Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses

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Abstract Pulses are valued for their protein content and are good source of bioactive phytochemicals. The objectives of this study were to estimate total phenolic content (TPC), flavonoid content and total antioxidant capacity (TAC) through six different assay methods from twenty seven different varieties of pulses and split pulses commonly consumed in India. TPC and flavonoid content ranged between 38.6–542.7 mg GAE/100 g and 18.3–344.7 mg RE/100 g respectively. TAC measured by FRAP (90.6–2773.5 mg TE/100 g), RPA (190.8–2128.4 mg TE/100 g), DPPH radical scavenging assay (RSA) (42.9–571.1 mg TE/100 g), ABTS RSA (4.5–194.9 mg TE/100 g), hydroxyl RSA (0.0–106.6 mg AAE/g) and nitric oxide (NO) RSA (11.9–43.7 mg AAE/100 g) varied widely among pulses and split pulses analysed in the present study. TAC measured using different methods showed positively strong and significant correlation with TPC as well as flavonoid content except NORSA, indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants. Food composition data obtained in this study would enrich the composition database at global level, which in turn will give in improving food and nutrition security of the population. These can be used for further research, diet formulation and epidemiological studies. From the results

it can be concluded that cow pea (red, small) had the highest TPC and TAC measured by all the parameters except NORSA.

Keywords Pulses · Split pulses · Total antioxidant capacity · Total phenolic content

Introduction

Among all the edible seeds, pulses are the best as they are nutritionally rich in proteins, essential vitamins and minerals, dietary fibers, have low glycemic index and gluten free. Further they are also rich in various active phytochemicals, e.g., isoflavones, coumestrol, phytate, saponins, lecithin, phytosterols and vitamin E (Prakash et al. 2007). The major polyphenolic compounds of pulses consist mainly of tannins, phenolic acids and flavonoids. The most abundant phenolic acid present in pulses is ferulic acid while flavonol glycosides, anthocyanin and tannins are mainly responsible for the attractive color of the seed coat (Singh et al. 2016a). There are three different parts recognised in the pulse seeds: cotyledon, seed coat and embryonic axis, which signify, approximately, 89, 10 and 1%, respectively, of the total seed weight. The cotyledon basically contains proteins and carbohydrates which are the main reserve substances. The seed coat, which acts as a defending barrier for the cotyledon, has the chief concentration of phenolic compounds (Dueñas et al. 2003). Epidemiological studies have shown correlations between the consumption of legumes (and their isoflavones such as daidzein, genistein, from soybean), and decreasing incidence of several diseases such as cancer, aging, and cardiovascular diseases (Russo et al. 2006). Tannins, phytic acid, saponins, and other factors such as polyphenols in

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legumes have been assumed to prevent chronic diseases (Kabagambe et al. 2005). It is well known that the mechanisms by which these pathologies develop generally involve oxidative alteration of physiologically critical molecules, including proteins, lipids, carbohydrates, and nucleic acids, along with modulation of gene expression and the inflammatory response. These oxidative damages could be retarded by endogenous enzymatic (catalase, superoxide dismutase and glutathione peroxidase) defence systems but these systems are not completely efficient, and hence, exogenous antioxidants require.

Pulses also hold an important part in the diet and consumed as staple food along with cereals in various parts of the world. India remains comparatively the largest producer of pulses globally (about 25%). India's total output in 2013, put at 18.4 million tonnes, principal pulse crops were chickpea, beans and pigeon peas. Moreover, in December 2013, the general assembly of the United Nations declared 2016 as the "International Year of Pulses" in order to increase awareness regarding production, encourage new research and development, and ultimately to avail consumption of pulses globally (FAOSTAT 2014). Traditionally in India, pulses are consumed as whole or in split form (with or without husk). The split form locally known as *dal* is very popular because splitting as well as dehulling of pulses greatly reduces its cooking time and improves the appearance, texture, palatability, digestibility and bioavailability of nutrients of the grain (Sethi et al. 2014).

There are two reports on quantification of phenolic acids, flavonoids and total antioxidant capacity among pulses and split pulses commonly consumed in India (Sreeramulu et al. 2009; Marathe et al. 2011). However, none of the earlier studies compared total antioxidant capacity using six different assay methods and their correlation using principal component analysis. Looking to the importance, objective of the present work was to evaluate the total phenolic content, flavonoid content and total antioxidant capacity using six different assay methods in 27 different Indian pulses and split pulses and to explore the relationship between total phenolic content, flavonoid content and total antioxidant capacity using six different assay in the samples.

