Fe^{2+} chelating capacity was measured by 2, 2'-bipyridyl competition assay (Yamaguchi et al. 2000). The reaction mixture contained 0.25 ml of 1 mM $FeSO_4$ 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^{2+} chelating activity using ethylenediamine tetra acetate (EDTA) as a standard. The results were expressed as mg EDTA equivalent/ g of seed extracts.

Results and discussion

Total phenol (TPC) and tannin (TC) contents Plant phenolics in general are highly effective free radical scavengers and antioxidants. The content of polyphenols in *D. lablab* raw and processed seeds was determined spectrophotometrically and the values are depicted in Table 1. The total phenolic contents ranged from 21.82 ± 0.43 to 39.25 ± 0.67 g gallic acid equivalent/100 g extract. *D. lablab* raw samples had the highest amount of total phenolics, followed by dry heated and pressure cooked samples. The tannin contents varied from 1.574 ± 0.38 to 1.809 ± 0.25 g tannic acid equivalent/100 g extract. The dry heated samples had highest tannin content amongst all the samples tested. Phenolic content in the lablab bean under pressure cooking, was reduced can be explained by (a) a lexiviation phenomenon that drives phenols into the cooking water

Table 1 Total phenolics and tannin content of raw and processed seed samples of *D. lablab*

Sample	Total phenolics (g GAE/100 g extract)	Tannins (g TAE/100 g extract)
Raw	39.3 ± 0.67^a	1.730 ± 0.13^c
Dry heated	27.6 ± 0.51^c	1.809 ± 0.25^a
Pressure cooked	21.8 ± 0.43^d	1.574 ± 0.38^c

Mean value ($n=3$), $\pm$ standard deviation. Different letters in the same column among each process and raw legume means significant difference ($P \leq 0.05$)

(Barroga et al. 1985; Siddhuraju and Becker 2003) (b) the seed phenols may bound to other compounds, resulting the formation of insoluble complexes (Fernandez-Orozco et al. 2003). Similarly the decrease in phenolic contents on the dry heat treatment of *Phaseolus vulgaris* was reported (Jiratanan and Liu 2004). The tannin contents of dry heated samples are found to be higher than in raw and pressure cooked samples that could be due to the solubility of phenolics and other aroma compounds (Siddhuraju et al. 2008).

High correlation coefficient was found between TPC and chelating capacity ($r^2=0.933$) and antihemolytic activity ($r^2=0.839$), but a poor correlation with HRSA, DPPH-RSA, TEAC, ferric ion reducing capacity and FTC assays. Furthermore, the content of tannins (TC) gave the well correlation ($r^2=0.644$ – 0.997) with all the antioxidant assays (Table 4). These low correlation values between total phenols and the antioxidative activity suggest that the major antioxidant compounds in studied seeds might be tannins. It was correspondence to the observation that lupin genotypes (Oomah et al. 2006) and cocoa beans (Othman et al. 2007) found no correlation between scavenging activity and total phenolic content. The unclear relationship between the antioxidant activity and the total phenolics may be explained as the total phenolics content does not incorporate all the antioxidants. In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants (Djeridane et al. 2006a).

DPPH radical-scavenging assay (DPPH-RSA) The scavenging of hydrogen radicals is one of the important mechanisms of antioxidation. In this study, DPPH was used to determine the free radical scavenging activity of the methanol extracts of the raw and processed seed samples of *D. lablab* (Table 2). The dry heated sample with lowest IC_{50} of 2.5 ± 0.17 μ g/ml showed higher DPPH radical scavenging effect, regardless of raw (4.9 ± 0.32 μ g/ml) and pressure cooked (5.7 ± 0.20 μ g/ml) sample extracts. Meanwhile, ascorbic acid, gallic acid, BHT and catechin serving as the positive controls exhibited an IC_{50} values of 11.5 ± 0.15 , 5.2 ± 0.07 , 15.3 ± 0.26 and 9.5 ± 0.22 μ g/ml, respectively. The DPPH-RSA of extracts from dry heated seed sample might have also been partly contributed by the maillard reaction products. Duenas et al. (2003) reported that proanthocyanidins from mocan seeds had high ability to quench DPPH and that their effect was dependent on the molecular weight, higher molecular weight compounds had a greater effect. The IC_{50} values for DPPH-RSA is well correlated with FTC method ($r^2=0.923$), TEAC ($r^2=0.998$), HRSA ($r^2=0.893$) and Ferric ion reducing capacity ($r^2=0.989$). No correlation was found with chelating capacity ($r^2=0.148$) and antihemolytic activity ($r^2=0.270$) (Table 4).

