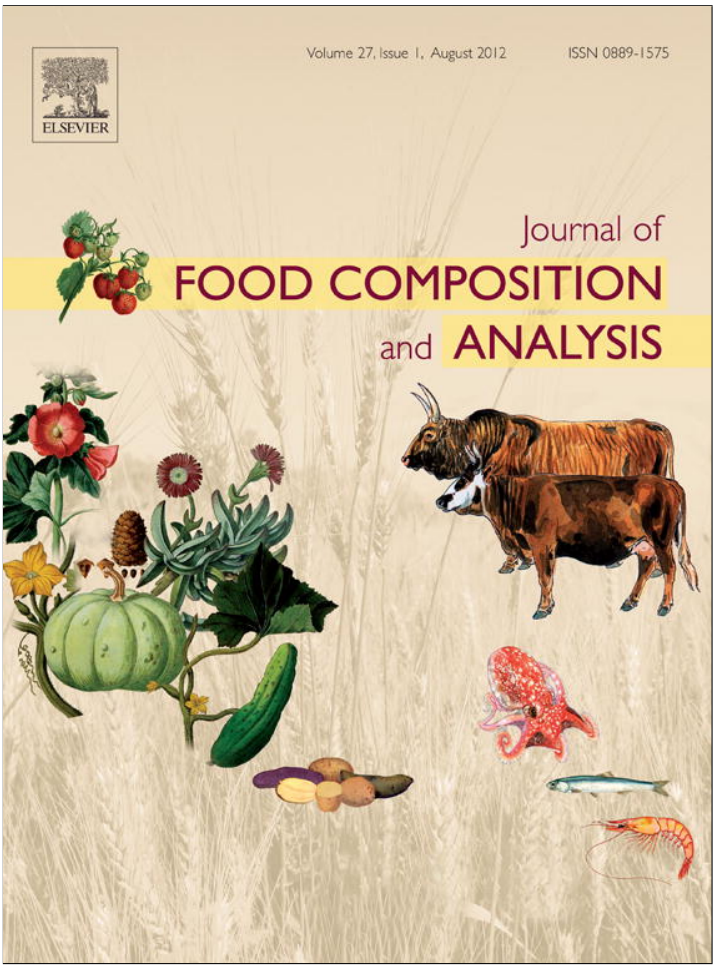

Journal of Food Composition and Analysis 27 (2012) 52–60

Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Total phenolic content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum* L. and *Pisum sativum* L.

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ARTICLE INFO

Article history:
Received 6 September 2011
Received in revised form 10 March 2012
Accepted 17 April 2012

Keywords:
Antioxidant activity
Cicer arietinum
Pisum sativum
Phenolics
Tannins
Processing
Free radical scavenging capacity
Metal chelating
Antinutrient
Food composition

ABSTRACT

Legumes are a rich source of proteins, dietary fiber, micronutrients and bioactive phytochemicals. The antioxidative properties and total phenolic contents of raw and processed (dry heating, autoclaving and soaking followed by autoclaving) seed extracts of *Cicer arietinum* and *Pisum sativum* were analyzed. The raw and processed seed samples were extracted with 80% methanol and 70% acetone separately and used for the evaluation of its antioxidant potential. Total phenolic and tannin content of raw and processed seed extracts ranged from 11.46–19.42 mg/g extract and 1.03–14.64 mg/g extract. In general, the raw seed extracts were the most potent antioxidant suppliers and free radical scavengers. Interestingly, among the various processing methods, dry heated sample registered higher DPPH (11.10 g extract/g DPPH) and ABTS (124,634 μmol/g extract) radical scavenging activity, metal chelating (2.34 mg EDTA/g extract) and inhibition of bleaching (70%). These results indicated that processing methods significantly changed contents and activities of antioxidant components of *C. arietinum* and *P. sativum*. Nonetheless, the dry heating processing method proved to be advantageous in retaining the integrity of the appearance and texture of the legume with greater retention of antioxidant components and activities.

1. Introduction

Free radicals contribute to several health disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, central nervous system injury, gastritis and cancer (Tepe et al., 2007). Due to environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals cause depletion of the immune system antioxidants and changes in gene expression, and they also induce abnormal proteins. The oxidation process is one of the most important methods for producing free radicals in food, drugs, and even living systems (Turkoglu et al., 2007). Today's hectic lifestyle causes an over-production of free radicals and reactive oxygen species. Natural antioxidants that protect from oxidative stress and associated diseases therefore play an important role in health care (Lopez et al., 2007).

Legumes are important sources of macronutrients and micronutrients, and have played an important role in the traditional diets of many regions throughout the world. In addition to their nutritional value, it has long been recognized that legumes are functional foods that both promote good health and have therapeutic properties (Geil and Anderson, 1994). Epidemiological studies have shown correlations between the consumption of foods with a high content of phenolics, such as fruits, vegetables, grains, and legumes, and decreasing incidence of several diseases, namely cancer, aging, and cardiovascular diseases (Anderson et al., 1999; Kushi et al., 1999; Miller et al., 2000; Kris-Etherton et al., 2002). Recently, legumes and pulses have gained in interest because they are excellent sources of bioactive compounds and can be important sources of ingredients for uses in functional foods and other applications.

Pulse crops include dry pea, chickpea, lentil and lupin, along with various types of dry beans such as kidney and lima beans. Pulse crops are an excellent source of protein, carbohydrates, and fiber, and provide many essential vitamins and minerals. Their highly nutritional properties have been associated with many beneficial health-promoting properties, such as managing high cholesterol and type-2 diabetes and in the prevention of various forms of cancer.

However, pulses and other leguminous crops also contain many antinutritional proteins, such as lectins, protease inhibitors and the non-antinutritional compounds, angiotensin I-converting enzyme (ACE) inhibitor. Various deleterious effects may occur following the ingestion of raw pulse seeds or flours, such as hemagglutination, bloating, vomitting and pancreatic enlargement, due to the activity of the anti-nutritional compounds inside the host. Conversely, anti-nutritional compounds in pulses may have many beneficial properties in the

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0889-1575/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2012.04.003

treatment and/or prevention of disease when properly processed (Roy et al., 2010).

Several reports have documented the antioxidant potential of polyphenols in fruits and vegetables and their implications in reducing the incidence of degenerative diseases (Kaur and Kapoor, 2001). However, studies on the processing of legumes are sparse, although a few short studies on screening of *in vitro* antioxidant activity of legumes have been documented (Latha and Daniel, 2001; Sreeramulu et al., 2009; Tsuda et al., 1993). Based on these considerations, the present study was undertaken to investigate the effects of dry heating, autoclaving and soaking followed by autoclaving on the phenolic compounds and antioxidant activities of chickpea and peas. This study will provide much beneficial information for the food and nutraceutical industry from legume seeds, and serve as a good base for other researchers to investigate legume antioxidants in future research.

