IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 4, Jul-Sep 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food And Nutritional Sciences

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.4, Iss.4, Jul-sep, 2015

Research Paper ***Open Access***

EVALUATION OF THE NUTRITIONAL ANTINUTRITIONAL AND ANTIOXIDANT PROPERTIES OF SELECTED COWPEA (*VIGNA UNGUICULATA*) CULTIVARS

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Received on: 5th August, 2015 **Accepted on: 18th September, 2015**

ABSTRACT

Four cultivars of cowpea *viz.* EC4216, BL2, Kohinoor and Gomati were analyzed for their proximate composition, antinutritional factors, minerals and antioxidant profile. Protein, lipid, crude fiber and ash content of all the cultivars were in the range of 27.63-39.44, 4.09-5.50, 6.27-6.88 and 3.72-5.13 g/100 g respectively. The investigated cowpea cultivars were rich in minerals like potassium, sodium, magnesium, iron, zinc, chromium, copper and boron. Methanolic extracts of BL2 cowpea cultivar showed highest TPC, radical scavenging activity and reducing capacity whereas Kohinoor exhibited highest TFC and FRAP values. The content of phytate, tannin and activity of trypsin inhibitor showed significant variation among the selected cultivars of cowpea. Antioxidant activity of cowpea cultivar showed their potential as ingredient in the development of functional food therefore their cultivation and utilization should be encouraged.

Key Word: Proximate, Mineral profile, Antioxidant activity, Antinutrients.

INTRODUCTION

Cowpea or *lobia* (*Vigna unguiculata*) is an important food and forage legumes in the semi-arid tropics that includes parts of Asia, Africa, Southern Europe, Southern United States and Central and South America. (Timko and Singh, 2008). Cowpea is also an important pulse and legume crop cultivated in India. Area under cowpea cultivation in India is 3.9 million hectares with a production of 2.21 million tons with the national productivity of 683 kg per hectare (Singh *et al*., 2012). Many cultivars of cowpea are commercially grown for its long green pods as a vegetable, for its dry seed as pulse and for its foliage as fodder of animals (Singh *et al*., 2006).

The nutritional content of cowpea varies mainly because of genetic background as well as climate, fertilization, season and agronomic practices (Kochhar *et al.*, 1988). Despite of all such nutritional attributes, like other legume seeds, cowpea also contains certain antinutritional factors (ANFs) like phytic acid, trypsin inhibitor, haemagglutinin, hydrogen cyanide, saponin, oligosaccharide, tannins and phenolic compounds. (Owolabi *et al.,* 2012; Udensi *et al.,* 2007).These antinutrients form complexes with minerals and protein, rendering them less soluble or less susceptible to enzymatic degradation and less available for absorption in body (Towo *et al*. 2003). Amongst all ANFs, phenolic compounds have also been found to possess antioxidant properties which are mainly due to their redox properties by which they can neutralize free radicals (Ogunlade *et al.*, 2014).

Sufficient data for nutrients and antinutrients are available for African cowpea varieties. Scanty information is available for antioxidant properties of cowpea. Limited literatures are there on Indian cowpea cultivars, regarding nutrient and antinutrients composition as well as antioxidant profile. Therefore present study was conducted on four selected cowpea cultivars grown in India namely EC -4216, BL-2, Kohinoor and Gomati. The objective of the study was designed to evaluate the variability among cowpea cultivars in terms of nutritional, antinutritional and antioxidant profile.

MATERIAL AND METHODS

MATERIALS

Four cultivars of cowpea were procured for the study namely EC-4216, BL-2, Kohinoor and Gomati from Division of Seed Technology, Indian Grassland and Forest Research Institute, Jhansi, Uttar Pradesh, India. Samples were grounded finely for further analysis.

METHODS

NUTRITIONAL ANALYSIS OF COWPEA CULTIVARS

PROXIMATE ANALYSIS

Finely grounded samples of cowpea cultivars were analyzed for their proximate composition i.e.

Moisture, Protein, Fat, Total Ash and Crude fiber using standard AOAC (2005) methods. . Total carbohydrate was calculated by differential method.

MINERAL ESTIMATION

Mineral analysis in selected cowpea samples were determined by using inductively coupled argon plasma-atomic emission spectroscopy (ICAP-AES) after digesting with 50% hydrochloric acid and concentrated nitric acid.