Table 2 EC₅₀ values of DPPH-RSA, antiradical activity, HRSA and ferric ion reducing capacity of *D. lablab* raw and processed samples

Sample	DPPH-RSA	Antiradical activity (A _{AR} , 1/EC ₅₀)	HRSA	Ferric reducing capacity
Raw	4.9±0.32 ^d	0.2±0.29 ^c	60.4±0.58 ^a	68.5±1.71 ^c
Dry heated	2.5±0.17 ^e	0.4±0.06 ^a	42.2±0.67 ^b	61.3±1.26 ^d
Pressure cooked	5.7±0.20 ^a	0.2±0.17 ^c	89.7±0.22 ^d	74.2±1.37 ^b
Ascorbic acid	11.5±0.15	0.1±0.06	16.2±0.36	10.8±1.01
Gallic acid	5.2±0.07	0.2±0.13	14.3±0.77	—
Catechin	9.5±0.22	0.1±0.08	19.3±0.21	26.9±2.25
BHT	15.3±0.26	0.1±0.03	43.7±0.19	44.0±1.58

Mean value ($n=3$), $\pm$ standard deviation. Different letters in the same column among each process and raw legume means significant difference ($P\leq 0.05$). EC₅₀ means the effective concentration of sample that can decrease DPPH concentration by 50%. EC₅₀ was expressed as μg sample/ml

Determination of trolox equivalent antioxidant capacity (TEAC) The trolox equivalent antioxidant capacity (TEAC) assay is widely applied to assess the amount of radicals that can be scavenged by an antioxidant, i.e. the antioxidant capacity (Arts et al. 2004). Table 3 presented the inhibition of ABTS⁺ radicals by the raw and processed seed samples of *D. lablab* and the inhibition was achieved at the concentration of 10 $\mu\text{g}/\text{ml}$. The dry heated sample extract of *D. lablab* demonstrated the highest radical scavenging activities when reacted with the ABTS⁺ radicals (4649.8 ± 38.4 $\mu\text{mol}/\text{g}$ DM) followed by raw (4161.3 ± 28.4 $\mu\text{mol}/\text{g}$ DM) and pressure cooked samples (3961.3 ± 31.7 $\mu\text{mol}/\text{g}$ DM). Hagerman et al. (1998) reported that high molecular weight phenolics (tannins) have a greater ability to quench free radicals (ABTS⁺) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups. Earlier, it was reported the ABTS⁺ radical cation scavenging activity in raw and dry heated samples of horse gram brown and black varieties (Siddhuraju and Manian 2007). On the other hand, the formation of tannin-protein complexes, both in insoluble and soluble complexes, as the result of conventional food/seed processing have also been shown to be potential free radical scavenger and radical sinks. Correlation analysis (Table 4) showed a strong correlation between TEAC values and IC₅₀ values of antioxidant activities (DPPH-RSA, $r^2=0.998$;

FRC, $r^2=0.995$; HRSA, $r^2=0.868$) and FTC method ($r^2=0.901$). No association was observed with CC and AHA activity.