Materials and methods

Chemicals

2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (A 1888), L-Ascorbic Acid (95210), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (D 9132), Gallic acid (G 7384), *N*-(1-naphthyl) ethylenediamine dihydrochloride (N 9125), 1,10-Phenanthroline (320056),

Rutin hydrate (R 5143), Sulphanilamide (33626), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (T 1253) were of Sigma Aldrich brand. Sodium Nitroprusside (dihydrate) (71778) and 6-Hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid (Trolox) (56510) were of Fluka brand (Sigma Aldrich, Bangalore, India). Other chemicals used were of analytical grade.

Sample preparation

Total twenty seven pulses and split pulse samples (500 g) were purchased from three different local shops of Anand (Gujarat, India). They were pooled, sieved, cleaned to remove broken seeds, damaged seeds and foreign materials. Then they were ground to flour using Kitchen grinder and used for extraction.

Sample extraction

Two grams of pulse and split pulse flour samples were extracted thrice in 80% aqueous methanol (pH 2.0) by shaking at room temperature for 90 min. Supernatants were centrifuged, filtered and the volume of each of these samples was made up to 50 ml with the solvent. The extracts were stored at -20°C for further analysis. The samples were extracted in duplicate batches with two separate purchases in the same season and four observations of two different studies were analyzed statistically.

Total phenolic content (TPC)

The TPC in extracts was quantified using Folin–Ciocalteu method (Singleton et al. 1999). Gallic acid was used as a standard for comparison and the results are expressed in milligrams of Gallic Acid Equivalent per 100 g sample (mg GAE/100 g).

Flavonoid content

Concentrated methanolic extracts was used for the estimation of flavonoid and the absorbance was read at 510 nm (Zhishen et al. 1999). Different aliquots of known concentration of rutin were treated as standard and the results are expressed as mg of Rutin Equivalent per 100 g sample (mg RE/100 g).

Ferric reducing antioxidant power (FRAP)

Total antioxidant capacity using FRAP assay was determined according to Benzie and Strain (1999). Trolox (water-soluble vitamin E analogue) was used as standard for the comparison and results are expressed as mg Trolox Equivalent per 100 g sample (mg TE/100 g).

Reducing power assay (RPA)

RPA assay was carried out according the method described by Oyaizu (1986). Different aliquots of Trolox were treated as standard and results are expressed as mg TE/100 g sample.

DPPH radical scavenging activity (DPPHRSA)

The antioxidant activity of the methanolic extracts of pulses and split pulses, on the basis of the scavenging activity of the stable 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals, was determined according to the technique reported by Brand-Williams et al. (1995). Percentage inhibition was calculated and results are expressed as mg TE/100 g sample.

ABTS radical scavenging activity (ABTSRSA)

The ABTS [2,2'-Azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt] radical scavenging activity assay was carried out using the modified autobleaching method as suggested by Re et al. (1999). The percentage inhibition was calculated and the results are expressed as mg TE/100 g of sample.

Hydroxyl radical scavenging activity (HRSA)

The scavenging activity (HRSA) of the extracts of pulses and split pulses on hydroxyl radical was measured using the modified method (Jin et al. 1996). In this system, hydroxyl radicals were generated by the Fenton reaction. The reaction mixture without any antioxidant was used as the negative control, and without H_2O_2 was used as the blank. The percentage HRSA was calculated. Different concentrations of L-Ascorbic Acid were used as standard and the results are expressed in mg L-Ascorbic Acid Equivalent per 100 g of sample (mg AAE/100 g).

Nitric oxide radical scavenging activity (NORSA)

Nitric oxide generated from sodium nitroprusside was measured by the Greiss reaction at 540 nm (Marcocci et al. 1994). Percentage nitric oxide radical scavenging activity (NORSA) was calculated. Different concentrations of L-Ascorbic Acid were treated as standard. The results are expressed in mg L-Ascorbic Acid Equivalent per 100 g of sample (mg AAE/100 g).

