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Biochemical and nutritional characterization of chickpea (*Cicer arietinum*) cultivars

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ABSTRACT

Thirty nationally released chickpea cultivars were evaluated for carbohydrate composition, soluble proteins, total lipids and anti-nutritional factors such as phenolic compounds, tannins, bound fructose of sucrose and raffinose family oligosaccharides, phytic acid, saponins, trypsin inhibitors and activities of enzymes related to them such as acid and alkaline phosphatases and α-galactosidase. Phytic acid showed a lot of diversity between cultivars and varied from 4.74-20.40 mg/g. Avrodhi, BG 256 and Virat were found to be nutritionally important as they had higher protein content (241.5 mg/g–261.5 mg/g) and starch content was found to be between 412.4 mg/g–485.5 mg/g. Avrodhi and BG 256 had lower content of tannin, phytic acid, saponin and trypsin inhibitors and the content of bound fructose of raffinose family oligosaccharides was found to be minimum in Avrodhi. Virat had the highest protein content among kabuli cultivars and it had lower amount of total phenols, flavonols, tannins and phytic acid. HC 1, BG 1053, Pant G 186 and PBG 1 had protein content between 200.0 mg/g–211.5 mg/g and had higher content of tannin, saponin, phytic acid and total phenols. Five cultivars namely HC 3, Vishal, ICCV 10, JG 315 and Saki 9516 had most of the anti-nutritional factors in medium content. Bound fructose of raffinose family oligosaccharides in kabuli cultivars were found to be in the range of 8.31-10.06 mg/g whereas in desi a lot of variation was observed and it ranged from 5.53 mg/g to 10.13 mg/g. All the cultivars were found to cluster in major four groups on the basis of principal component analysis. The result showed the diversity between nutritional and antinutritional factors in the cultivars that could be further used by plant breeders to develop superior genotypes.

Key words: Antinutritional, *Cicer arietinum*, Nutritional , Pulses, Quality

Legumes are one of the most nutritious plant food but the presence of antinutritional factors limit their biological value and acceptance as food (Aguilera *et al.* 2009). Protein quality of leguminous seeds does not reach the same level as in animal products because of presence of antinutritional traits, i.e. phytic acid, saponins, raffinose family oligosaccharides (RFOs), trypsin inhibitors, phenolic compounds and tannins in the seeds. These antinutritional factors are toxic or indigestible when ingested regularly in large amounts over a long period of time (Wang *et al*. 2003, Jain *et al*. 2009).

Raffinose family oligosaccharides are indigestible and cause flatulence as α-galactosidase enzyme responsible for their digestion is absent in human intestine (Wang *et al.* 2003). Protease inhibitors and tannins inhibit the digestibility of protein and starch, whereas, phytic acid impairs the dietary mineral absorption and utilization by chelating trace and macro elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract (Adegunwa *et al.* 2012, Khandelwal *et al*. 2010, Gebrelibanos *et al.* 2013). Saponins cause lysis of red blood cells and have harmful effects on the intestinal mucosa. Saponins also reduce the absorption of nutrients either directly by binding with or by inactivating enzymes involved in the digestion process and cause systemic toxicity (Alexander *et al.* 2009). Saponins have also been found to lower cholesterol, prevent cancer and advantageous in many forms, hence becoming health promoting parameter also (Vasistha and Srivastava 2011).

Pulses are consumed after cooking, soaking and heating. Different processing methods improve the nutritional quality of food legumes and grains to various extents (Rehman and Shah 2005, Vasudeva and Vishwanathan 2010). However there are different reports about the effect of cooking on quality of pulses. Although heat treatment significantly improve protein quality in pulses by destruction or inactivation of heat labile antinutritional factors but chemical composition of pulses and its nutritive value is also affected by cooking as it markedly decreased the level of some essential amino acids (Wang *et al.* 2009, 2010). Long soaking times apart from being inconvenient to consumers also

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produces potentially harmful microbial proliferation (Zamindar *et al.* 2011). Cooking results significant reduction in phytic acid, tannin and raffinose family oligosaccharides in pulses (Wang *et al.* 2008). Mohamed *et al.* (2011) reported that fermentation, germination in combination with dehulling and cooking caused decrease in phytic acid content. However there are conflicting reports about the processing methods on the content of phytic acid. It was reported that the content of phytic acid was found to be resistant to different processing methods in kidney beans (Yasmin *et al.* 2008). Cooking had no effect on phytic acid content in beans and chickpea (Wang *et al.* 2010). Trypsin inhibitor elimination would reduce the overall level of sulphur containing amino acids. So, from the nutritional point of view the development of low trypsin inhibitor mutants or identification of cultivars having low trypsin inhibitor is very good approach (Bansal *et al.* 2011).