Hydroxyl radical scavenging activity (HRSA) Among the oxygen free radicals, hydroxyl radical is an extremely reactive formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochstein and Atallah 1988). Table 2 shows the radical scavenging activity of the raw and processed seed extracts of *D. lablab* and reference antioxidants on the hydroxyl radicals (OH[•]). The IC₅₀ values obtained for raw (60.4 ± 0.58 $\mu\text{g}/\text{ml}$) and pressure cooked sample (89.7 ± 0.22 $\mu\text{g}/\text{ml}$) extracts were different from the IC₅₀ values of dry heated sample (42.1 ± 0.67 $\mu\text{g}/\text{ml}$). The IC₅₀ values of reference standards ascorbic acid, gallic acid, catechin and BHT were found to be 16.2 ± 0.36 , 14.3 ± 0.77 , 19.3 ± 0.21 and 43.7 ± 0.19 $\mu\text{g}/\text{ml}$, respectively. As compared by their IC₅₀ values, raw and pressure cooked samples showed less hydroxyl radical scavenging activity than dry heated sample and reference standards. These results show that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Similarly, raw and dry heated seed extracts of horse gram found to have potent hydroxyl radical scavenging activity and this might have

Table 3 Antioxidant activities in linoleic acid emulsion system, antihemolytic activity, TEAC and chelating capacities of *D. lablab* raw and processed samples

Sample	Linoleic acid peroxidation (%)	Antihemolytic activity (%)	TEAC ($\mu\text{mol}/\text{g}$ DM)	Chelating capacity (mg EDTA/g extract)
Raw	97.2±1.02 ^b	59.6±1.53 ^a	4161.3±28.4 ^d	74.2±1.37 ^c
Dry heated	98.4±1.48 ^a	56.4±1.09 ^c	4649.8±38.4 ^c	68.5±1.71 ^a
Pressure cooked	95.6±0.98 ^d	49.2±1.18 ^b	3961.3±31.7 ^e	61.3±1.26 ^d
BHT	94.5±1.64	57.3±1.73	—	—

Mean value ($n=3$), $\pm$ standard deviation. Different letters in the same column among each process and raw legume means significant difference ($P\leq 0.05$)

Table 4 Linear correlation coefficients between composition and antioxidant capacity (Panel A) and linear correlation coefficients amongst the different antioxidant methods (Panel B)

TPC Total phenol content; TC Tannin content; DPPH Diphenyl picryl hydrazyl; TEAC Trolox equivalent antioxidant capacity; HRSA Hydroxyl radical scavenging activity; FTC Ferric thiocyanate method; FRC Ferric ion reducing capacity; AHA Antihemolytic activity; CC Chelating capacity on Fe^{2+}

	DPPH	TEAC	HRSA	FTC	FRC	AHA	CC
Panel A							
TPC	0.018	0.009	0.203	0.163	0.027	0.839	0.933
TC	0.858	0.830	0.997	0.989	0.879	0.645	0.995
Panel B							
DPPH	1.000						
TEAC	0.998	1.000					
HRSA	0.893	0.868	1.000				
FTC	0.923	0.901	0.997	1.000			
FRC	0.989	0.995	0.911	0.938	1.000		
AHA	0.270	0.236	0.594	0.542	0.298	1.000	
CC	0.149	0.122	0.443	0.392	0.171	0.977	1.000

been attributed to combined effects of Maillard reaction products (Siddhuraju and Manian 2007). The antioxidant properties of faba bean tannins indicated that the antioxidant activity was accounted for by the direct interaction of tannin with hydroxyl radical rather than to a metal chelating activity (Carbonaro et al. 1996). The antioxidant activity of this assay correlated well with DPPH-RSA ($r^2=0.893$), TEAC ($r^2=0.868$), FTC method ($r^2=0.997$), Ferric ion reducing capacity ($r^2=0.911$) and antihemolytic activity ($r^2=0.593$), for the chelating capacity ($r^2=0.443$) no association could be found (Table 4).