ANTIOXIDANT ANALYSIS OF COWPEA CULTIVARS

TOTAL PHENOLIC CONTENT

Analysis of Total Phenolic Content (TPC) was determined by Folin Ciocalteau method as described in ISO 14502-1:2005 (E). 1ml of methanolic extracts of cowpea seeds were taken in a test tube and 5 ml of diluted Folin Ciocalteau reagent (1:10 with distilled water) was added. Thereafter 4 ml of Sodium Carbonate (7.5% w/v) was also added and mixed together. The tubes were kept in dark at room temperature for 60 minutes. The absorbance was measured in UV visible spectrophotometer (Thermo Scientific, model-Evolution600) at 765 nm against blank as standard. A standard curve was prepared with “Gallic acid” and results were expressed in terms of mg per 100g of polyphenol present in the sample.

RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of the extracts was measured using the DPPH (1, 1- diphenyl 2-picrylhydrazyl) method of Sanja et al.,(2009) with slight modification. 10 mg of cowpea seed flour was mixed with 10 ml acidified methanol and heated at 40° C in water bath for 20 minutes. 100 µl of sample extract thus prepared was in a test tube and diluted with 2.9 ml of pure methanol. Sample mix was mixed with 150 µl of DPPH solution (4.3 mg in 3.3 ml methanol) which also served as a control with same concentration. It was then incubated for 15 minutes in dark and the decrease in absorbance was measured at 515 nm with the help of UV visible spectrophotometer. The % radical scavenging activity was calculated using following formula:

Control Absorbance-Sample Absorbance/Control Absorbance × 100

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

200µl of methanolic extract of each cowpea seed flour sample was mixed with 1.3 ml of freshly prepared FRAP reagent and kept for incubation at 37° C for 30 minutes. Absorption of samples was measured at 595 nm using spectrophotometer. The absorbance changes in the test mixture were compared with standard mixture of heptahydrate ferrous sulphate (0.1 mM/L – 1.0 mM/L). FRAP values were expressed as mMol of Fe (II) equivalent/ g flour. (Sutharut and Sudarat, 2012).

REDUCING CAPACITY

Determination of reducing capacity was carried out as described by Sharma and Gujral (2011). Briefly, 2 ml of cowpea seed extract was mixed with phosphate buffer (2.5 ml pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of trichloroacetic acid (TCA) (10%) was added to the mixture and it was centrifuged at 10000 g for 10 min. Supernatant so collected was mixed with 2.5 ml deionized water and ferric chloride solution (0.5 ml. 0.1%, w/v) and the absorbance of the solution were measured at 700 nm. The measurement was compared to a standard curve of freshly prepared ascorbic acid solution, and final results were expressed as micromoles of ascorbic acid equivalents (AAE)/g of flour.

TOTAL FLAVONOID CONTENT

Ethanolic extracts (2 ml) was mixed with 150 µl of sodium nitrite (5%). After 5 minutes, 150µl of aluminium chloride (10%) was added. After 10 minutes interval 1 ml of 1M sodium hydroxide and 1.2 ml of distilled water were added in the mixture. The mixture was vortexed and after 10 minutes incubation absorbance was read at 510 nm by spectrophotometer. A calibration curve was prepared using a standard solution of quercetin (0.05-0.5 mg/ml). Final results were expressed as mg quercetin equivalents/g (QE) of sample. (Boateng et al., 2008).

ANTINUTRITIONAL ANALYSIS OF COWPEA CULTIVARS

TANNIN

Tannin content in cowpea was determined by Folin-Denis method as described by Sadasivum and Manickam (2005). Color intensity was measured at 700 nm after 30 minutes of incubation period. Standard graph was prepared by using 0-100 µg tannic acid. Tannin content of the samples was calculated as per cent (%) tannic acid from the standard graph.

PHYTATE

Phytate content is determined by colorimetric method as described in Sadasivam and Manickam (2005). 3% TCA was used for extracting the phytate and was precipitated as ferric phytate which was then converted into ferric hydroxide and soluble sodium phytate by adding sodium hydroxide in boiling condition. Hot nitric acid was added to it and solution was diluted. Colour of solution was developed using potassium thiocyanate and its intensity was read immediately at 480nm.The absorbance of iron content so determined was used for calculating phytate phosphorus content assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. Ferric Nitrate was used to make standard curve.