2. Materials and methods

2.1. Chemicals

Ferric chloride, 2,2′-diphenyl-1picrylhydrazyl (DPPH), potassium persulfate, 2,2 azinobis (3-ethylbenzo-thiozoline-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, ethylene diamine tetra acetic acid (EDTA), and disodium salt were obtained from HiMedia, Merck or Sigma. All other reagents used were of analytical grade.

2.2. Seed samples and processing

Two varieties of seeds of chick pea (*Cicer arietinum*) and green pea (*Pisum sativum*) were purchased from Coimbatore, Tamil Nadu, India. The seeds (200 g) were dry heated in a hot air oven at 150 °C for 20 min and the seeds were allowed to cool to room temperature. For the treatment by autoclaving, the seeds (200 g) were soaked in distilled water (seed:water, 1:10, 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 121 °C. The seeds autoclaved directly without soaking were also included in this study. After hydrothermal process the autoclaved water was decanted and seeds were dried at 45 °C. The raw and processed seed samples were ground to a fine powder (particle size of about 0.25 mm) and stored in separate screw capped bottles for further analysis.

2.3. Solvent extraction

After defatting by petroleum ether, the raw and processed ground seed samples (15 g) were extracted by stirring with 105 mL 80:20 (methanol:H_2O) at 25 °C for 48 h and filtering through Whatman No. 4 filter paper. The residues were re-extracted with an additional 75 mL of methanol, as described above, for 3 h. The solvent of the combined extract was evaporated under low temperature at 40 °C in incubator respectively. The remaining residues, after methanol extraction and air drying, were extracted by stirring with 105 mL 70:30 (acetone:H_2O) (v/v) at 25 °C for 48 h and filtering through Whatman No. 4 filter paper. The solvent of extract was evaporated under low temperature at 40 °C in an incubator (NSW make, New Delhi). The extract thus obtained was used directly for total phenolic and tannin estimation and also for the assessment of antioxidant activity through various *in vitro* assays. From the extract, a known volume was taken, dried in an oven at incubator temperature of 40 °C (until sample getting a constant weight) and the recovery percent was calculated as equation:

$$\text{Recovery}\,\% = \frac{(\text{Extract} + \text{container in g}) - (\text{Empty container in g})}{\text{Sample weight (g)}} \times 100$$

2.4. Estimation of total phenolics and tannins

The total phenolic content was determined according to Folin–Ciocalteu method (FCM) described by (Siddhuraju and Becker, 2003). FCM actually measures a sample's reducing capacity and can be considered as another antioxidant (electron transfer) capacity assay. For the assay, aliquots (100 μL) of extracts were taken in test tubes and the volume was made up to 1 mL with distilled water. Then 0.5 mL of Folin–Ciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE). Tannins in the extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a 100 mm × 12 mm test tube and to this 1.0 mL of distilled water and then 1.0 mL of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4 °C for 4 h. Then the sample was centrifuged (3000 × *g* 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 phenol content of the supernatant was measured, as mentioned above and expressed as the content of non-tannin phenolics on a dry matter basis (Siddhuraju and Manian, 2007). From the above results, the tannin content of the sample was calculated as follows:

$$\text{Tannin}\,(\%) = \text{Total phenolics}\,(\%) - \text{Non-tannin phenolics}\,(\%)$$

2.5. Total flavonoids

The total flavonoid content was measured by a spectrophotometric assay (Zhishen et al., 1999): 1 mL aliquot of standard solution of rutin at different concentrations (0–100 mg/L, external calibration with $n = 6$ concentrations) or sample was added to 10 mL volumetric flasks containing 4 mL water. At the onset of the experiment, 0.3 mL of 5% $NaNO_2$ was added to the flask. After 5 min, 3 mL of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. The absorbance of the mixture was determined at 510 nm *vs.* the prepared blanks. Total flavonoid content was expressed as mg rutin equivalents (RUT) per g extract.

2.6. Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of raw and processed chick pea and green pea seed samples was estimated according to the procedure described by Benzie and Strain (1996) as modified by Pulido et al. (2000). FRAP reagent (900 μL), prepared freshly and incubated at 37 °C, was mixed with 90 μL of distilled water and 30 μL of test sample, or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 °C for 30 min in a water bath. The FRAP reagent contained 2.5 mL of 20 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of

20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of incubation the absorbance readings were taken immediately at 593 nm using a spectrophotometer. Methanolic solutions of known Fe(II) concentration ranging from 100 to 2000 μmol/L ($FeSO_4 \cdot 7H_2O$) were used for plotting the calibration curve. The parameter Equivalent Concentration (EC_1) was defined as the concentration of antioxidant has a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4 \cdot 7H_2O \cdot EC_1$ was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/L concentration of Fe(II) solution determined using the corresponding regression equation.

2.7. Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex according to the method of Prieto et al. (1999). An aliquot of 100 μL of sample solution was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 mL vial. The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as μmol of ascorbic acid equivalents/g extract (ascorbic acid equivalent antioxidant activity) using the calibration curve of ascorbic acid. Linearity range of the calibration curve was 0.05–0.25 μM.

2.8. Metal chelating activity

The extracts (100 μL) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The results were expressed as mg EDTA equivalent/g extract using the calibration curve of EDTA. Linearity range of the calibration curve was 0.5–2.5 μg (Dinis et al., 1994).

2.9. Stable free radical scavenging activity using DPPH$^\bullet$ method

The antioxidant activities of the raw and processed chick pea and green pea seed extracts, ASC, TAN, RUT, QUE and BHA were measured in terms of hydrogen donating or radical scavenging ability, using the DPPH method (Brand-Williams et al., 1995) modified by Sanchez-Moreno et al. (1998). A methanolic 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 the decrease in absorbance at 515 nm was determined at the end of incubation period with a spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the samples and was calculated using the following equation:

$$(\%)\ \text{radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The antioxidant activity of the extract was as expressed as g extract/g DPPH hydrogen donating ability.

2.10. Total antioxidant activity assay by radical cation (ABTS$^{\bullet+}$)

ABTS was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at temperature for 12–16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89, v/v) and equilibrated to 30 °C to give an absorbance at 734 nm of 0.700 ± 0.02 in a 1 cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 μL aliquot of each dilution into the assay, they produced between 20 and 80% inhibition of the blank absorbance. After the addition of 1 mL diluted ABTS solution to 10 μL of samples or Trolox standards (final concentration 0–15 μM) in ethanol OD (optical density) was taken at 30 °C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration (Re et al., 1999) described by Siddhuraju and Becker (2003). The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μmol/g sample extracts using the calibration curve of Trolox. Linearity range of the calibration curve was 0.25–1.25 mm/L. The total antioxidant activities of ASC, BHA, RUT and TAN were also measured by ABTS method for comparison.