Statistical analysis

The data were subjected to Analysis of variance (ANOVA) and the significance of mean differences was determined

by Duncans' post hoc test ($p \leq 0.05$) using SPSS version 20.0. The results are expressed as mean $\pm$ standard deviation (SD) of four observations. Principal component analysis (PCA) was also carried out by SPSS 20.0.

Results and discussion

Total phenolic content, flavonoid content and total antioxidant capacity (FRAP, RPA, DPPHRSA and ABTSRSA) of Indian pulses and split pulses are summarized in Table 1.

Total phenolic content

Different phenolic compounds in food matrix samples have different responses in the Folin-Ciocalteu method depending on the number of phenolic groups they have (Singleton et al. 1999). Various polyphenolic compounds have been reported to exhibit antioxidant activities because of the reactivity of the phenolic moiety, scavenging free radicals via electron donation or hydrogen donation (Jayaprakasha and Patil 2007). The mean values of total phenolic content equivalent to gallic acid of different pulses and split pulses extracts varied between 38.6 and 542.7 mg/100 g range. Cow pea (red, small) had significantly ($p \leq 0.05$) higher TPC while redgram dal had lower TPC compared to other studied pulses and split pulses. The analyzed pulses and split pulses showed following rank for TPC: Cow pea (red, small) > Kidney bean (red, small) > Kidney bean (beige-mottled) > Horse gram > -Soya bean > Lentil > Black gram > Black gram dal (with husk) > Kidney bean (maroon, big) > Green gram > -Green gram dal (with husk) > Chick pea (brown) > Lentil dal > Chick pea (green) > Peas (white) > Chick pea (cream) > Bengal gram dal > Moth bean > Red gram > Peas (green) > Black gram dal (without husk) > Field bean (brown) > Green gram dal (without husk) > Field bean (cream) > Field bean (white) > Cow pea (white, big) > Redgram dal. Split pulses (dal) contains comparatively lower amount of TPC than their whole counterpart. In the study of Sreeramulu et al. (2009), total phenolic content of pulses and legumes ($n = 11$) showed a wide range from 62 to 418 mg GAE/100 g, with highest content in black gram dal while green gram dal had the least content. In green gram dal (without husk), we found 55.2 mg GAE/100 g TPC whereas Sreeramulu et al. (2009) detected 62.4 mg GAE/100 g. According to a recent study (Sreeramulu et al. 2013), the values ranged from 41.3 to 284.3 mg GAE/100 g of TPC among pulses and legumes and reported highest value in green gram (whole) while lowest for green gram dal. Approximately similar results observed for kidney bean, black gram dal and green gram

Table 1 Total phenolic content, flavonoid content and total antioxidant capacity of common Indian pulses and split pulses