TRYPSIN INHIBITOR

Trypsin inhibitor (TI) content was determined according to the method of Kakade et al., (1974) as modified by Hammerstrand et al., (1981) using BAPNA (N-a-Benzoyl-DL-Arginine p- nitroanilide) as a substrate.

STATISTICAL ANALYSIS

All the determinations were carried out in triplicates and results were presented as mean along with their standard deviation (mean±SD). Statistical package of Social Sciences (SPSS), software programme for windows (version 16.0), was used for conducting one way analysis of Variance (ANOVA) and Duncan's procedure was used to compare means and for determining significant difference at 5% significance level (p<0.05).

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION

The results of proximate analysis of selected cowpea cultivars are presented in Table 1. Moisture content of cowpea cultivars ranged between 7.64-10.73 g/100 g. Protein content in all cultivars ranged between 27.63-39.44 g/100 g on dry weight basis (dwb). BL2 cultivar contained highest amount of protein followed by Kohinoor, EC4216 and Gomati. BL2 had shown significant varietal difference in comparison with other cultivars. These cultivars contain higher protein when compared with commonly consumed pulses in India like green gram, lentil, chickpea, cowpea (Ghavidel and Prakash, 2007) and some Nigerian cowpea varieties (Chinma et al., 2008 and Owolabi et al., 2012). However, BL2 cultivar contains high protein which is in accordance with protein content of 101 cowpea genotypes (Celestine *et al.,* 2013). This is suggests that this cultivar is richer source of protein than other cultivars. Fat content varied between 4.09-5.50 g/100 g (dwb). Gomati cultivar had the highest fat content but there was no significant difference among other cowpea cultivars. These values are closer to lipid content of five Nigerian cowpea varieties (Owolabi *et al.*, 2012). Total ash content ranged between 3.72-5.13 g/100 g (dwb) and its highest content was found in Gomati cultivar succeeded by Kohinoor, EC4216 and BL2 respectively. Data obtained for total ash were similar to values reported by Owolabi et al. (2012). Crude fiber content ranged between 6.27-6.88 g/100 g (dwb). EC4216 cultivar ranked highest for fiber while BL2 ranked last. The fairly high fiber content of cowpea cultivars make them nutritionally important from the dietary point of view because high dietary fiber intake was found inversely associated with coronary heart disease and long term daily intake of fiber decreases risk of all-cause mortality with increasing age (Streppel *et al.*, 2008). Carbohydrate was calculated by differential method using mean values of the proximate constituents and highest value was obtained for EC4216 cultivar (46.79 g/100g) and lowest in BL2 cultivar (38.55 g/100g).

Table 1 Proximate Composition of Cowpea Cultivars (dwb)

Cultivars	Moisture g/100g	Protein g/100g	Fat g/100g	Total Ash g/100g	Fiber g/100g	Carbohydrate* g/100g
EC-4216	10.5 ± 0.05^{b}	28.02 ± 0.91^{a}	4.09 ± 0.52^{a}	3.72 ± 0.26^{a}	6.88 ± 0.13^{a}	46.79
BL -2	7.64 ± 1.50^{a}	39.44 ± 0.53^{b}	4.14 ± 0.83^{a}	3.96 ± 0.31^{a}	6.27 ± 0.37^{a}	38.55
Kohinoor	10.73 ± 0.55^{b}	29.12 ± 2.24^{a}	4.48 ± 0^{a}	4.85 ± 2.12^{a}	6.30 ± 0.74^{a}	44.52
Gomati	9.24 ± 2.06^{ab}	27.63 ± 0.90^{a}	5.50 ± 0.95^{a}	5.13 ± 1.38^{a}	6.61 ± 0.11^{a}	45.89

[a] Means followed by same letter within a column do not differ significantly (p< 0.05).
Note: dwb- dry weight basis; * values are calculated using mean values of other proximate constituents

MINERAL COMPOSITION

The evaluation of minerals of four cowpea cultivars was done in the present study which is graphically exhibited as Macro Minerals and Micro Minerals (Figure 1 and 2).