2.11. Nitric oxide scavenging activity assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci et al., 1994). Various concentrations of samples and sodium nitroprusside (SNP, 5 mM final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 mL were incubated at 25 °C for 150 min. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1% sulfanilamide and 0.1% naphthylethylene diamine dihydrochloride in 5% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 540 nm. The total antioxidant activities of ASC (500 μg) and QUE (500 μg) were also measured by nitric oxide scavenging method for comparison. The % nitric oxide scavenging activity was calculated by the following equation:

$$(\%)\ \text{Nitric oxide scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.12. Superoxide anion radical scavenging activity assay

The method used by Martinez et al. (2001) for determination of the superoxide dismutase was followed with modification (Dasgupta and De, 2004) in the riboflavin-light-nitroblue tetra zolium (NBT) system (Beauchamp and Fridovich, 1971). Each 3 mL of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, NBT (75 μM) and 1 mL of sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp. The total antioxidant activities of BHA (150 μg), CAT (150 μg), TRO (150 μg) and RUT (150 μg) were also measured by superoxide anion radical scavenging method for comparison. The % superoxide anion radical scavenging activity was calculated by the following equation:

(%) superoxide anion radical scavenging activity

$$= \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.13. Inhibition of β-carotene bleaching

Two milliliters of a solution of β-carotene in chloroform (2 mg/10 mL) was pipetted into a flask containing 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45 °C for 4 min, and 100 mL of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. A 4.8 mL aliquot of the emulsion was added to a tube containing 0.2 mL of the antioxidant solution at 500 mg/L, and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene (Taga et al., 1984). The tubes were placed in a water bath at 50 °C, and the absorbance measurements were conducted again at 15 min intervals up to 120 min. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula:

$$\text{AA} = \left[1 - \frac{A_0 - A_t}{A_0' - A_t'}\right] \times 100,$$

where A_0 and A_0' are the absorbance of values measured at zero time of the incubation for test sample and control, respectively and A_t and A_t' are the absorbances measured in the test sample and control, respectively, after incubation for 120 min. The total antioxidant activities of BHA (50 μg), RUT (50 μg) and TRO (50 μg) were also measured by inhibition of β-carotene bleaching method for comparison.

2.14. Hydroxyl radical scavenging activity

The scavenging activities of the extracts of raw and processed seed samples on hydroxyl radical were measured according to the method of Klein et al. (1991). Various concentrations (100, 200 and 300 μg) of extracts were added to 1.0 mL of iron–EDTA solution (0.13% ferrous ammonium sulfate 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). 3 mL of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following equation:

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

2.15. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test ($P < 0.05$) using SPSS (Version 13.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determinations ± standard deviation. Pearson's correlation test was conducted to determine the linear correlations among variables.

3. Results and discussion

3.1. Extract yield percentage and phenolic content of seed extracts

The extract yields, total phenolics and tannins of raw and processed *C. arietinum* and *P. sativum* seed samples are presented in Table 1. The extract yield percent of the differentially processed seeds ranged from 1.33 to 28.06%. The maximum extract yield was obtained for the CDA (28.06%) followed by CDM (23.80%). On the other hand, the highest concentrations of total phenolics (19.42 mg TAN/g extract) were recorded for the raw seed extracts than the processed seed extracts in *C. arietinum.* Conversely, soaking followed by autoclaving showed highest phenolic content (18.59 mg TAN/g extract) than the raw seed extracts in *P. sativum.* The tannin content of the differentially processed seeds ranged from 1.03 to 14.64 mg TAN/g extract. The highest concentrations of tannin were recorded for the CDM (14.64 mg TAN/g extract) than the raw seed extracts. The findings of the present study are similar to the Siddhuraju and Becker (2007) which found that maximum extract yield was obtained for the extracts of dry heated

Table 1
Solvent extract recovery percentage, total phenolic, tannin and flavonoid contents of raw and processed seed extracts from *C. arietinum* and *P. sativum*.

Samples	Extract yield (%)	Total phenolics (mg/g extract)	Tannins (mg/g extract)	Flavonoids (mg/g extract)
CRM	4.0	$19.42^{a} \pm 2.72$	$10.85^{abc} \pm 1.46$	ND
CDM	23.80	$19.18^{a} \pm 1.26$	$14.64^{a} \pm 1.69$	ND
CAM	1.33	$16.39^{abc} \pm 1.70$	$11.48^{ab} \pm 0.75$	ND
CSAM	1.40	$12.91^{bc} \pm 0.33$	$7.15^{bcd} \pm 0.37$	ND
CRA	9.0	$18.64^{ab} \pm 5.53$	$9.48^{abc} \pm 5.59$	$10.65^{a} \pm 1.43$
CDA	28.06	$14.64^{abc} \pm 1.37$	$5.52^{cde} \pm 1.31$	$8.47^{b} \pm 0.86$
CAA	6.66	$11.46^{c} \pm 0.91$	$3.39^{de} \pm 0.99$	$8.77^{b} \pm 1.12$
CSAA	2.73	$13.19^{bc} \pm 0.36$	$7.66^{bcd} \pm 0.62$	$8.16^{b} \pm 0.26$
PRM	17.26	$12.88^{bc} \pm 0.68$	$6.85^{bcd} \pm 1.01$	ND
PDM	3.60	$14.12^{abc} \pm 2.53$	$9.08^{abc} \pm 2.12$	ND
PAM	1.93	$17.64^{ab} \pm 0.36$	$12.41^{ab} \pm 0.38$	ND
PSAM	2.40	$18.59^{ab} \pm 6.11$	$13.61^{a} \pm 5.91$	ND
PRA	21.53	$12.91^{bc} \pm 0.86$	$1.03^{e} \pm 0.18$	$7.93^{b} \pm 0.37$
PDA	7.20	$14.12^{abc} \pm 2.53$	$6.88^{bcd} \pm 3.08$	$8.83^{b} \pm 0.47$
PAA	3.20	$17.52^{ab} \pm 1.87$	$9.80^{abc} \pm 1.86$	$9.26^{b} \pm 0.29$
PSAA	4.13	$18.59^{ab} \pm 6.11$	$11.83^{ab} \pm 6.17$	$8.44^{b} \pm 0.48$

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. CRM, *Cicer arietinum* raw methanol extract; CDM, *Cicer arietinum* dry heated methanol extract; CAM, *Cicer arietinum* autoclaving methanol extract; CSAM, *Cicer arietinum* soaking followed by autoclaving methanol extract; CRA, *Cicer arietinum* raw acetone extract; CDA, *Cicer arietinum* dry heated acetone extract; CAA, *Cicer arietinum* autoclaving acetone extract; CSAA, *Cicer arietinum* soaking followed by autoclaving acetone extract; PRM, *Pisum sativum* raw methanol extract; PDM, *Pisum sativum* dry heated methanol extract; PAM, *Pisum sativum* autoclaving methanol extract; PSAM, *Pisum sativum* soaking followed by autoclaving methanol extract; PRA, *Pisum sativum* raw acetone extract; PDA, *Pisum sativum* dry heated acetone extract; PAA, *Pisum sativum* autoclaving acetone extract; PSAA, *Pisum sativum* soaking followed by autoclaving acetone extract.

samples. The results on the variation of TPC by processing are in good agreement with those reported by Ismail et al. (2004), which reported that thermal treatment decreased the TPC in all vegetables, and these results also exhibited trends similar to those of previous reports (Xu and Chang, 2008a,b).

