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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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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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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