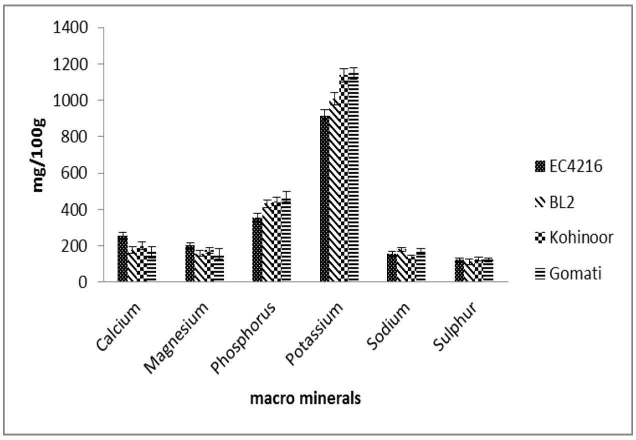

Figure 1 Macro Minerals Content in Cowpea Cultivars

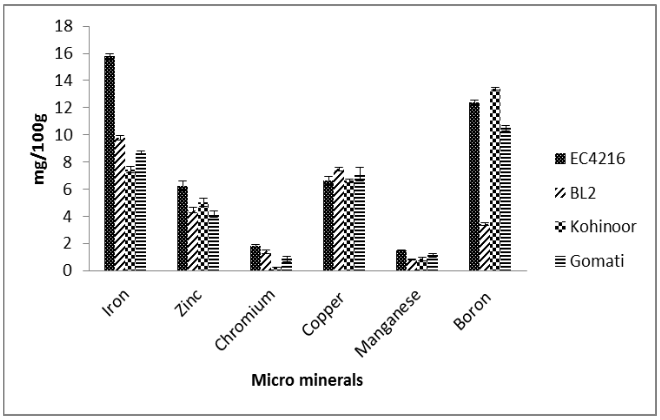

Figure 2 Micro Mineral Content of Cowpea Cultivars

MACRO MINERALS

Analysis of Macro Minerals like Calcium, Magnesium, Phosphorus, Potassium, Sodium and Sulphur revealed that Ca and Mg levels were observed maximum in EC4216 cultivar. P and K values were found utmost in

Gomati cultivar while Na and S levels were highest in BL2 and Kohinoor cultivar respectively. Ca levels in all the studied cultivars were lower than values of certain Nigerian cowpea cultivars. However, Mg levels in the present study were significantly higher when compared with Mg values of the same study (Chinma *et al.*, 2008). Phosphorus content of the studied cultivars ranged between 353.9-469.8 mg/100g and similar kind of results were obtained by Timorácká et al., (2011) on eleven varieties of white and red kidney beans. Ca, Mg and P are essential for maintenance of healthy bones and also act as catalyst in certain biochemical pathways (Satyanarayana 2002). K and Na values varied from 915-1148 mg/100g and 138.1-179.5 mg/100g respectively. K values of all cultivars of cowpea obtained in the present study were in agreement with Carvalho et al. (2012) who worked upon 30 Brazillian cowpea genotypes. Notwithstanding, Na content in present study was reported higher than those of Brazillian cowpea genotypes. Na and K are major body electrolytes and regulate salt and water balance (Gopalan, 2012). Sulphur content in the present cultivar ranged between 112.9-126.2 mg/100g which is comparatively a lower value with respect to S value of cowpea reported by Gopalan (2012).

MICRO MINERALS

Micro Minerals assessment of selected cowpea cultivar showed that Iron, Zinc, Chromium and Manganese were concentrated maximum in EC4216 cultivar while Copper and Boron content were higher in BL2 and Kohinoor cultivars. The iron content in the studied cowpea cultivars varied from7.41-15.78 mg/100g which was slightly higher than the values reported by Chinma et al., (2008). Zn, Cr and Cu concentrations in studied samples of cowpea cultivars ranged between 4.18-6.25, 0.26-1.81 and 6.61-7.48 mg/100g respectively. These reported values were higher than micro minerals values of pulses and legumes reported by Gopalan (2012). Nevertheless, Mn levels (0.82-1.5 mg/100g) in the samples were closer to the values reported by Gopalan (2012). Boron content was found between 3.42-13.36 mg/100g. Since B is an important micronutrient for plants, which is present in soil, is required for nitrogen fixation in legumes, bestowing a positive effect on growth and yield of legumes like cowpea (Subhasinghe et al., 2003) therefore its presence has been marked in the studied samples. All these micro minerals discussed above have essential role in our body functions because they act as cofactor, by their presence in certain enzymes and biomolecules and they help in certain metabolic cycles occurring in the body (Satyanarayana, 2002).