These significant losses could be attributed to water-soluble phenolics leaching into soaking and cooking water before and drying thermal processing as well as breakdown of phenolics during processing. Similarly, the decrease in total phenolic content on heat treatment of *Beta vulgaris* and *Phaseolus vulgaris* was reported by Jiratavan and Liu (2004). However, processing does not always result in the destruction of the antioxidant components. In some cases, processing factors can induce the formation of compounds (Manzocco et al., 1998; Nicoli et al., 1997). The findings of the present study are in good agreement with Siddhuraju and Becker (2001) which observed that total phenolics were significantly higher after soaking and dry heating compared to cooking or autoclaving of both raw and presoaked seeds. It is important to also note that the rate of increase in phenolic contents primarily depends on the type of legume and the preparation procedure used. Hence toasting rather than boiling or cooking yielded higher phenolic and flavonoid content in dry beans (Boateng et al., 2008). The binding between phenolics and the protein matrix might account for the enhancement of antioxidant capacity in peas. This is because a phenolic–protein interaction is able to stabilize the protein and its antioxidant capacity is increased during heating (Tsai and She, 2006). The extractable total phenolics and tannins of the dry heated samples were found to be higher than in raw samples that could be due to the solubility of phenolics and other aroma compounds (Siddhuraju et al., 2008).

3.2. Total flavonoid content

Flavonoids are widespread plant secondary metabolites, including flavones, flavanols, and condensed tannins. Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress. *In vitro*, flavonoids from several plant sources have shown free-radical scavenging activity and protection against oxidative stress (Xu et al., 2007). However, there were only a few reports on the identification and quantification of flavonoids in food legumes, including common beans (Hempel and Bohn, 1996; Beninger et al., 1999; Romani et al., 2004) and peas (Troszynska et al., 2002). The total flavonoid content of raw and processed *C. arietinum* and *P. sativum* seed samples are presented in Table 1. The flavonoid content of the differentially processed seeds ranged from 7.93 to 10.65 mg rutin/g extract. The maximum flavonoid content was obtained for the CRA (10.65 mg rutin/g extract) followed by PDA (8.83 mg rutin/g extract). The significant solid losses by soaking and boiling samples could be attributed to the diffusion of water soluble components, into soaking and cooking water. In addition, thermal treatments also could break the glucosides of flavonoids to form alglycones which possess higher antioxidant properties. The findings of the present study result is good agreement with Xu and Chang (2007) which suggested that acetone was the best among the six selected solvents for extracting flavonoids from yellow pea, green pea and chick pea. Interestingly, our results on flavonoid content were higher in most cases than that reported previously in legumes (Heimler et al., 2005; Xu et al., 2007). The differences between current results and previous report may be attributed to the differences in the sources of the samples.

Table 2
FRAP, phosphomolybdenum assay, metal chelating activity, DPPH• radical and ABTS•+ cation radical scavenging activity of raw and processed seed extracts from *C. arietinum* and *P. sativum*.

Samples	FRAP (μg extract/mmol Fe(II))[A]	Phosphomolybdenum (μmol ascorbic acid equ/g extract)	Metal chelating (mg EDTA equ/g extract)	IC_{50} of DPPH (g extract/g DPPH)[B]	TRO equ (μmol/g extract)[C]
CRM	$1517.57^{c} \pm 161.35$	$557.91^{l} \pm 35.65$	$2.10^{c} \pm 0.30$	$12.17^{c} \pm 7.79$	$2547.90^{b} \pm 565.66$
CDM	$1437.63^{cd} \pm 327.03$	$1184.87^{ghij} \pm 174.31$	$0.46^{gh} \pm 0.19$	$68.00^{ab} \pm 83.43$	$726.61^{b} \pm 170.74$
CAM	$2053.59^{a} \pm 9.16$	$752.62^{kl} \pm 65.54$	$0.37^{gh} \pm 0.02$	$35.80^{bc} \pm 16.33$	$3294.12^{b} \pm 1744.40$
CSAM	$1679.70^{bc} \pm 130.27$	$898.32^{jkl} \pm 107.64$	$1.08^{def} \pm 0.18$	$82.06^{a} \pm 38.49$	$2864.09^{b} \pm 515.15$
CRA	$758.88^{f} \pm 75.75$	$1034.35^{hijk} \pm 294.30$	$1.28^{de} \pm 0.09$	$3.72^{c} \pm 0.60$	$6285.33^{b} \pm 697.32$
CDA	$771.89^{f} \pm 24.19$	$1314.69^{efghi} \pm 138.80$	$1.34^{de} \pm 0.37$	$11.10^{c} \pm 1.52$	$124634.79^{a} \pm 17122.71$
CAA	$966.42^{ef} \pm 31.96$	$1356.80^{efgh} \pm 189.95$	$0.97^{ef} \pm 0.65$	$13.57^{c} \pm 0.95$	$4660.08^{b} \pm 276.44$
CSAA	$1039.29^{ef} \pm 51.47$	$915.58^{ijkl} \pm 104.56$	$1.58^{d} \pm 0.46$	$14.36^{c} \pm 1.05$	$4381.83^{b} \pm 525.18$
PRM	$2187.79^{a} \pm 239.66$	$1633^{def} \pm 152.53$	$1.43^{de} \pm 0.03$	$13.67^{c} \pm 0.45$	$6155.37^{b} \pm 1142.56$
PDM	$1973.30^{a} \pm 273.12$	$1713.79^{de} \pm 180.25$	$1.13^{def} \pm 0.01$	$15.58^{c} \pm 1.14$	$3907.54^{b} \pm 687.27$
PAM	$1558.15^{c} \pm 37.19$	$1336.32^{efghi} \pm 32.11$	$1.02^{ef} \pm 0.16$	$19.73^{c} \pm 1.58$	$1517.10^{b} \pm 544.04$
PSAM	$1919.20^{ab} \pm 198.12$	$1247.02^{fghij} \pm 420.80$	$1.79^{fg} \pm 0.01$	$19.17^{c} \pm 0.71$	$1276.79^{b} \pm 904.09$
PRA	$825.70^{f} \pm 174.74$	$1733.81^{de} \pm 542.97$	$1.47^{de} \pm 0.29$	$29.40^{c} \pm 0.26$	$4163.34^{b} \pm 726.08$
PDA	$1417.14^{cd} \pm 97.86$	$1525.97^{efg} \pm 150.211$	$2.34^{c} \pm 0.31$	$13.49^{c} \pm 0.62$	$2977.92^{b} \pm 227.92$
PAA	$1222.51^{de} \pm 333.97$	$1418.95^{efgh} \pm 172.26$	$0.70^{h} \pm 0.13$	$15.93^{c} \pm 2.80$	$5551.76^{b} \pm 727.96$
PSAA	$1508.33^{c} \pm 158.68$	$2010^{d} \pm 499.31$	$0.98^{ef} \pm 0.10$	$17.32^{c} \pm 1.16$	$2415.10^{b} \pm 447.34$
α-TOC	–	–	$12.67^{a} \pm 0.25$	–	–
BHA	$2.94^{g} \pm 0.631$	$4915.37^{a} \pm 197.881$	$10.49^{b} \pm 0.06$	$0.14^{c} \pm 0.01$	$1196.54^{b} \pm 112.90$
TAN	$1.77^{g} \pm 0.140$	$3585.55^{b} \pm 77.213$	–	$0.10^{c} \pm 0.002$	$1373.33^{b} \pm 115.61$
TRO	$1.56^{g} \pm 0.139$	$2655.51^{c} \pm 63.892$	–	–	–
RUT	$5.88^{g} \pm 0.987$	$1545.5^{efg} \pm 14.860$	–	$0.20^{c} \pm 0.03$	$791.67^{b} \pm 42.61$
ASC	$1.38^{g} \pm 0.178$	–	–	$0.14^{c} \pm 0.002$	$1093.33^{b} \pm 191.78$
QUE	–		–	$0.06^{c} \pm 0.001$	–