Common name	Total phenolic content (mg GAE/100 g)	Flavonoid content (mg RE/100 g)	Total antioxidant capacity (mg TE/100 g)			
			FRAP	RPA	DPPHRSa	ABTSRSa
Black gram	162.9 ± 3.4 ⁱ	116.1 ± 6.6 ^{gh}	448.5 ± 7.9 ^h	400.3 ± 26.7 ^{ijk}	107.1 ± 1.9 ^{ij}	29.4 ± 0.7 ^h
Chick pea, brown	94.1 ± 1.3 ^f	23.6 ± 4.3 ^{ab}	311.8 ± 25.8 ^{ef}	469.3 ± 41.5 ^l	82.9 ± 1.8 ^{efg}	15.7 ± 0.3 ^{ef}
Chick pea, green	83.5 ± 2.5 ^{def}	38.5 ± 1.8 ^{abc}	238.3 ± 5.9 ^{bcd}	447.4 ± 10.2 ^{kl}	72.9 ± 0.7 ^{de}	20.5 ± 0.8 ^{fg}
Chick pea, cream	81.8 ± 4.9 ^{cdef}	29.3 ± 5.9 ^{abc}	221.1 ± 13.6 ^{bcd}	289.5 ± 25.8 ^{def}	57.6 ± 6.4 ^{bc}	4.9 ± 0.2 ^{ab}
Cow pea, red, small	542.7 ± 28.5 ⁿ	344.7 ± 40.6 ^k	2773.5 ± 145.2 ⁿ	2128.4 ± 41.7 ^q	571.1 ± 21.4 ^q	194.9 ± 12.8 ⁿ
Cow pea, white, big	39.9 ± 2.2 ^a	24.0 ± 1.4 ^{ab}	235.9 ± 25.2 ^{bcd}	219.3 ± 12.5 ^{abc}	74.0 ± 5.7 ^{de}	6.6 ± 1.2 ^{abc}
Field bean, brown	62.4 ± 9.3 ^b	30.8 ± 9.5 ^{abc}	287.6 ± 50.5 ^{de}	296.1 ± 37.5 ^{defg}	87.1 ± 3.6 ^{efgh}	22.5 ± 4.4 ^g
Field bean, cream	42.0 ± 5.1 ^a	20.2 ± 3.3 ^a	360.9 ± 55.0 ^{fg}	190.8 ± 18.1 ^a	60.6 ± 15.2 ^c	6.1 ± 0.7 ^{ab}
Field bean, white	41.4 ± 2.8 ^a	18.3 ± 3.9 ^a	195.4 ± 32.5 ^b	234.7 ± 19.5 ^{abcd}	49.3 ± 2.2 ^{ab}	8.6 ± 1.1 ^{abcd}
Green gram	123.1 ± 10.9 ^g	45.9 ± 6.2 ^{cd}	209.4 ± 47.4 ^{bc}	326.8 ± 35.9 ^{efgh}	186.4 ± 4.9 ^{lm}	11.9 ± 0.6 ^{cde}
Horse gram	238.1 ± 4.2 ^{kl}	90.6 ± 3.2 ^f	821.9 ± 26.5 ^k	1289.6 ± 59.9 ^p	112.2 ± 1.0 ^j	156.3 ± 3.5 ^m
Kidney bean, beige-mottled	246.1 ± 7.9 ^l	217.6 ± 27.0 ⁱ	1085.9 ± 65.7 ^l	1162.4 ± 145.8 ^o	281.9 ± 7.4 ^o	82.9 ± 4.9 ^k
Kidney bean, red, small	335.9 ± 23.8 ^m	257.2 ± 17.7 ^j	1285.2 ± 71.5 ^m	1149.2 ± 68.3 ^o	311.7 ± 3.7 ^p	96.2 ± 6.1 ^l
Kidney bean, maroon, big	144.3 ± 5.5 ^h	133.4 ± 13.2 ^h	553.1 ± 65.7 ⁱ	594.4 ± 46.6 ^m	173.6 ± 6.5 ^k	39.1 ± 5.6 ⁱ
Lentil	183.5 ± 25.5 ^j	101.9 ± 7.2 ^{ef}	714.1 ± 29.9 ^j	695.2 ± 33.1 ⁿ	210.4 ± 6.4 ⁿ	60.3 ± 2.9 ^j
Moth bean	70.5 ± 5.1 ^{cde}	58.7 ± 7.2 ^{de}	407.9 ± 12.9 ^{gh}	241.3 ± 16.8 ^{abcd}	65.6 ± 1.9 ^{cd}	9.2 ± 1.0 ^{abcd}
Peas, green	65.9 ± 1.6 ^{bc}	26.5 ± 4.5 ^{abc}	215.7 ± 49.6 ^{bcd}	276.3 ± 15.2 ^{cdef}	81.9 ± 2.7 ^{ef}	5.1 ± 0.1 ^{ab}
Peas, white	82.2 ± 5.4 ^{cdef}	45.7 ± 3.9 ^{cd}	232.9 ± 37.6 ^{bcd}	436.5 ± 37.5 ^{ijkl}	89.3 ± 3.6 ^{efgh}	6.5 ± 0.3 ^{abc}
Red gram	68.5 ± 7.9 ^{bcd}	34.0 ± 2.2 ^{abc}	382.8 ± 18.7 ^{gh}	265.4 ± 22.8 ^{bcd}	93.7 ± 7.8 ^{gh}	29.3 ± 2.4 ^h
Soya bean	226.8 ± 5.9 ^k	37.5 ± 1.7 ^{abc}	412.5 ± 3.5 ^{gh}	325.8 ± 3.9 ^{efgh}	110.6 ± 13.3 ^j	11.9 ± 0.6 ^{cde}
Split pulses (<i>dal</i>)						
Bengal gram dal	81.1 ± 3.9 ^{cdef}	33.4 ± 2.1 ^{abc}	201.6 ± 10.9 ^b	337.8 ± 23.2 ^{fghi}	63.9 ± 1.4 ^{cd}	7.4 ± 0.6 ^{abcd}
Black gram dal, with husk	150.3 ± 11.2 ^{hi}	116.3 ± 16.9 ^{gh}	412.5 ± 25.5 ^{gh}	361.9 ± 16.6 ^{ghi}	97.8 ± 6.4 ^{hi}	30.5 ± 3.3 ^h
Black gram dal, without husk	65.8 ± 2.6 ^{bc}	40.9 ± 13.0 ^{bcd}	211.7 ± 18.5 ^{bc}	198.5 ± 14.0 ^{ab}	42.9 ± 3.6 ^a	6.4 ± 0.7 ^{abc}
Green gram dal, with husk	119.7 ± 11.1 ^g	37.5 ± 7.9 ^{abc}	278.2 ± 24.2 ^{cde}	377.2 ± 32.2 ^{hij}	191.3 ± 2.0 ^m	12.9 ± 0.5 ^{de}
Green gram dal, without husk	55.2 ± 8.8 ^{ab}	25.5 ± 3.2 ^{ab}	90.6 ± 6.7 ^a	337.8 ± 20.9 ^{fghi}	179.3 ± 4.9 ^{kl}	4.5 ± 0.2 ^a
Lentil dal	85.8 ± 10.0 ^{ef}	71.1 ± 7.5 ^e	174.3 ± 5.3 ^b	262.1 ± 51.9 ^{bcd}	112.9 ± 9.4 ^j	10.5 ± 0.5 ^{bcde}
Red gram dal	38.6 ± 4.6 ^a	23.6 ± 2.0 ^{ab}	218.8 ± 27.5 ^{bcd}	212.7 ± 4.4 ^{abc}	44.6 ± 1.4 ^a	12.1 ± 0.3 ^{cde}
Range	38.6–542.7	18.3–344.7	90.6–2773.5	190.8–2128.4	42.9–571.1	4.5–194.9