ANTIOXIDANT PROFILE OF COWPEA CULTIVARS

TOTAL PHENOLIC CONTENT (TPC)

Total phenolic compounds varied significantly in the analyzed samples of cowpea. The maximum and minimum total polyphenols levels were 187.33 mgGAE/100g (BL-2 cultivar) and 78.3 mgGAE/100g (Gomati cultivar) (Table 2). The TPC values were closer to the values reported by Noubissié et al., (2012) on dehulled cowpea. Other studies reviewed for TPC value of cowpea and other legumes are variable [14, 28-30] than present results. This may be due to certain influencing factors such as assay method and conditions (eg. type of extraction, solvent), type of standard used and type of legume sample used (variety, color , maturity).

RADICAL SCAVENGING ACTIVITY

The capacity of methanolic sample extract to scavenge DPPH radical can be expressed as its magnitude of antioxidation ability which is due to hydrogen donating atoms of sample extract or antioxidant compound into its non-radical form (DPPH-H). The observed change in the free radical scavenging ability of cowpea cultivars extracts is presented in Table 2. Radical scavenging activity percentage in the studied cowpea cultivars ranged between 67 to 88.9 %. Extract obtained from BL2 cultivar showed maximum percentage of radical scavenging ability whereas Gomati cultivar extract showed minimum activity when compared with other cultivars. Similar results were obtained by Noubissié et al., (2012) and Sharma et al., (2013) on antioxidant activity of certain indigenous chickpea cultivars.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ferric reducing antioxidant potential of cowpea cultivars is exhibited in Table 2. FRAP was recorded highest in case of EC4216 cultivar, followed by Kohinoor, BL2 and Gomati cultivars. These results were closer to chickpea FRAP values reported by Sharma et al., (2013) and also with coloured rice FRAP values reported by Sutharut and Sudarat (2012). However, the values reported by Zia-Ul-Haq et al., (2013) on Pakistani cowpea cultivars were higher than the present FRAP values and this difference may be due to varietal differences.

Table 2 Antioxidant Profile of Cowpea Cultivars

Cultivars	Total Phenolic Content (mg/100g)	Antiradical activity %	Ferric Reducing Antioxidant Power (mmol Fe (II) Eq/g	Reducing capacity (µmol AAE/g	Total Flavonoid Content (mg QE/g)
EC-4216	147.33 ± 2.49^{c}	87.5 ± 4.5^{c}	7.89 ± 0.62^{c}	98 ± 1^{b}	1.026 ± 0.04^{b}
BL -2	187.33 ± 3.29^{d}	88.9 ± 4.8^{c}	6.79 ± 0.36^{b}	105 ± 1^{c}	0.843 ± 0.012^{a}
Kohinoor	113.39 ± 2.39^{b}	79.7 ± 2.9^{b}	7.36 ± 0.32^{bc}	103 ± 2.08^{c}	1.160 ± 0.07^{c}
Gomati	78.3 ± 0.47^{a}	67.4 ± 1.92^{a}	5.09 ± 0.20^{a}	90 ± 1.73^{a}	1.053 ± 0.02^{b}

[a] Means followed by same letter within a column do not differ significantly ($p < 0.05$).

REDUCING CAPACITY

Reducing power is a sensitive method for the semi-quantitative determination of dilute concentrations of polyphenolics which participate in the redox reaction. Increased absorbance of the reaction mixture indicated increased reducing capacity of the sample extracts. The results of this research showed that the reducing capacity of BL2 variety was highest (105.0 µmol/AAE/g) while Gomati exhibited lowest (90.0µmol/AAE/g) reducing capacity (Table 2). The reported range of value of reducing capacity was higher when compared with reducing capacity of some indigenous chickpea cultivars. (Sharma et al., 2013). However, the present results were similar with certain Canadian wheat cultivars milling fractions (flour and semolina), which varied between 99-131 µmol/AAE/g of defatted material (Liyana-Pathirana and Shahidi, 2007).

TOTAL FLAVONOID CONTENT

The total flavonoid content (TFC) is the overall estimation of broad group of flavonoids which are considered as free radical acceptors. In the present research total flavonoid content (TFC) in selected cowpea seeds have been analyzed and it was found that Kohinoor cultivar of cowpea contains maximum TFC (1.16 mg QE/g) while BL2 contains minimum TFC (0.843 mg QE/g), as given in Table 2. Similar kind of TFC results were obtained from previous studies on raw pinto beans (Akilloglu and Karakaya 2010), raw chickpea cultivars (Tiznado et al., 2013) and raw soybeans and mung beans (Lee et al., 2011). These results are suggestive of the fact that cowpea seeds contains significant amount of certain flavonoids like quercetin and myricetin, as reported by Wang et al., (2006), which in present study are expressed as the total flavonoid content.