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($P < 0.05$) different. CRM, *Cicer arietinum* raw methanol extract; CDM, *Cicer arietinum* dry heated methanol extract; CAM, *Cicer arietinum* autoclaving methanol extract; CSAM, *Cicer arietinum* soaking followed by autoclaving methanol extract; CRA, *Cicer arietinum* raw acetone extract; CDA, *Cicer arietinum* dry heated acetone extract; CAA, *Cicer arietinum* autoclaving acetone extract; CSAA, *Cicer arietinum* soaking followed by autoclaving acetone extract; PRM, *Pisum sativum* raw methanol extract; PDM, *Pisum sativum* dry heated methanol extract; PAM, *Pisum sativum* autoclaving methanol extract; PSAM, *Pisum sativum* soaking followed by autoclaving methanol extract; PRA, *Pisum sativum* raw acetone extract; PDA, *Pisum sativum* dry heated acetone extract; PAA, *Pisum sativum* autoclaving acetone extract; PSAA, *Pisum sativum* soaking followed by autoclaving acetone extract; BHA, Butylated hydroxyl anisole; TAN, Tannic acid; TRO, Trolox; RUT, Rutin; ASC, Ascorbic acid; QUE, Quercetin; α-TOC, α-Tocopherol.

[A] Ferric reducing/antioxidant power assay (concentration of substance having ferric-TPTZ reducing ability equivalent to that of 1 mM Fe(II)).

[B] g of extract required to decrease one g of the initial DPPH concentration by 50%.

[C] Total antioxidant activity (μmol equivalent Trolox performed by using ABTS radical cation).

3.3. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was originally developed by Benzie and Strain (1996), to measure reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals. The reaction measures reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a colored product. The reducing activities of raw and processed *C. arietinum* and *P. sativum* seed samples are presented in Table 2. Significant differences ($P < 0.05$) in FRAP values were found among the raw and processed samples. The ferric reducing ability of the extracts revealed that all of them gave good FRAP activity (758–2187 mmol Fe(II)/μg extract). Among the treatments, the highest activity was noted for PRM (2187 mmol Fe(II)/μg extract) followed by CAM (2053 mmol Fe(II)/μg extract). FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples (Halvorsen et al., 2006; Pellegrini et al., 2003; Guo et al., 2003). The major finding of the present study is similar to Xu and Chang (2008a,b) which reported that regular boiling processes decrease FRAP values in peas, lentils and black common beans. Tsai and She (2006) concluded that there was a change in the phenolic compounds after heating which contributed to the increase in reducing potential. Furthermore, thermal processing could lead to the formation of novel compounds with antioxidant activity or the release of bound phenolic compounds (Nicoli et al., 1997; Dewanto et al., 2002a,b).

3.4. Phosphomolybdenum assay

The phosphomolybdate method has been routinely used to evaluate the antioxidant capacity of extracts (Prieto et al., 1999). In the presence of extracts, Mo(VI) is reduced to Mo(V) and forms a green colored phosphomolybdenum V complex, which shows a maximum absorbance at 695 nm. The assay being simple and independent of other antioxidant measurements commonly employed, its application was extended to plant polyphenols. The reducing activities of raw and processed *C. arietinum* and *P. sativum* seed samples are presented in Table 2. Among the treatments, the highest activity was noted for PSAA (2010 μmol ascorbic acid/g extract), followed by PRA (1733 μmol ascorbic acid/g extract). The findings of the present study are contrary to Sowndhararajan et al. (2011) reported that the raw seed extract exhibited higher phosphomolybdenum activity. Differences between our results and previous reports may be attributed partly to the differences in sources, extraction medium and chemical nature of the compounds present within the extracts. The differential response of the extracts in various antioxidant tests may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occur at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants (Loo et al., 2008).

3.5. Metal chelating activity

Fe^{2+} chelation is an important antioxidative mechanism which retards metal-catalyzed oxidation (Kehrer, 2000). The effective Fe^{2+} chelators afford protection against oxidative damage by removing Fe^{2+} that may otherwise participate in HO generating Fenton type reactions. This in turn can give protection against oxidative damage by inhibiting production of ROS and lipid peroxidation. The Fe^{2+} chelating capacity of various legumes was determined by measuring the iron–ferrozine complex and results are summarized in Table 2. Among the treatments, the highest activity was noted for PDA (2.34 mg EDTA/g extract), followed by CRM (2.10 mg EDTA/g extract). However, in *P. sativum*, dry heated sample exhibited high metal chelating activity than raw samples that could be due to the formation of aroma compounds. On the other hand, CRM exhibited higher metal chelating activity than processed samples. Interestingly, these results are similar to the observations of Siddhuraju (2006), which reported higher metal chelating capability for the raw seeds of *V. aconitifolia* than processed ones. Our results on variation of metal chelating activity is in good agreement with those reported by Han and Baik (2008) which found that chickpeas (1.0 mg EDTA/g extract) and peas (3.2 mg EDTA/g extract) exhibited greater chelating activities than lentils. It was reported that the chelating agents, which forms bonds with metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gulcin et al., 2004).