Antioxidant activity in linoleic acid emulsion system The ferric thiocyanate method determines the antioxidant activity with the measurement of the amount of peroxides formed in a linoleic acid emulsion of antioxidant during incubation (Erkan et al. 2008). The inhibitory effect of the extracts from *D. lablab* raw and processed seed samples on the peroxidation of linoleic acid at concentration of 250 $\mu\text{g/ml}$, in comparison to BHT was measured using the ferric thiocyanate (FTC) method (Table 3). Each extract showed strong antioxidant activity in inhibition of linoleic acid peroxidation. From the FTC results, the inhibition of peroxidation in linoleic acid system of raw, dry heated and pressure cooked samples were found to be $97.2\pm1.02\%$, $98.4\pm1.48\%$ and $95.6\pm0.98\%$, respectively at after incubation of 54 h. These values were higher than that of BHT ($94.5\pm1.64\%$). The seed coat extracts containing phenolic substances from red and black beans (Tsuda et al. 1994) and peanut seed testa (Yen et al. 2005) have been reported to have a strong antioxidant activity against lipid peroxidation. Similarly, extract of roasted followed by defatted peanut kernels displayed most remarkable antioxidative activity on linoleic acid emulsion system (Hwang et al. 2001). The correlations (Table 4) between FTC method and DPPH-RSA, HRSA, TEAC, antihemolytic activity, chelating capacity and ferric ion reducing capacity was $r^2=0.923$, $r^2=0.997$, $r^2=0.901$, $r^2=0.542$, $r^2=0.392$ and $r^2=0.938$, respectively.

Ferric ion reducing capacity (Fe^{3+} to Fe^{2+}) The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada et al. 1992). As the concentration increased from 50 to 200 $\mu\text{g/ml}$, there was an increase in absorbance with the raw and processed samples of *D. lablab* and the IC_{50} values were measured and presented in Table 2. The IC_{50} values of ferric ion reducing capacity of samples and standards were found to be in the following order: ascorbic acid (10.8 ± 1.01 $\mu\text{g/ml}$) > catechin (26.9 ± 2.25 $\mu\text{g/ml}$) > BHT (44 ± 1.58 $\mu\text{g/ml}$) > dry heated (61.3 ± 1.26 $\mu\text{g/ml}$) > raw (68.5 ± 1.71 $\mu\text{g/ml}$) > pressure cooked (74.2 ± 1.37 $\mu\text{g/ml}$). The dry heated samples had potent reducing power than raw and pressure cooked samples. The stability of antioxidant potential of dry heated samples might partly be due to the formation of products from Maillard reaction. Tsai and She (2006) concluded that there was a change in the phenolic compounds after heating which contributed to the 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). Upon comparing the correlation coefficients (Table 4) between ferric ion reducing capacity with DPPH-RSA, TEAC, FTC, chelating capacity, HRSA and antihemolytic activity were found to be $r^2=0.989$, 0.995 , 0.938 , 0.171 , 0.911 and 0.297 , respectively.

Antihemolytic activity Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lysis in cow blood (Manian et al. 2008). Table 3 depicts the antihemolytic properties of raw and processed seed samples of *D. lablab* as well as BHT. The antihemolytic activity of raw sample was outstanding, compared with dry heated,

pressure cooked samples and BHT. At a concentration of 50 µg/ml, the antihemolytic activity of raw, dry heated and pressure cooked samples were $59.6 \pm 1.53\%$, $56.4 \pm 1.09\%$ and $49.2 \pm 1.18\%$, respectively, while that of BHT was $57.3 \pm 1.73\%$ only. It was speculated that excellent antihemolytic power of raw samples presented in this study may be due to the abundant total phenolic compounds. Moreover, the RBC hemolysis is a more sensitive system for evaluating the antioxidant properties of the phytochemicals. Similarly, a highly significant efficiency in inhibiting radical induced red blood cell hemolysis was also observed for *Oudneya africana*, *Artemisia arborescens* and *Globularia alpyum* whose activities were nearly similar to caffeic acid (Djeridane et al. 2006b). Data from this study (Table 4) showed a good correlation between antihemolytic activity with chelating capacity ($r^2=0.977$), HRSA ($r^2=0.594$) and FTC ($r^2=0.542$) methods. However, no correlation was found with other assays like DPPH-RSA, TEAC and ferric reducing capacity.