ANTINUTRITIONAL CONTENT OF COWPEA CULTIVARS

PHYTATE CONTENT

The phytate content of different cowpea cultivar is shown in Table 3. It varied from 650-1131.34 mg/100g (dwb). These values are closer to those reported by Lestienne et al., (2005) and Rehman and Shah (2005) on phytate content of various legumes. High range of phytate were observed among cowpea cultivars and the phytate content was significantly higher (p<0.05) in Gomati cultivar compared to the other studied cultivars. This suggests that phytate concentrations in the raw cowpea is high enough to form a strong complex with iron due to its chelating property and therefore can lower bioavailability of micro minerals (Towo et al., 2003). However, phytic acid could be useful as an antioxidant for preserving biological material because chelating capacity of phytic acid with metals like iron, zinc and calcium can help prevent oxidation of such samples (Graf et al., 1987)

Table 3 Antinutritional Content of Cowpea Cultivars

Cultivars	Phytate content (mg/100g)	Tannin content (%)	Trypsin Inhibitor Content (mg/g)
EC-4216	650.0±12[a]	0.36±0.015[a]	21.77±0.32[a]
BL-2	900.0±18[b]	0.78±0.043[d]	37.08±0.02[c]
Kohinoor	883.23±15[b]	0.50±0.015[b]	31.18±1.18[b]
Gomati	1131.34±19[c]	0.72±0.039[c]	32.51±4.10[bc]

[a] Means followed by same letter within a column do not differ significantly (p< 0.05).

TANNIN CONTENT

Table 3 summarizes tannin percentage in cowpea cultivars. Highest tannin content was found in BL-2 cultivar (0.78%) and lowest was in case of EC-4216 cultivar (0.36%). It must be noted that tannin content of BL-2 cultivar was also significantly higher than EC-4216 cultivar (p<0.05). The results are closer to raw tannin percentage of vegetable cowpea (Udensi *et al.*, 2007). Tannins are categorized as phenolic compounds which are produced as secondary metabolites. It also inhibits digestive enzymes thus lowering absorption of important nutrients especially protein and starch (Towo et al., 2003). The results suggest variability in tannin content amongst studied cowpea cultivars indicating high tannin presence can result in reduced bioavailability of protein. Being phenolic compound, tannins are soluble in water so it can be eliminated by various food processing methods.

TRYPSIN INHIBITOR CONTENT

Highest TIC was found in BL2 cultivar and least in EC4216. Table 3 presents data on Trypsin Inhibitor Content (TIC) of selected cowpea cultivars. TIC values were similar as reported by Kansal et al., (2008) and Kantha et al., (1986) on chickpea and winged beans respectively. Trypsin inhibitor is a type of protease inhibitor that reduces the biological activity of trypsin enzyme responsible for breakdown of different types of proteins during digestion resulting in diminished absorption of proteins.

CONCLUSIONS

The results of the present study reveal that four selected cultivars of cowpea (*Vigna unguiculata*) seeds grown in India are valuable sources of nutrients due to high protein, fiber and moderate carbohydrate with adequate quantity of macro and micro minerals. Certain minerals like potassium, sodium, magnesium, iron, zinc, chromium, copper and boron were found in appreciable amount in the present work and these have important role in metabolic processes of the body. Antioxidant analysis of cowpea cultivars revealed significant differences in total phenolic content, radical scavenging, FRAP, reducing capacity and total flavonoid content. BL2 cultivar was found to have highest antioxidant properties for most of the parameters indicating cowpea as a natural source of antioxidants against reactive oxygen species which are produced as a result of lipid peroxidation and various other mechanisms in body. A major limiting aspect of cowpea is the presence of antinutritional factors like phytate, tannins and trypsin inhibitor which need to be minimized through processing prior to consumption.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. G. K. Rai, Director, Institute of Professional Studies, University of Allahabad for providing necessary facilities for this research work. Special thanks to Central Testing Laboratory, Department of Soil Science, Punjab Agricultural University, Ludhiana for ICAP-AES testing of samples. Financial support was given by University Grants Commission is also duly acknowledged to carry out the study.

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