3.6. Free radical scavenging activities on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$

It is generally recognized that free radicals produced in the body are partly associated with the etiology of cancers and other chronic diseases. Dietary antioxidants, capable of scavenging free radicals, are able to reduce the risk of the disease. Therefore, it is important to determine the radical scavenging effect of antioxidants in legumes. DPPH and ABTS free radical scavenging capacities of the extracts from raw and processed legume samples are presented in Table 2. Significant differences ($P < 0.05$) in DPPH values were found both in raw and processed legumes, among most processing treatments of *C. arietinum* and *P. sativum*. A lower value of IC_{50} indicates a higher antioxidant activity. Among the treatments, CRA (3.72 g extract/g DPPH) had the highest DPPH free radical scavenging ability followed by the CDA (11 g extract/g DPPH). On the other hand, PDA (13.49 g extract/g DPPH) exhibited highest radical scavenging activity.

In general, loss of DPPH was partly due to soluble antioxidants in leaches water and heat effect. The results on variation of DPPH activity by processing are also in good agreement with those reported by Xu and Chang (2008a) and Han and Baik (2008) which found that thermal treatment decreased the free radical scavenging activity in legumes. Interestingly, our results are similar to Siddhuraju and Manian (2007) reported that extracts obtained from raw and dry heated seeds of horse gram (black variety) showed the highest DPPH radical scavenging activity. On the other hand, the DPPH radical scavenging efficiency of extracts from dry heated seed samples can be partly attributed to Maillard reaction products other than the phenolic constituents because they also effectively participate as a radical scavengers (Siddhuraju et al., 2008).

ABTS is one of the radicals generally used for testing the preliminary radical scavenging activity of a compound or plant extract. The ABTS$^{\bullet+}$, generated from oxidation of ABTS by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxyl radicals) (Leong and Shui, 2002). Among the treatments, CDA (124,634 μmol/g extract) exhibited the greatest scavenging ability followed by CRA (6285 μmol/g extract) and PRM (6155 μmol/g extract). Similar observations indicating that the dry heated samples shows high radical scavenging activity in indigenous legumes (Siddhuraju et al., 2008), horse gram (Siddhuraju and Manian, 2007) and *Tamarindus indica* (Siddhuraju, 2007) were also reported. Hagerman et al. (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS$^{\bullet+}$) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups.

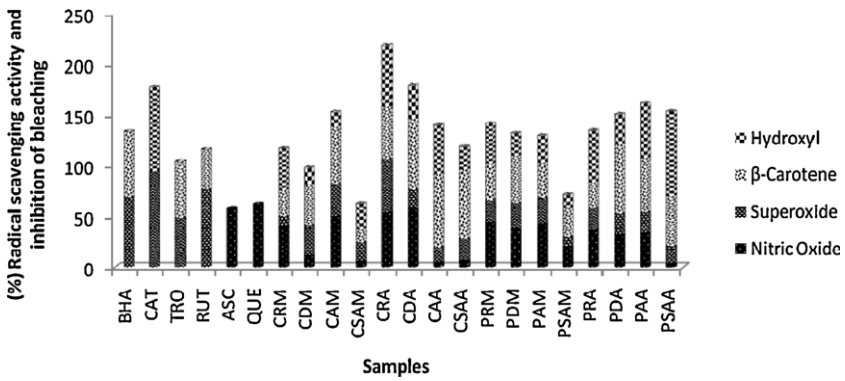

Fig. 1. Nitric oxide, superoxide, hydroxyl radical scavenging activity and inhibition of β-carotene bleaching of raw and processed seed extracts from *C. arietinum* and *P. sativum*. Values are means of triplicate determinations ± standard deviation. BHA, Butylated hydroxyl anisole; CAT, Catechin; TRO, Trolox; RUT, Rutin; ASC, Ascorbic acid; QUE, Quercetin; CRM, *Cicer arietinum* raw methanol extract; CDM, *Cicer arietinum* dry heated methanol extract; CAM, *Cicer arietinum* autoclaving methanol extract; CSAM, *Cicer arietinum* soaking followed by autoclaving methanol extract; CRA, *Cicer arietinum* raw acetone extract; CDA, *Cicer arietinum* dry heated acetone extract; CAA, *Cicer arietinum* autoclaving acetone extract; CSAA, *Cicer arietinum* soaking followed by autoclaving acetone extract; PRM, *Pisum sativum* raw methanol extract; PDM, *Pisum sativum* dry heated methanol extract; PAM, *Pisum sativum* autoclaving methanol extract; PSAM, *Pisum sativum* soaking followed by autoclaving methanol extract; PRA, *Pisum sativum* raw acetone extract; PDA, *Pisum sativum* dry heated acetone extract; PAA, *Pisum sativum* autoclaving acetone extract; PSAA, *Pisum sativum* soaking followed by autoclaving acetone extract.

3.7. Nitric oxide scavenging activity

Nitric oxide (NO) is a defense molecule with cytotoxic, microbiocidal and microbiostatic activities. SNP will release nitric oxide when dissolved in PBS solution and react with oxygen to form nitrite. SNP solution under aerobic conditions, in the presence of various extracts with Griess reagent, can be used to evaluate the scavenging effect on nitric oxide of the extract. Among the various samples, CDA (58%) exhibited high scavenging activity followed by raw (54%) and other processing samples (Fig. 1). Interestingly, in *P. sativum* the PRM (45%) showed high scavenging activity whereas positive controls such as ascorbic acid and quercetin exhibited 57% and 62% activity. These results are similar to that *C. sappan* extracts and it was found to be an efficient scavenger of ABTS, superoxide anion radical and nitric oxide in a dose dependent manner (Saenjum et al., 2010).

3.8. Superoxide anion ($O_2^{\bullet-}$) radical scavenging activity

Superoxide anions ($O_2^{\bullet-}$) are the most common free radicals whose concentration increase under conditions of oxidative stress and are generated either by auto-oxidation processes or by enzymes and produce other cell damaging free radicals and oxidizing agents (Liu and Ng, 2000). The raw and processed seed samples of *C. arietinum* and *P. sativum* were assessed and the results are given in Fig. 1. CRA (51%) exhibited high scavenging activity when compared to the other processing methods. Interestingly, the methanol extract of both the legumes of dry heated samples showed high scavenging activity compared to the raw and autoclaved samples. The lower activity of hydrothermally processed samples may be attributed to the partial loss of active phytocompounds in the seeds during the soaking as well as decanting of autoclaved liquid. The results are in accordance with those of Siddhuraju and Becker (2007) which found that the raw and dry heated samples exhibited high scavenging activity rather than the autoclaved samples.