Chelating capacity on Fe^{2+} Ferrous ions, commonly found in the food system are considered to be the most effective prooxidants (Yamaguchi et al. 1998). 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^{2+} ion chelating effect and the activity was expressed as mg EDTA equivalent. At the dosage level of 250 µl examined, the raw samples showed a chelating capacity of 74.2 ± 1.37 mg EDTA/g extract followed by dry heated (68.5 ± 1.71 mg EDTA/g extract) and pressure cooked (61.3 ± 1.26 mg EDTA/g extract) samples. This finding implied that the methanol extract of raw seed sample exhibiting the highest Fe^{2+} ion chelating ability when compared with that of processed seed 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) tannin content also showed a significant Fe^{2+} chelating effect. Amongst the methods used for quantifying antioxidant activities, the correlation between chelating capacity and antihemolytic activity, HRSA and FTC method (Table 4) was $r^2=0.977$, $r^2=0.443$ and $r^2=0.392$, respectively. No correlation was found between chelating capacity with ferric ion reducing capacity, DPPH-RSA and TEAC methods.

Conclusion

In conclusion, the raw and dry heated samples of *D. lablab* possess potent antioxidant activity than the pressure cooked 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. Since dry beans contain compounds other than fiber that may have significant antioxidant potential, it will be useful to investigate their potential in alleviating diseases and maximizing their use in food industry.

Acknowledgement The authors are grateful to the Management, Karpagam Educational Institutions for generous support and encouragement to carry out the study.

References

- Amarowicz R, Karamac M, Shahidi F (2003) Antioxidant activity of phenolic fractions of lentils (*Lens culinaris*). J Food Lipids 10:1–10
- Arts MJTJJ, Dallinga S, Voss HP, Haenen GRMM, Bast A (2004) A new approach to assess the total antioxidant capacity using the TEAC assay. Food Chem 88:567–570
- Barroga FB, Laurena AC, Mendoza EMT (1985) Polyphenols in mungbean; determination and removal. J Agric Food Chem 33:1006–1009
- Boateng J, Verghese M, Walker LT, Ogutu S (2008) Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). LWT Food Sci Technol 41:1541–1547
- Carbonaro M, Virgili F, Carnovale E (1996) Evidence for protein-tannin interaction in legumes: Implications in the antioxidant properties of faba bean tannins. LWT Food Sci Technol 29:743–750
- Chau CF, Cheung PCK, Wong YS (1998) Hypocholesterolemic effects of protein concentrate from three Chinese indigenous legume seeds. J Agric Food Chem 46:3698–3701
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006a) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 97:654–660
- Djeridane A, Yousfi M, Nadjemi B, Vidal N, Lesgards JF, Stocker P (2006b) Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. Eur Food Res Technol 224:801–809
- Duenas M, Hernandez T, Estrella I, Rabanal R (2003) Phenolic composition and antioxidant activity of mocan seeds (*Visnea mocanera* L.f.). Food Chem 82:373–379
- Duranti M (2006) Grain legume proteins and nutritional properties. Fitoterapia 77:67–82
- Erkan N, Ayranci G, Ayranci E (2008) Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem 110:76–82
- Fernandez-Orozco R, Zielinski H, Piskula MK (2003) Contribution of low-molecular-weight antioxidants to the antioxidant capacity of raw and processed lentil seeds. Nahrung Food 47:291–299
- Granito M, Paolini M, Perez S (2008) Polyphenols and antioxidant capacity of *Phaseolus vulgaris* stored under extreme conditions and processed. LWT Food Sci Technol 41:994–999
- Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW (1998) High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 46:1887–1892