Currently, the focus is on developing high yielding varieties to fill the gap between demand and supply of food legumes. The efforts put by plant breeders in developing high yielding varieties of legumes will be of little significance if it does not fit in with the consumer preference and there is need to identify the quality variations in the cultivars (Suneja *et al.* 2011; Celestine *et al* 2012). Grain legume germplasms constitute 15% of 7.4 million accession preserved globally. But the primary focus has been on morphoagronomic traits and therefore limited data on grain quality are available (Upadhyaya *et al.* 2011). As nutritive quality of legumes depends both on nutritional and antinutritional factors, so there arises a need to evaluate legumes for nutritional and antinutritional factors so as to identify the genotypes with high nutritional and low antinutritional factors to involve in breeding programme to develop superior genotypes as well as promoting their consumption. Therefore in present study, an attempt has been made to evaluate chickpea cultivars recommended at national level for their nutritional and antinutritional factors as the information on quality aspects is lacking for these cultivars.

MATERIALS AND METHODS

The present investigation was carried out on 30 chickpea (25 desi and 5 kabuli) cultivars obtained from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The seeds of chickpea were crushed to fine powder with mortar and pestle and the contents were passed through 80µm sieve to have uniform powder which was stored for extraction and assay of various components in triplicates.

Total soluble sugars were extracted from 200 mg crushed powder by crushing with 80% ethanol followed by 70% ethanol (Kaur *et al.* 2000). From the pooled extract, total soluble sugars were estimated (Dubois *et al.* 1956). Total bound fructose was determined after destroying the free fructose with 30% NaOH by resorcinol HCl procedure. The sugar free residue was dried at 60°C and used for the estimation of starch (Kaur *et al.* 2000). Total proteins were extracted from powdered seeds with 0.1 N NaOH and estimated by the method of Lowry *et al.* (1951). Total lipids were extracted from chickpea seed powder with chloroform: methanol (2:1) mixture for six hours at 4°C followed by extraction again with chloroform : methanol mixture. The contents after filtering through G-1 sintered glass funnel were put in separatory funnel, 1/5th volume of 0.9% saline solution was added to it. After shaking vigorously, lower layer of chloroform containing lipids was collected followed by two washings with chloroform, and evaporated on a flash evaporator. Lipids were redissolved in chloroform and their known volume was dried at 50°C to determine the total lipid content.

Antinutritional factors such as phenolic compounds, tannins, bound fructose of raffinose family oligosaccharides, saponins, phytic acid and trypsin inhibitors were extracted and estimated as described previously (Suneja *et al.* 2011). Phenolic compounds were extracted by refluxing seed powder with 80% aqueous methanol. The refluxed material after filtration was used to estimate total phenols, o-dihydroxyphenols and flavonol. Tannins were estimated using Folin-Denis reagent along with tannic acid (10-100 µg) as standard, the intensity of colour was measured at 700 nm. The total bound fructose of sucrose and raffinose series oligosaccharides was determined after destroying free fructose with 30% NaOH by resorcinol–HCl procedure. Saponins were extracted with acetone and later with methanol from the 500 mg seed powder and estimated. The phytic acid was extracted from the 100 mg powered seeds with 1.2% HCl and precipitated with ferric chloride and organic phosphorous was estimated. Trypsin inhibitor was extracted from powdered seeds (100 mg) with 0.1 M phosphate buffer (pH 7.5) containing 0.1 M NaCl and estimated by assaying the bovine trypsin inhibition by using N a-benzoyl-DL-arginine p-nitroanilide (BAPNA) as a substrate. One inhibitor unit is defined as the quantity of inhibitor which inhibits 50% of trypsin activity.

100 mg of chickpea seed powder was extracted with 3 ml of ice cold 0.1M sodium acetate buffer (pH 5.0) for acid phosphatase and α-galactosidase and 0.1M Tris HCl buffer (pH 8.0) for alkaline phosphatase and centrifuged at 10 000 × g for 15 minutes at 4°C. Acid and alkaline phosphatase enzymes were assayed by using 2.2 nM p-nitrophenyl phosphate as a substrate while that of α-galactosidase was determined by using 12.5mM p-nitrophenyl-α-D-galactopyranoside (p-NPG) as a substrate in 0.05M sodium acetate buffer (pH 5.0). After required incubation for 30 minutes at 37°C, the reaction was terminated by adding 0.2 N NaOH. The intensity of yellow colour (developed due to the liberation of p-nitrophenol) was measured at 420 nm. A standard curve of p-nitrophenol (0-02-0.08 µmole) was prepared simultaneously to calculate the amount of p-nitrophenol formed during enzyme reaction (Suneja *et al.* 2011). Means and standard deviations were calculated for various nutritional and antinutritional factors. Factorial CRD was used to determine significant differences among cultivars and parameters and to find interaction of cultivars

Table 1 Distribution of total sugars (mg/g), starch (mg/g), protein (mg/g) and lipids (%) in chickpea cultivars