Values are mean ± SD; n = 4

GAE gallic acid equivalent, RE rutin equivalent, TE trolox equivalent

Columns with different letters are significantly different ($p \leq 0.05$) from each other

dal (without husk) (Sreeramulu et al. 2013) in present study. Marathe et al. (2011) also reported higher TPC (2.0–6.4 mg GAE/g) for cow pea (red and brown), soybean, common bean (black, red, brown, and beige), peanut, fenugreek and horse gram. They suggested that, the outer layers of seed i.e., seed coat usually contain a higher amount of polyphenolic compounds as expected from their

protective function in the plants. Most of the light color seed coat varieties (except soybean) having low phenolics, while not all legumes with dark color seed coat contained high amount of phenolics. They also observed that seed size could also significantly influence the TPC and hence the antioxidant activity. This may perhaps owing to the fact that the ratio of surface area (seed coat area)/g, of seeds

vary inversely with seed size. Thus, bigger seeds have lesser amount of seed coat and phenolics. In the present study, higher phenolic content observed in split pulses with husk than without husk. One of the previous studies observed high antioxidant activity mainly in the seed coat due to the essential fact that large amounts of phenolic compounds, mainly flavonoids, are located in this part of the seed (Nilsson et al. 2004) and further they can be also used as natural antioxidants.

Flavonoid content

Flavonoids are secondary metabolites in plants which make up the largest group among phenolics. The flavonoid content of pulses and split pulses extracts ranged from 18.3 to 344.7 mg RE/100 g. Cow pea (red, small) showed a significantly ($p \leq 0.05$) higher flavonoid content as observed in TPC where as lowest value was observed in field bean (white). In the present study, we found 23.5 mg RE/100 g in Chick pea (brown), 45.9 mg RE/100 g in green gram and 37.5 mg RE/100 g in Soya bean. Approximately similar values were also observed by Gujral et al. (2013) for the said samples. However, Duenas et al. (2006) showed that flavones, flavonols, and proanthocyanidins from methanolic extracts of the seed coat and cotyledon of lentils and dark peas contributed the most antioxidant capacity to the seed coat, whereas the major antioxidant contributor of the cotyledon was the flavonoid catechin.