3.9. Inhibition of β-carotene bleaching

The bleaching inhibition, measured by the peroxidation of β-carotene, is presented in Fig. 1. The linoleic acid free radical attacks the highly unsaturated β-carotene. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system (Jayaprakasha et al., 2001). Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, samples retained their color and thus absorbance for a longer time. Hence this system is often employed to evaluate the efficacy of unknown antioxidative compounds even if it describes only specific properties related to specific type of radicals used in assessment (Madhujith et al., 2004). Bleaching inhibition in the presence of different extracts CAA (71%), CDA (70%), PDA (68%) and CSAA (67%) showed high antioxidant activity than positive controls such as BHA (64%), TRO (55%) and RUT (40%). In both the plant samples, the processed samples showed higher activity when compared to the raw samples. The findings of the present study is contrary to Anwar et al. (2007), four cultivars of *Vigna radiata* exhibited lower antioxidant activity namely M-1 (49.8%) and NM-92 (56.7%). This variation might have been due to the difference in procedures adopted, extraction medium and chemical nature of the compounds present within the extracts.

3.10. Hydroxyl radical scavenging activity

Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. Hydroxyl (OH^-) radicals are extremely reactive and may be generated in the human body under physiological conditions, where they can react with non-selective compounds such as proteins, DNA, unsaturated fatty acids and almost every biological membrane. Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are thought to initiate cell damage *in vivo* (Rollet-Labelle et al., 1998). The hydroxyl radical scavenging abilities of raw and processed seed samples of *C. arietinum* and *P. sativum* are shown in Fig. 1. Among the various samples, PSAA (83%) showed high radical scavenging activity when compared to other processed samples. Interestingly, the raw sample exhibited high radical scavenging activity in *C. arietinum* and *P. sativum* methanol extract. Our findings of the present study are contrary to the earlier reports Siddhuraju (2006, 2007) and Siddhuraju and Becker (2007) which found that the dry heated sample exhibited high scavenging activity rather than raw and autoclaved sample. The findings of the present study are in good agreement with the raw seed extracts of *M. uniflorum* (brown and black varieties) and *D. lablab* had the highest hydroxyl radical scavenging activity (Siddhuraju et al., 2008). The differences between current results and previous report

may be attributed to the differences in the sources of the samples. The results on legumes are in agreement with an earlier report (Mustafa et al., 2006), in which no significant correlation was observed between the total phenolic content and antioxidant activity among 92 plant extracts. The lack of correlation could be due to different responses of different phenolic compounds in different assay systems. Specifically, no correlation among total phenolic content, DPPH and ABTS was reported in the wheat extracts (Liangli et al., 2002). However, an holistic analysis of phenolic compounds needs to be carried out to explain the poor correlation of total phenolic content and antioxidant activity in legumes.

4. Conclusion

In summary, the processing methods, namely dry heating, autoclaving and soaking followed by autoclaving, significantly affected the total phenolic contents and antioxidant activities in all samples. The changes depended on the type of legume and processing conditions. These changes in the overall antioxidant properties of processed legumes could be attributed to the synergistic combinations or counteracting of several types of factors, including oxidative reaction, leaching of water-soluble antioxidant compositions, formation or breakdown of antioxidant compositions, and solid losses during processing. However, dry heat processes caused smaller losses in total phenolic content and antioxidant activity. Therefore, this viable processing technique will considered for the formulation of therapeutic supplementary foods for the vulnerable groups in future. To better understand the role and fate of natural and heat-induced antioxidants on food stability and human health, the preceding chemical composition research was performed to investigate the molecular mechanisms responsible for loss or formation of antioxidants and interactions between natural and heat-induced antioxidants and their effects on the overall antioxidant properties of cooked legumes.

References

Anderson, J.W., Smith, B.M., Washnock, C.S., 1999. Cardiovascular and renal benefits of dry bean and soybean intake. American Journal of Clinical Nutrition 70, 464S–474S.

Anwar, F., Latif, S., Przybylski, R., Sultana, B., Ashraf, M., 2007. Chemical composition and antioxidant activity of seeds of different cultivars of *Mungbean*. Journal of Food Science S503–S510.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44, 276–287.

Beninger, C.W., Hosfield, G.L., Bassett, M.J., 1999. Flavonol composition of three genotypes of dry bean (*Phaseolus vulgaris* L.) differing in seed coat color. Journal of the American Society for Horticultural Science 124, 514–518.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239, 70–76.

Boateng, J., Verghese, M., Walker, L.T., Ogutu, S., 2008. Effect of processing contents in selected dry beans (*Phaseolus* spp L.). LWT – Food Science and Technology 41, 1541–1547.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology 28, 25–30.

Dasgupta, N., De, B., 2004. Antioxidant activity of *Piper betel* L. leaf extract *in vitro*. Food Chemistry 88, 219–224.

Dewanto, V., Wu, X.Z., Liu, R.H., 2002a. Processed sweet corn has higher antioxidant activity. Journal of Agricultural and Food Chemistry 50, 4959–4964.

Dewanto, V., Wu, X.Z., Adom, K.K., Liu, R.H., 2002b. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50, 3010–3014.

Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M., 1994. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 315, 161–169.

Geil, P.B., Anderson, J.W., 1994. Nutrition and health implications of dry beans: a review. Journal of the American College of Nutrition 13, 549–558.

Gulcin, I., Beydemir, S., Alici, H.A., Elmasta, M., Buyukokuroglu, M.E., 2004. *In vitro* antioxidant properties of morphine. Pharmacological Research 49, 59–66.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., Jiang, Y., 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research 23, 1719–1726.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riecel, T.L., 1998. High molecular weight polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry 46, 1887–1892.

Halvorsen, B.L., Carlsen, M.H., Phillips, K.M., Bohn, S.K., Holte, K., Jacobs Jr., D.R., Blomhoff, R., 2006. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. American Journal of Clinical Nutrition 84, 95–135.

Han, H., Baik, B.K., 2008. Antioxidant activity and phenolic content lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and Soybeans (*Glycine max*), and their quantitative changes during processing. International Journal of Food Science and Technology 43, 1971–1978.

Heimler, D., Vignolini, P., Dini, M.G., Romani, A., 2005. Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. Journal of Agricultural and Food Chemistry 53, 3053–3056.

Hempel, J., Bohn, H., 1996. Quality and quantity of prevailing flavonoid glycosides of yellow and green French beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry 44, 2114–2116.

Ismail, A., Marjan, Z.M., Foong, C.W., 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry 87, 581–586.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chemistry 73, 285–290.

Jiratavan, T., Liu, R.H., 2004. Antioxidant activity of processed table beets (*Beta vulgaris* var, conditiva) and green beans (*Phaseolus vulgaris* L.). Journal of Agriculture and Food Chemistry 52, 2659–2670.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables – the millennium's health. International Journal of Food Science and Technology 36, 703–725.

Kehrer, J.P., 2000. The Haber–Weiss reaction and mechanisms of toxicity. Toxicology 149, 43–50.