Cultivars	Total sugars	Starch	Total proteins	Total lipids
Avrodhi	61.8 ±1.4	412.4 ± 24.9	261.5 ± 5.5	3.2
BG 256	62.5 ± 7.6	447.0 ± 25.8	241.5 ± 8.5	4.2
BG 372	65.1 ± 3.5	395.8 ± 12.5	232.5 ± 15.5	2.4
DCP 923	63.3 ± 1.7	403.1 ± 17.0	270.5 ± 4.5	3.2
Digvijay	72.0 ± 6.5	420.8 ± 37.5	251.5 ± 10.5	4
Dahood yellow	60.4 ± 1.8	350.5 ± 11.9	233.5 ± 19.5	2.8
GNG 469	59.7 ± 3.7	471.3 ± 18.2	255.0 ± 6.0	3.8
HC 1	66.9 ± 1.2	441.1 ± 11.9	211.5 ± 13.5	2.4
HC 3	73.0 ± 1.6	474.9 ± 9.2	233.0 ± 7.0	2.9
HC 5	71.0 ± 2.7	392.7 ± 11.4	255.5 ± 7.5	3.1
ICCV 10	69.7 ± 1.7	427.1 ± 13.5	241.5 ± 11.5	4.1
JG 11	68.3 ± 4.1	420.3 ± 14.0	242.5 ± 8.5	3.2
JG 74	67.9 ± 1.5	406.7 ± 9.7	237.5 ± 5.5	2.5
JG 315	70.3 ± 7.4	386.9 ± 8.9	233.0 ± 8.0	3.1
KWR 108	73.6 ± 1.6	423.4 ± 12.6	213.0 ± 10.0	3.4
Pant G 186	59.9 ± 1.5	394.3 ± 22.3	203.5 ± 9.5	3.1
Radhay	69.7 ± 2.3	448.4 ± 10.6	169.0 ± 6.0	3.9
RSG 888	64.3 ± 5.2	409.8 ± 13.0	188.0 ± 13.0	2.9
RSG 931	68.5 ± 1.9	424.5 ± 34.8	202.0 ± 8.0	1.8
Saki 9516	71.9 ± 2.8	469.3 ± 35.9	206.5 ± 5.5	4.5
Vijay	66.3 ± 1.6	412.5 ± 13.5	265.0 ± 26.0	4
Vishal	66.9 ± 2.8	474.4 ± 28.6	249.5 ± 8.5	4.7
PBG 1	69.3 ± 1.4	316.1 ± 19.8	201.5 ± 10.5	1.7
GPF 2	54.3 ± 1.8	375.5 ± 17.8	195.0 ± 7.0	6.6
PDG 3	61.7 ± 1.6	330.2 ± 33.3	183.5 ± 08.5	3
ICCV 2	72.9 ± 1.4	433.3 ± 12.1	289.5 ± 6.5	2.7
JKG 1	72.9 ± 1.9	418.7 ± 10.0	193.5 ± 12.5	3.4
L 550	63.2 ± 1.8	464.5 ± 19.4	189.0 ± 21.0	3.9
Virat	71.7 ± 2.5	485.5 ± 23.9	242.5 ± 17.5	4.3
BG 1053	49.5 ± 1.6	415.6 ± 10.4	200.0 ± 10.0	4.5
Mean	66.283	418.22	226.383	3.443

Values are mean±SD of triplicate samples estimated in triplicates

with parameters. Multivariate Principal Component Analysis was also applied to group cultivars into separate clusters and to find out those antinutritional factors which shared a contribution towards the variation observed in the content of antinutritional factors in chickpea seeds.

RESULTS AND DISCUSSION

Nutritional composition of chickpea seeds

Total sugars were found in the range of 49.5 - 73.6 mg/g seeds with mean value of 66.28 mg/g (Table 1). The carbohydrates of legumes are source of energy (Panhwar 2005). The average starch content was found to be 413.60 mg/g and 443.52 mg/g in desi (S.No. 1-25) and kabuli (S.No. 26-30) cultivars respectively. Starch content was found to be more in kabuli as compared to desi cultivars. Wang *et al*. (2010) also observed similar results in chickpea. Our results are in consistent with those found by Trichopoulou and Georga (2004) who reported that chickpea seeds contain about 438 mg/g starch content. Ozer *et al.* (2010) reported 45.34% starch in chickpea genotypes.

Pulses offer a relatively cheaper source of protein than milk, cheese, cashew, almond, meat and fish (Jain *et al.* 2009). Total soluble protein content varied from 169.0 to 289.5 mg/g seeds with mean value of protein content was found to be 226.38 mg/g. All the cultivars had protein content greater than 200 mg/g except for seven cultivars comprising four desi (Radhay, RSG 888, GPF 2 and PDG 3) and three kabuli cultivars (JKG 1, L 550 and BG 1053) which had protein content less than 200 mg/g. Chickpea seeds, on an average contains 230 mg/g proteins (ICRISAT 2007). Legume seeds contain 200-250 mg/g protein which is 2-3 times higher than that of cereals (Khalil *et al.* 2006). Pulses are primary source for nutritional and functional proteins and provide well balanced essential amino acid profile when blended with cereal proteins and other foods rich in sulphur containing amino acid and tryptophan (Boye *et al*. 2010).