- Hochstein P, Atallah AS (1988) The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. *Mutat Res* 202:363–375
- Hwang JY, Shue YS, Chang HM (2001) Antioxidant activity of roasted and defatted peanut kernels. *Food Res Int* 34:639–647
- Jiratanan T, Liu RH (2004) Antioxidant activity of processed table beets (*Beta vulgaris* var. *conditiva*) and green beans (*Phaseolus vulgaris* L.). *J Agric Food Chem* 52:2659–2670
- Kaur CH, Kapoor H (2002) Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Technol* 37:153–161
- Kikuzaki H, Nakatani N (1993) Antioxidant effects of some ginger constituents. *J Food Sci* 58:1407–1410
- Klein SM, Cohen G, Cederbaum AI (1991) Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry* 20:6006–6012
- Makkar HPS, Becker K, Abel H, Pawelzik E (1997) Nutrient contents, rumen protein degradability and antinutritional factors in some colour and white-flowering cultivars of *Vicia faba* beans. *J Sci Food Agric* 75:511–520
- Manian R, Anusuya N, Siddhuraju P, Manian S (2008) The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem* 107:1000–1007
- Naim M, Gestetner B, Bondi A, Birk Y (1976) Antioxidative and antihemolytic activities of soybean isoflavones. *J Agric Food Chem* 24:1174–1177
- Obboh G (2006) Antioxidant properties of some commonly consumed and underutilized tropical legumes. *Eur Food Res Technol* 224:61–65
- Oomah BD, Tiger N, Olson M, Balasubramanian P (2006) Phenolics and antioxidative activities in narrow leafed lupins (*Lupinus angustifolius* L.). *Plant Foods Hum Nutr* 61:91–97
- Othman A, Ismail A, Abdul Ghani N, Adenan I (2007) Antioxidant capacity and phenolic content of cocoa beans. *Food Chem* 100:1523–1530
- Oyaizu M (1986) Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 44:307–315
- Pastor-Cavada E, Juan R, Pastor JE, Alaiz M, Vioque J (2009) Antioxidant activity of seed polyphenols in fifteen wild *Lathyrus* species from South Spain. *LWT Food Sci Technol* 42:705–709
- Re R, Pellegirini N, Progettente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med* 26:1231–1237
- Rocha-Guzman NE, Gonzalez-Laredo RF, Ibarra-Perez FJ, Nava-Berumen CA, Gallegos-Infante JA (2007) Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. *Food Chem* 100:31–35
- Sanchez-Moreno C, Larrauri JA, Saura-Calixto F (1998) A procedure to measure the antiradical efficiency of polyphenols. *J Sci Food Agric* 76:270–276
- Shimada K, Fujikawa K, Yahara NT (1992) Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem* 40:945–948
- Siddhuraju P, Becker K (2003) Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. *utilis*) extracts and certain non-protein amino/ imino acids through *in vitro* models. *J Sci Food Agric* 83:1517–1524
- Siddhuraju P, Manian S (2007) The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chem* 105:950–958
- Siddhuraju P, Maheshu V, Loganayaki N, Manian S (2008) Antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from processed indigenous legumes, *Macrotyloma uniflorum* (Lam.) Verdc. and *Dolichos lablab* L. *Food* 2:159–167
- Tharanathan RN, Mahadevamma S (2003) Grain legumes- a boon to human nutrition. *Trends Food Sci Technol* 14:507–518
- The Wealth of India (1992) Raw Materials 3, CSIR, New Delhi, India, p 59–67
- Tsai PJ, She CH (2006) Significance of phenol-protein interactions in modifying the antioxidant capacity of peas. *J Agric Food Chem* 54:8491–8494
- Tsuda T, Ohshima K, Kawakishi S, Osawa T (1994) Antioxidant pigments isolated from the seeds of *Phaseolus vulgaris* L. *J Agric Food Chem* 42:248–251
- Xu BJ, Yuan SH, Chang SKC (2007) Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *J Food Sci* 72: S167–S177
- Yamaguchi T, Takamura H, Matoba T, Terao J (1998) HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem* 62:1201–1204
- Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa K (2000) Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit rind. *J Agric Food Chem* 48:180–185
- Ye XY, Wang HX, Ng TB (2000) Dolichin, a new chitinase-like antifungal protein isolated from field beans (*Dolichos lablab*). *Biochem Biophys Res Commun* 269:155–159
- Yen WJ, Chang LW, Duh PD (2005) Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. *LWT Food Sci Technol* 38:193–200