Ferric reducing antioxidant power

Total antioxidant capacity can be evaluated from different plant foods by various assay methods. It is now getting more importance, since it has been found that phenolic compounds are one of the most effective non-nutrient antioxidants. The total antioxidant capacity estimation by FRAP assay measures the effect of antioxidant of any substance in the reaction medium as reducing ability of the complex Fe(III)-TPTZ (2,4,6-tris(2-pyridyl)-s-triazine). Ferric reducing antioxidant power of pulses and split pulses varied in the range of 90.6–2773.5 mg TE/100 g which are presented in Table 1. A significantly ($p \leq 0.05$) higher FRAP value was found in cow pea (red, small), while least value was in green gram dal (without husk) compared to other pulses and split pulses. Cow pea (red, small) > Kidney bean (red, small) > Kidney bean (beige-mottled) > Horse gram > Lentil > Kidney bean (maroon, big) > Black gram > Black gram dal (with husk) > Soya bean > Moth bean > Red gram > Field bean (cream) > Chick pea (brown) > Field bean (brown) > Green gram dal (with husk) > Chick pea (green) > Cow pea (white, big) > Peas (white) > Chick pea (cream) > Redgram dal > Peas (green) > Black gram dal (without

husk) > Green gram > Bengal gram dal > Field bean (white) > Lentil dal > Green gram dal (without husk) was the order of FRAP values among pulses and split pulses studied. The results showed appreciable amount of antioxidant activity of pulses and split pulses. Sreeramulu et al. (2013) and Sreeramulu et al. (2009) reported ferric reducing antioxidant power of pulses and legumes and observed highest value in rajma and the lowest in green gram dhal. Similarly, the highest FRAP value was also noticed for kidney bean next to cow pea (red, small) and least value in green gram dal (without husk) among all studied pulses and split pulses in the present study. The higher FRAP of cowpea and kidney bean is attributed to their higher flavonoids and phenolic acids content. The highest FRAP value of cow pea could be due to the presence of higher amount of flavonols (quercetin, myricetin and kaempferol) in red variety. Earlier study reported the higher flavonols content in red variety of cow pea compared to black, light brown and white variety. In this study we also found higher flavonoid content in cowpea red variety. The same explanation is also true for kidney bean. The third highest FRAP was found in horse gram which could be due to higher content of phenolic acids. A previous study reported higher amount of cinnamic acid derivatives (caffeic acid, chlorogenic acid, ferulic acid and p-coumaric acid) in cotyledon and embryonic axe compared to benzoic acid derivatives (gallic acid and protocatechuic acid) in phenolic acid group. They also reported comparatively less amount of flavonoids in horse gram. However, values differed from the reported value as in the present study it was expressed in mg Trolox equivalent.

Reducing power assay

RPA measures the reductive ability of antioxidant to transform Fe^{+3} to Fe^{+2} . The reducing properties are generally related with the presence of reductones (Duh 1998), which have been shown to exert antioxidant action by breaking the free radical chain by hydrogen-donating capability (Gordon and Galli 1990). All the pulses and split pulses studied showed reducing power assay values in the range of 190.8–2128.4 mg TE/100 g (Table 1). Compared to all pulses and split pulses, cow pea (red, small) had significantly ($p \leq 0.05$) higher RPA value where as lowest was found in field bean (cream). Only one Indian study reported (Sreeramulu et al. 2009) reducing power among commonly consumed pulses and legumes and observed a wide range between 77 and 489 mg AAE/100 g with lowest in black gram dal and highest in rajmah. Similar trend also observed in the present study but the values are differed as they used 60% acidic methanol as a solvent for extraction and expressed the results in Ascorbic acid equivalent.

DPPH radical scavenging activity

DPPHRSA of pulses and split pulses ranged between 42.9 and 571.1 mg TE/100 g (Table 1). Cow pea (red, small) showed significantly ($p \leq 0.05$) higher DPPHRSA, while the lowest was observed for black gram dal (without husk). Approximately similar values observed in the present study as obtained by Sreeramulu et al. (2013) for black gram dal (without husk) and redgram dal. In peas, we found 81.9 mg TE/100 g DPPHRSA where as Sreeramulu et al. (2009) detected 88 mg TE/100 g DPPHRSA which is in the agreement with the present study. Slightly higher values observed for the rest of the samples compared to other reported values for DPPHRSA among pulses and split pulses. Marathe et al. (2011) also studied DPPH free radical scavenging activity of different varieties of legumes.