Lipid content in chickpea seeds were found to be 1.7 – 6.6% in desi and 2.7-4.5% in kabuli cultivars (Table 1). The lowest lipid content was found to be in PBG 1 and RSG 931 (1.7-1.8%) and the highest in GPF 2 (6.6%). Ozer *et al.* (2010) reported that chickpea seeds contain 5.14% lipids.

Antinutritional composition of chickpea seeds

Although chickpea seeds are of high nutritional value as they are good source of carbohydrates and proteins but presence of antinutritional factors (ANFs) limit their nutritional value . Eight antinutritional factors namely total phenols, flavonols, o-dihydroxyphenols, tannins, raffinose family oligosaccharides, saponins, phytic acid and trypsin inhibitors and related enzymes were estimated in 30 chickpea cultivars (Table 2).Total average phenol content was found to be lower in kabuli (1.33 mg/g seeds) than desi cultivars (1.67 mg/g). Desi cultivars showed a lot of variation in total phenol content as DCP 923 had higher total phenol content (2.26 mg/g) and JG 315 had the lowest content (0.93 mg/g). Total phenols in chickpea were reported to be present in the range of 1.0 – 6.0 mg/g (Horn-Ross *et al.* 2000). The average content of flavonols in chickpea seeds were found to be 1.00 mg/g in desi and 0.43 mg/g in kabuli cultivars. All the kabuli cultivars were found to have lower amount of flavonols as compared to desi cultivars. Mean value of o-dihydroxyphenols was found to be 0.208 mg/g in desi and 0.228 mg/g in kabuli cultivars. Phenolic compounds have been reported to lower the activity of digestive enzymes such as amylase, trypsin and chymotrypsin and could also damage the mucosa of digestive tract (Ramakrishna *et al.* 2006).

The average content of tannins was found to be 7.66 mg/g seeds in all the test cultivars. In desi cultivars the average content was 7.92 mg/g and in kabuli cultivars it was 6.36 mg/g seeds (Table 2). All the kabuli cultivars had lower tannin content as compared to desi cultivars. Two desi cultivars namely Avrodhi and Vishal were found to have the lowest tannin content. Higher tannin content was reported in desi chickpea than kabuli chickpea seeds (Maheri-Sis *et al.* 2008). The antinutritional and toxic effects

Table 2 Distribution of phenolic components (mg/g) bound fructose of raffinose series oligosaccharides (mg/g), saponins (mg/g), phytic acid (mg/g) and trypsin inhibitor (IU/g) in chickpea cultivars