Klein, S.M., Cohen, G., Cederbaum, A.I., 1991. Production of formaldehyde during metabolism of dimethyl sulphoxide by radical generating system. Biochemistry 20, 6006–6012.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113, 71S–88S.

Kushi, L.H., Meyer, K.A., Jacobs, D.R., 1999. Cereal, legumes, and chronic disease risk reduction: evidence from epidemiologic studies. American Journal of Clinical Nutrition 70, 451S–458S.

Latha, S., Daniel, M., 2001. Phenolic antioxidants of some common pulses. Journal of Food Science and Technology 38, 272–273.

Leong, L.P., Shui, G., 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry 76, 69–75.

Liangli, Yu., Scott, H., Jonathan, P., Mary, H., John, W., Ming, Q., 2002. Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry 50, 1619–1624.

Liu, F., Ng, T.B., 2000. Antioxidant and free radical scavenging activities of selected medicinal herbs. Life Sciences 66, 725–735.

Loo, A.Y., Jain, K., Darah, I., 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chemistry 107, 1151–1160.

Lopez, V., Akerreta, S., Casanova, E., Garcia-Mina, J.M., Cavero, R.Y., Calvo, M.I., 2007. In vitro antioxidant and anti-rhizopus activities of lamiaceae herbal extracts. Plant Foods for Human Nutrition 62, 151–155.

Madhujith, T., Naczk, M., Shahidi, F., 2004. Antioxidant activity of common beans (*Phaseolus vulgaris* L.). Journal of Food Lipids 11, 220–233.

Manzocco, L., Anese, M., parpinel, M., 1998. Antioxidant properties of tea extracts as affected by processing. LWT – Food Science and Technology 31, 694–698.

Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., Packer, L., 1994. The Nitric oxide scavenging property *Ginkgo biloba* extract EGb76J. Biochemical and Biophysical Research Communications 201, 748–755.

Martinez, A.C., Marcelo, E.L., Marco, A.O., Moacyr, M., 2001. Differential responses of superoxide dismutase in freezing resistant *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. Plant Science 160, 505–515.

Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M., 2000. Whole-grain products and antioxidants. Cereal Foods World 45, 59–63.

Mustafa, O., Neil Reese, R., Artemio Jr., Z.T., Joseph, C., Scheernens, A., Raymond, M., 2006. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) methods. Journal of Agricultural and Food Chemistry 54, 1151–1157.

Nicoli, M.C., Anese, M., Parpinel, M., Franceschi, S., Lerici, C.R., 1997. Loss and/or formation of antioxidants during food processing and storage. Cancer Letters 114, 71–74.

Pellegrini, N., Serafini, M., Colombi, B., Rio, D.D., Salvatore, S., Bianchi, M., Brighenti, F., 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. The Journal of Nutrition 133, 2812–2819.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 269, 337–341.

Pulido, R., Bravo, L., Saura-Calixto, F., 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 48, 3396–3402.

Re, R., Pellegirini, N., Proteggente, A., Pannaala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity Appling an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26, 1231–1237.

Rollet-Labelle, E., Grange, M.J., Elbim, C., Marquetty, C., Goqgerot-Pocidalo, M.A., Pasquier, C., 1998. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. Free Radical Biology and Medicine 24, 563–572.

Romani, A., Vignolini, P., Galardi, C., Mulinacci, N., Benedettelli, S., Heimler, D., 2004. Germplasm characterization of Zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. Journal of Agricultural and Food Chemistry 52, 3838–3842.

Roy, F., Boye, J.I., Simpson, B.K., 2010. Bioactive proteins and peptides in pulse crops: pea, chickpea and lentil. Food Research International 43, 432–442.

Saenjum, C., Chaiyasut, C., Kadehumsang, S., Chansakaow, S., Suttajit, M., 2010. Antioxidant activity and protective effect on DNA damage of *Caesalpinia sappan* L. extract. Journal of Medicinal Plants Research 4, 1594–1600.

Sanchez-Moreno, C., Larrauri, J.I., Saura-Calixto, F.A., 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 76, 270–276.

Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic regions of Drumstick tree (*Moringa olifera Lam.*) leaves. Journal of Agricultural and Food Chemistry 51, 2144–2155.

Siddhuraju, P., 2006. The antioxidant activity and free radical scavenging capacity of phenolics of raw and dry heated moth bean (*Vigna aconitifolia*) (Jacq.) Marechal seed extracts. Food Chemistry 99, 149–157.

Siddhuraju, P., 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT Food Science and Technology 40, 982–990.

Siddhuraju, P., Becker, K., 2001. Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens* Var. utilis. Journal of Agricultural and Food Chemistry 49, 3058–3067.

Siddhuraju, P., Becker, K., 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chemistry 101, 10–19.

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 Chemistry 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.

Sowndhararajan, K., Siddhuraju, P., Manian, S., 2011. Antioxidant and free radical scavenging capacity of the underutilized legume, *Vigna vexillata* (L.) A. rich. Journal of Food Composition and Analysis 24, 160–165.

Sreeramulu, D., Reddy, V.K., Raghunath, M., 2009. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. Indian Journal of Biochemistry and Biophysics 46, 112–115.

Taga, M.S., Miller, E.E., Pratt, D.E., 1984. Chia seeds as a source of natural lipid antioxidants. Journal of the American Oil Chemists Society 61, 928–931.

Tepe, B., Eminagaoglu, O., Akpulat, H.A., Aydin, E., 2007. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *amasiaca* (Freyn & Bornm.) Bornm. Food Chemistry 100, 985–989.

Troszynska, A., Estrella, I., Lopez-Amores, M.L., Hernandez, T., 2002. Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. Lebensmittel Wissenschaft Technologie 35, 158–164.

Tsai, P.J., She, C.H., 2006. Significance of phenol–protein interactions in modifying the antioxidant capacity of peas. Journal of Agricultural and Food Chemistry 54, 8491–8494.

Tsuda, T., Makino, Y., Kato, H., Osawa, T., Kawakishi, S., 1993. Screening for antioxidative activity of edible pulses. Bioscience, Biotechnology and Biochemistry 57, 1606–1608.

Turkoglu, A., Duru, M.E., Mercan, N., Kivrak, I., Gezer, K., 2007. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. Food Chemistry 101, 267–273.

Xu, B.J., Chang, S.K.C., 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science 72, S159–S166.

Xu, B.J., Chang, S.K.C., 2008a. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chemistry 110, 1–13.

Xu, B.J., Chang, S.K.C., 2008b. Total phenolic content and antioxidant properties of Eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. Journal of Food Science 73, H19–H27.

Xu, B.J., Yuan, S.H., Chang, S.K.C., 2007. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. Journal of Food Science 72, S167–S177.

Zhishen, J., Mangcheng, T., Jianming, W., 1999. The determinating of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64, 555–559.