ABTS radical scavenging activity

The antioxidant capacity of plant foods can be measured using autobleaching of a preformed solution of ABTS radical cation. It can be prepared using $K_2S_2O_8$ as an oxidant. The blue-green colour of aqueous ABTS solution is formed by the loss of an electron by the nitrogen atom of ABTS (2,2-azinobis(3-ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom). The ABTSRSA was found in the range of 4.5–194.9 mg TE/100 g (Table 1), with the highest in cow pea (red, small) and the lowest in green gram dal (without husk). According to Marathe et al. (2011), legumes with high TPC like, cowpea (red and brown), soybean, common beans (black, brown, beige and red), horse gram and fenugreek confirmed high antioxidant activity. Similarly, in the present study, highest ABTSRSA observed in the extracts which had higher TPC namely cow pea (red, small), horse gram and kidney bean.

Hydroxyl radical scavenging activity

The range for HRSA among pulses and split pulses was observed between 0.0 and 106.6 mg AAE/g and graphically presented in Fig. 1. A significantly ($p \leq 0.05$) highest HRSA was noticed in cow pea (red, small) whereas field bean (white) did not show any activity (for this analysis, from field bean (white) sample, we used 40 mg flour/ml concentration and did not show any inhibition of hydroxyl radicals). The results showed moderate level of HRSA in pulses and split pulses. Cow pea (red, small) had appreciable amount of antioxidant activity and thus excellent for free hydroxyl radical scavenging. Siddhuraju (2006) found that both raw and dry heated seed extracts of moth bean had the highest hydroxyl radical scavenging activity and

suggested that xylose and lysine maillard reaction products had dose dependant scavenging activity on hydroxyl radical which might be accredited to the pooled effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen (Yen and Hsieh 1995). HRSA of chickpea protein hydrolysate (CPH) using 1,10 phenanthroline reagent was reported by Li et al. (2008) and suggested that, OH radical is the most spontaneous free radical and can be formed from superoxide anion and hydrogen peroxide, in the presence of metal-ions, such as copper or iron. When a hydroxyl radical reacts with aromatic compounds, it can add on across a double bond, resulting in hydroxycyclohexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxy radical, or decompose to phenoxyl-type radicals by water elimination (Lee et al. 2004).

Nitric oxide radical scavenging activity

Nitric oxide is a vital chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes (Lata and Ahuja 2003). Nitric oxide is a free radical and has an unpaired electron. It can react rapidly in the intracellular environment to form nitrate, nitrite and s-nitrosothiols which acts as free radicals. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. Nitric oxide causes DNA damage via peroxynitrite. Diazotization takes place between nitrite and sulphanilamide, this diazotized product is coupled with naphthylene diamine to form chromophore, which is reduced by antioxidant when measured at 546 nm (Sreejayan and Rao 1997). Spectrophotometric assay using the Griess reagent is used extensively to quantify Nitric oxide radical scavenging activity (NORSA) in plant foods. The NORSA among pulses and split pulses ranged between 11.9 and 43.7 mg AAE/100 g (Fig. 2). Interestingly, a significant ($p \leq 0.05$) higher NORSA was observed in lentil while the activity was the least in horse gram. The results of the present study showed noticeable amount of free nitric oxide radical scavenging activity and thus these natural plant extracts or its products may counteract the ill-effect of nitric oxide in vivo.

Correlation between TPC, flavonoid content and total antioxidant capacity

Correlation between TPC versus flavonoid content and TAC measured using different methods was studied. A positively strong and significant ($p \leq 0.01$) correlation can be observed between TPC and TAC measured using different parameters such as flavonoid ($R^2 = 0.829$), FRAP ($R^2 = 0.882$), RPA ($R^2 = 0.855$), DPPHRSA

Fig. 1 Hydroxyl radical scavenging activity of common Indian pulses and split pulses ($n = 27$). Values are mean $\pm$ standard deviation of four observations. Bars with different letters are significantly different ($p \leq 0.05$) from each other