Cultivars	Total phenols	Flavonols	o-dihydroxy phenol	Tannins	Bound fructose of raffinose series oligosaccharides	Saponins	Phytic acid	Trypsin inhibitor
Avrodhi	1.90 ± 0.14	0.76 ± 0.07	0.20 ± 0.04	5.73 ± 0.62	6.56 ± 0.12	14.74 ± 2.9	7.67 ± 0.52	264.6 ± 64.4
BG 256	1.81 ± 0.12	0.62 ± 0.04	0.18 ± 0.02	7.10 ± 0.11	9.27 ± 0.36	13.14 ± 1.9	11.84 ± 1.03	230.2 ± 11.8
BG 372	2.04 ± 0.15	0.89 ± 0.09	0.20 ± 0.03	7.46 ± 0.14	7.25 ± 0.34	18.42 ± 2.1	7.16 ± 0.55	271.6 ± 9.1
DCP 923	2.26 ± 0.12	0.98 ± 0.04	0.22 ± 0.03	7.75 ± 0.25	7.02 ± 0.38	18.64 ± 3.4	15.86 ± 0.39	297.7 ± 2.1
Digvijay	1.86 ± 0.13	0.74 ± 0.11	0.18 ± 0.02	7.72 ± 0.25	8.89 ± 0.57	21.28 ± 2.3	17.29 ± 1.19	297.3 ± 7.6
Dahood Yellow	2.12 ± 0.23	0.96 ± 0.04	0.22 ± 0.04	8.22 ± 0.46	7.09 ± 0.92	9.29 ± 1.1	18.10 ± 0.80	311.7 ± 2.3
GNG 469	1.84 ± 0.18	0.53 ± 0.07	0.22 ± 0.03	6.53 ± 0.43	6.15 ± 0.34	10.23 ± 1.4	16.30 ± 0.65	291.7 ± 13.4
HC 1	2.31 ± 0.16	1.63 ± O.30	0.30 ± 0.03	8.91 ± 0.28	7.41 ± 0.19	16.39 ± 2.1	20.40 ± 0.96	280.8 ± 4.2
HC 3	1.84 ± 0.13	0.81 ± 0.13	0.16 ± 0.02	6.90 ± 0.41	7.11 ± 0.36	18.26 ± 3.2	11.58 ± 0.80	259.5 ± 9.7
HC 5	1.52 ± 0.15	0.76 ± 0.07	0.26 ± 0.06	8.33 ± 0.72	6.03 ± 0.70	30.58 ± 4.4	11.73 ± 0.92	289.1 ± 8.3
ICCV 10	1.41 ± 0.09	1.01 ± 0.23	0.22 ± 0.02	8.11 ± 0.79	8.05 ± 0.29	18.75 ± 2.0	18.42 ± 0.66	285.6 ± 7.4
JG 11	0.94 ± 0.05	0.69 ± 0.02	0.21 ± 0.04	6.41 ± 1.06	9.31 ± 0.49	14.52 ± 2.1	14.14 ± 1.26	292.9 ± 11.8
JG 74	2.14 ± 0.11	0.86 ± 0.07	0.20 ± 0.02	9.13 ± 0.86	6.18 ± 0.58	12.04 ± 2.9	11.60 ± 1.63	302.2 ± 2.9
JG 315	0.93 ± 0.05	0.90 ± 0.02	0.29 ± 0.01	8.95 ± 0.87	6.99 ± 0.26	15.45 ±2.5	15.05 ± 0.92	283.1 ± 3.4
KWR 108	1.33 ± 0.04	1.01 ± 0.16	0.26 ± 0.04	7.95 ± 0.48	7.54 ± 0.28	14.63 ± 1.4	17.40 ± 0.66	277.1 ± 4.7
Pant G 186	2.20 ± 0.07	1.66 ± 0.06	0.23 ± 0.04	10.01 ± 1.69	5.53 ± 0.11	18.04 ± 1.3	8.09 ± 0.56	301.8 ± 3.5
Radhay	1.69 ± 0.12	0.79 ± 0.14	0.13 ± 0.02	8.06 ± 1.11	9.31 ± 0.55	14.19 ± 2.9	9.79 ± 1.82	298.1 ± 6.4
RSG 888	1.75 ± 0.09	0.77 ± 0.02	0.12 ± 0.01	8.66 ± 0.32	7.76 ± 0.55	13.03 ± 1.1	16.51 ± 0.65	300.0 ± 3.3
RSG 931	1.80 ± 0.10	0.94 ± 0.03	0.17 ± 0.02	8.99 ± 0.51	5.58 ± 0.94	17.65 ± 0.9	6.82 ± 0.92	295.9 ± 1.5
Saki 9516	1.14 ± 0.04	0.86 ± 0.05	0.18 ± 0.04	7.99 ± 0.65	7.91 ± 0.31	26.29 ± 2.7	5.60 ± 0.95	301.2 ± 6.5
Vijay	1.83 ± 0.14	1.54 ± 0.08	0.12 ± 0.01	6.67 ± 0.95	10.13 ± 0.19	26.02 ± 2.4	5.89 ± 0.89	296.0 ± 2.3
Vishal	1.26 ± 0,09	0.82 ± 0.10	0.14 ± 0.01	5.59 ± 0.90	7.38 ± 0.22	26.45 ± 2.5	9.12 ± 0.47	294.3 ± 2.3
PBG 1	1.45 ± 0.12	1.78 ± 0.15	0.29 ± 0.04	9.63 ± 0.51	6.15 ± 0.18	28.49 ± 4.8	11.67 ± 0.69	254.4 ± 6.6
GPF 2	1.25 ± 0.08	1.15 ± 0.25	0.29 ± 0.05	8.62 ± 0.60	6.34 ± 0.18	35.14 ± 1.2	4.74 ± 1.03	272.4 ± 19.9
PDG 3	1.35 ± 0.11	1.68 ± 0.29	0.25 ± 0.03	8.73 ± 0.24	7.11 ± 0.31	17.21 ± 1.4	11.80 ± 0.67	286.2 ± 2.0
ICCV 2	1.74 ± 0.09	0.43 ± 0.05	0.21 ± 0.05	6.36 ± 0.69	8.31 ± 0.18	15.07 ± 1.4	18.74 ± 0.33	292.8 ± 9.8
JKG 1	1.19 ± 0.08	0.40 ± 0.05	0.20 ± 0.03	6.90 ± 0.86	10.06 ±0.34	28.54 ± 1.5	12.04 ± 0.45	266.5 ± 4.1
L 550	1.41 ± 0.05	0.54 ± 0.06	0.27 ± 0.02	5.99 ± 0.35	10.06 ± 0.56	17.76 ± 4.2	18.20 ± 0.77	241.6 ± 7.5
Virat	1.22 ± 0.15	0.37 ± 0.02	0.17 ± 0.02	6.96 ± 0.39	9.77 ± 0.68	24.64 ± 4.9	7.64 ± 0.45	277.2 ± 6.3
BG 1053	1.12 ± 0.02	0.41 ± 0.04	0.29 ± 0.03	5.61 ± 1.09	9.32 ± 0.18	31.40 ± 1.2	17.77 ± 0.78	257.1 ± 13.3
Mean	1.621	0.91	0.211	7.66	7.719	19.542	12.632	282.353

Values are mean±SD of triplicate samples estimated in triplicates. One inhibitor unit for trypsin inhibitor is the quantity of inhibitor that inhibits 50% of bovine trypsin activity.

of tannins, have been categorized as: depression of food intake, formation of the less digestible tannin-dietary protein complexes, inhibition of digestive enzymes, increased excretion of endogenous protein, digestive tract malfunctions and toxicity of absorbed tannin or its metabolites (Champ 2002, Akinyede *et al.* 2005).Tannins have been reported to inhibit the digestive enzymes and thereby lower the digestibility of most important nutrients especially protein and starch (Khattab and Arntfield 2009). Tannins interact with both enzyme and non-enzyme proteins to form tannin-protein complexes resulting in inactivation of digestive enzymes and protein digestibility (Khandelwal *et al.* 2010). The presence of tannins in foodgrains lower feed efficiency, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter excretion of protein and essential amino acids (Ekop *et al.* 2008).

Bound fructose of sucrose and raffinose series oligosaccharides varied from 5.53 mg/g (Pant G 186) to 10.13 mg/g (Vijay) with mean value of 7.71 mg/g (Table 2). Kabuli cultivars had higher content of bound fructose of sucrose and raffinose series oligosaccharides whereas desi cultivars had a lot of variation in content as in them it ranged from 5.53 (Pant G 186) to 10.13 mg/g (Vijay) Presence of RFOs in legumes is one of the main reason for its non- acceptability in animal and human nutrition because they lack α-galactosidase enzyme to break α (1→ 6) linkage in RFOs (Wang *et al.* 2003).

Saponin content was found to be higher in kabuli cultivars as compared to desi cultivars as mean value of saponin content was 18.75 mg/g and 23.48 mg/g respectively in desi and kabuli cultivars (Table 2). Saponin content in chickpea was reported to be about 25mg/g of dry weight.

Table 3 Distribution of acid phosphatase, alkaline phosphatase and α-galactosidase in chickpea cultivars