($R^2 = 0.794$), ABTSRSA ($R^2 = 0.780$) and HRSA ($R^2 = 0.963$) except NORSa among common Indian pulses and split pulses studied. This suggests that phenolics and flavonoid compounds mainly present in pulses may be responsible for their antioxidant activity. These results on correlation are in agreement with several reported studies. Pulses with the highest TPC exert the highest antioxidant

capacity as measured by FRAP, DPPH radical scavenging and ORAC (Yeo and Shahidi 2015) as well as ABTSRSA (Marathe et al. 2011). Moreover, in the previous study we also showed a strong and positive relationship between TPC, flavonoid content and TAC measured using different methods (except NORSa) for rayan fruit and seed (Parikh and Patel 2016). The distinct association between the

Fig. 2 Nitric oxide radical scavenging activity of common Indian pulses and split pulses ($n = 27$). Values are mean $\pm$ standard deviation of four observations. Bars with different letters are significantly different ($p \leq 0.05$) from each other

bioactive compounds and antioxidant activity might be related to the presence of various active compounds in the plant, which gave diverse trends of antioxidant activity. Synergistic effects of the different compounds, experimental conditions and mechanisms of antioxidant reactions method used may affect this association. Besides, most of the methods have their own limitations in the determination of TAC (Jayaprakasha and Patil 2007).

Principal component analysis

Principal component analysis (PCA) was also applied to the obtained data to determine the interrelationships among different parameters studied of Indian pulses and split pulses as well as similarities and differences among samples on the basis of the analyzed parameters. Pulses contain several types of antioxidants and in varying proportions. The effects of antioxidants are not necessarily additive, given the fact that they most likely use different reaction mechanisms with respect to the eight different methods used. The results of the PCA showed that two of the eight principal component axes had eigenvalues over one. The eigenvalues of the correlation matrix for PC 1 and PC 2 were 6.405 and 1.072 respectively. The principal axis 1 (PC 1) accounted 80% of the variance where as principal axis 2 (PC 2) for 13.4% and altogether accounted for 93.4% of the total variance of the data matrix. The PCA produced principal component score plot revealed that all the variables were positively associated and also showed good correlations between them (except NORSA). They were found to be similarly loaded on PC1 with the exception of NORSA, which imply that phenolic compounds are mainly responsible for the antioxidant activity of pulses and split pulses. A recent study also carried out PCA and observed good correlation amongst TPC, flavonoid content, DPPH and ABTS antioxidant activity among studied fruits and vegetables (Singh et al. 2016b). Beside PC2, NORSA get high loading while TPC had low loading, which also indicated that phenolic compounds might not be good nitric oxide radical scavenger. Such correlations among TPC, flavonoid content and TAC measured using different parameters are similar to the conclusion illustrated from the correlation analysis.

PCA loading plot showing similarities and differences among Indian pulses and split pulses on the basis of the analyzed parameters. Most of the sample sites located near the origin represents the mean antioxidant activity of all the samples. Black gram, kidney bean (red, small), kidney bean (maroon, big) and lentil formed a distinct group on the positive side of the score plot while chick pea (green), chick pea (cream), bengal gram dal, field bean (cream) formed a cluster on negative side of the score plot. The results were compatible with the several reported studies,

where the coloured pulses have better phenolic concentrations; cow pea (red, small) was much more marked than the white one. Furthermore, black gram dal (with husk) plotted on the positive side of the score plot where as black gram dal (without husk) located on the opposite side; it may be due to the fact that outer layer of the seed (husk) contains the phenolic compounds mainly flavonoids. PCA showed that lentil and horse gram located at the opposite side of the PC2, the NORSA might be the major responsible for the difference between them. This was because lentil exhibited the highest NORSA while horse gram had the lowest. As concerned to PC1, several samples were located at the right of the plot while some on the left of the score plot. Suggesting that cow pea (red, small) had the largest positive score while field bean (white) had the largest negative score, which also highlighted their significant differences in antioxidant activity.

Conclusion

The current study determined TPC, flavonoid content and TAC using different methods of common Indian pulses and split pulses. Results indicated that phenolic compounds are the major contributors to the antioxidant properties of pulses and split pulses. Incorporation of pulses in daily diet can be beneficial for health and used as a functional food for the prevention of non communicable diseases (NCDs). Food composition data obtained in this study would enrich the composition database at global level, which in turn will guide in improving food and nutrition security of the population. These can be used for further research, diet formulation and epidemiological studies. From the results it can be concluded that cow pea (red, small) had the highest total phenolic and flavonoid content as well as total antioxidant capacity measured by all the parameters except NORSA.

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