Cultivars	Acid phosphatase activity (n moles p-nitrophenol formed min/g)	Specific activity of acid phosphatase (enzyme activity mg/protein)	Alkaline phosphatase activity (n moles p-nitrophenol formed min/g)	Specific activity of alkaline phosphatase (enzyme activity mg/protein)	α-galactosidase activity (n moles min/g)	Specific activity of α-galactosidase (enzyme activity mg/protein)
Avrodhi	542.5 ± 24.6	20.9 ± 1.3	371.0 ± 31.1	3.7 ± 0.3	400.7 ± 88.6	15.1 ± 3.5
BG 256	704.5 ± 78.2	28.4 ± 3.2	376.7 ± 32.2	4.3 ± 0.3	323.2 ± 18.1	12.8 ± 1.1
BG 372	611.2 ± 37.7	24.7 ± 2.1	353.2 ± 24.0	4.4 ± 0.2	299.0 ± 11.4	12.0 ± 0.4
DCP 923	689.7 ± 21.7	22.3 ± 1.7	384.0 ± 25.5	4.0 ± 0.2	532.7 ± 25.5	17.2 ± 0.8
Digvijay	607.7 ± 28.6	22.9 ± 1.0	439.2 ± 14.5	4.4 ± 0.3	350.2 ± 15.0	13.2 ± 1.0
Dahood Yellow	607.5 ± 25.0	24.0 ± 1.1	214.5 ± 16.5	2.3 ± 0.1	222.5 ± 12.6	8.8 ± 0.2
GNG 469	654.2 ± 22.2	19.3 ± 1.2	467.5 ± 24.7	4.8 ± 0.1	388.5 ± 35.5	11.4 ± 0.6
HC 1	470.2 ± 33.6	19.5 ± 2.9	389.5 ± 22.0	4.0 ± 0.2	240.0 ± 25.8	10.0 ± 0.3
HC 3	445.0 ± 62.9	19.3 ± 2.2	333.5 ± 16.6	3.5 ± 0.2	246.5 ± 14.6	10.8 ± 0.9
HC 5	573.7 ± 29.5	20.1 ± 0.9	440.2 ± 32.7	4.1 ± 0.2	307.5 ± 14.5	10.7 ± 0.3
ICCV 10	611.0 ± 17.5	19.5 ± 1.0	475.0 ± 31.4	3.9 ± 0.2	327.5 ± 12.6	11.5 ± 0.3
JG 11	726.0 ± 37.1	25.8 ± 3.5	387.0 ± 26.6	3.8 ± 0.2	254.5 ± 18.7	9.0 ± 0.5
JG 74	387.7 ± 27.7	22.9 ± 0.8	327.2 ± 18.7	3.5 ± 0.2	174.5 ± 16.6	10.2 ± 0.7
JG 315	576.5 ± 12.8	20.0 ± 0.9	408.0 ± 78.6	4.0 ± 0.5	416.5 ± 52.5	14.4 ± 1.3
KWR 108	517.0 ± 12.5	17.8 ± 1.4	421.2 ± 31.7	4.0 ± 0.3	358.7 ± 29.7	12.3 ± 1.1
Pant G 186	737.2 ± 56.0	33.7 ± 6.6	494.2 ± 45.4	4.9 ± 0.5	306.7 ± 39.7	14.0 ± 2.5
Radhay	851.2 ± 72.0	26.1 ± 1.3	475.7 ± 17.1	4.3 ± 0.2	388.0 ± 45.3	11.8 ± 0.2
RSG 888	584.7 ± 56.2	22.6 ± 2.6	398.0 ± 26.4	3.7 ± 0.2	279.2 ± 19.1	10.8 ± 0.5
RSG 931	556.5 ± 27.5	23.4 ± 1.4	346.5 ± 19.9	3.4 ± 0.2	253.0 ± 18.5	10.6 ± 0.3
Saki 9516	625.7 ± 13.4	25.0 ± 0.9	387.5 ± 13.2	3.6 ± 0.2	268.7 ± 14.4	10.7 ± 0.6
Vijay	862.0 ± 11.8	28.2 ± 2.0	473.0 ± 26.4	3.9 ± 0.2	388.7 ± 16.0	12.7 ± 0.4
Vishal	654.5 ± 61.7	26.3 ± 2.5	493.5 ± 23.2	3.7 ± 0.2	314.0 ± 16.7	12.5 ± 0.2
PBG 1	637.2 ± 32.3	27.8 ± 1.5	383.2 ± 18.4	4.0 ± 0.1	247.7 ± 11.1	10.8 ± 0.9
GPF 2	601.2 ± 17.3	30.2 ± 1.4	369.0 ± 19.6	4.3 ± 0.2	282.0 ± 19.3	14.2 ± 0.7
PDG 3	556.5 ± 10.4	22.0 ± 3.5	340.0 ± 34.5	3.8 ± 0.2	265.0 ± 17.6	10.5 ± 2.0
ICCV 2	690.0 ± 20.2	18.9 ± 0.5	518.0 ± 25.9	3.7 ± 0.1	368.5 ± 53.7	10.0 ± 1.1
JKG 1	654.5 ± 58.8	16.8 ± 1.2	402.7 ± 70.4	3.4 ± 0.5	508.0 ± 79.1	13.3 ± 3.2
L 550	771.5 ± 61.0	18.0 ± 1.4	467.7 ± 40.4	3.6 ± 0.2	522.0 ±19.0	11.8 ± 0.2
Virat	933.0 ± 45.5	22.0 ± 1.2	477.7 ± 26.3	3.3 ± 0.3	476.5 ± 39.2	11.2 ± 0.8
BG 1053	809.0 ± 98.1	19.4 ± 0.9	473.5 ± 12.1	3.4 ± 0.1	426.2 ± 47.6	10.2 ± 0.5
Mean	641.636	22.926	409.596	3.856	3.856	11.81

Values are mean±SD of triplicate samples estimated in triplicates

(Kerem *et al.* 2005). Jain *et al.* (2009) reported that saponin content in chickpea, mungbean and pigeonpea ranged from 0.05-0.23%.

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential and nutritionally important divalent cations such as Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} and forms insoluble complexes, thereby making minerals unavailable for absorption (Raboy 2002). The average value of phytic acid in 30 cultivars is 12.72 mg/g seeds (Table 2). The average amount of phytic acid was higher in kabuli (14.87 mg/g seeds) than desi (12.18 mg/g seeds) cultivars. A lot of variation was observed in phytic acid content in the cultivars as highest content was found in HC 1 (20.4 mg/g) and lowest in GPF 2 (4.74 mg/g). Nikolopoulou *et al.* (2007) reported that phytate level varies in legumes with variety, cultivar type and soil type. Hidvegi and Lasztity (2002) reported that legumes have higher phytic acid content up to 1.75g/100 g as compared to cereals (1.42 g/100g). Phytic acid cannot be digested by non-ruminant livestock and humans (Brinch-Pedersen *et al.* 2002). Phytic acid phosphorus that is excreted in animal waste can cause pollution of water resources (Raboy 2001). Low phytic acid in crop plants is the main viable mode to decrease its content in our food.

The average content of trypsin inhibitor was found to be 282.3 IU/g seeds in 30 chickpea cultivars. Dahood yellow was found to contain the highest amount of trypsin inhibitor (311.7 IU/g seeds) and the lowest amount was found in BG 256 (230.2 IU/g). The average value of enzyme activities of acid and alkaline phosphatase were found to be 641.63 and 409.59 n mole p-nitrophenol formed/min/g respectively in chickpea cultivars (Table 3). Mean value of enzyme activity of α- galactosidase was found to be 313.42 n moles/min/g and 460.24n moles/min/g in desi and kabuli cultivars respectively. Average amount of all the enzyme activities were higher in kabuli cultivars.

There was significant relationship found within cultivars, parameters and significant interaction was observed between cultivars and parameters. Interaction of starch with the cultivars was found to be maximum (418.22 mg/g) and minimum with o-dihydroxyphenol (0.21 mg/g) (Table 4). The relationships between various antinutritional factors,protein and starch are shown in Table 5. Value of correlation coefficient varied from –0.521 (between α-galactosidase and tannin) to –0.616 (between protein and acid phosphatase and between starch and tannin). Total phenol showed positive correlation with saponin, trypsin inhibitor and negative correlation with bound fructose of raffinose series oligosaccharides at different levels of significance. Our results are consistent with Shim *et al.* (2003) who also reported positive correlation of total phenol and saponin. Phytic acid showed significantly negative correlation with saponin ($r = –0.394$, $P < 0.05$). Proteins were positively correlated with total phenol ($r = 0.406$, $P < 0.02$) and negatively correlated with tannins ($r = –0.520$, $P < 0.01$). Starch showed positively significant correlation with tannin ($r = 0.616$, $P < 0.01$).

Multivariate Principal Component Analysis was used

Table 4 Mean and CD(5%) values of factors A(Cultivars) &B (Parameters) in chickpea cultivars

Factor-A(Cultivars)	Mean
Avrodhi	168.00
BG 256	173.54
BG 372	162.27
DCP 923	192.40
Digvijay	178.33
Dahood Yellow	146.18
GNG 469	187.83
HC 1	154.09
HC 3	150.86
HC 5	170.63
ICCV 10	193.99
JG 11	178.09
JG 74	174.12
JG 315	138.98
KWR 108	173.06
Pant G 186	184.01
Radhay	166.72
RSG 888	198.12
RSG 931	181.66
Saki 9516	196.00
Vijay	162.33
Vishal	156.34
PBG 1	170.05
GPF 2	215.34
PDG 3	201.12
ICCV 2	185.56
JKG 1	154.92
L 550	157.63
Virat	147.94
BG 1053	192.63
CD (P=0.05)	0.14
Factor-B (Parameters)	
Total phenols(mg/g)	1.62
Flavonols(mg/g)	0.91
O-dihdroxy phenol (mg/g)	0.21
Tannins(mg/g)	7.66
Trypsin inhibitor TIU/g)	282.35
Saponins(mg/g)	19.54
Phytic acid(mg/g)	12.63
Raffinose family oligosaccharides	7.72
Acid phosphatase activity (n moles/p-nitrophenol formed min/g/1 seeds	641.63
Alkaline phosphatase activity (n moles p-nitrophenol formed min/g/seeds)	409.59
α-galactosidase activity (n moles min/g/seeds)	337.89
Total sugars (mg/g)	66.28
Starch(mg/g)	418.22
Total proteins(mg/g)	226.38
CD(P=0.05)	
Factor-A(Genotypes)	0.136
Factor-B(Parameters)	0.935
A*B(Genotypes*Parameters)	0.512

Table 5 Correlation coefficient between various antinutritional factors, protein and starch

	Total phenols	Flavonols	o–dihydroxy phenols	Tannins	Phytic acid	Saponin	Bound fructose of raffinose series oligo–saccharides	Trypsin inhibitor	Acid phos–phatase	Alkaline phos–phatase	α–Galacto–sidase	Starch	Protein
Total phenols		0.348	–0.187	–0.025	0.107	0.548**	–0.394*	0.401*	–0.16	–0.192	–0.093	0.109	0.406*
Flavonols			0.228	0.442*	–0.152	–0.031	–0.452*	0.256	–0.189	–0.047	–0.377*	–0.45	–0.098
o–dihydroxy phenols				0.248	0.345	0.22	0.263	–0.315	–0.21	0.029	0.008	–0.441*	–0.179
Tannins					–0.105	0.117	–0.397*	0.035	–0.403*	–0.235	–0.521**	0.616**	–0.52**
Phytic acid						–0.394*	0.15	0.008	–0.139	–0.096	0.149	0.025	0.125
Saponin							0.181	–0.355	0.173	0.095	0.076	–0.294	–0.29
Bound fructose of raffinose series oligosaccharides								–0.355	0.586**	0.09	0.458*	0.298	–0.121
Trypsin inhibitor									–0.006	–0.081	–0.151	0.085	0.345*
Acid phosphatase										0.428*	0.596**	0.267	0.616**
Alkaline phosphatase											0.329	0.299	0.0076
α– Galactosidase												0.347	0.194
Starch													0.313
Protein													

Values with *and ** are significant at 0.05 and 0.01 level of significance respectively.

to cluster the cultivars on the basis of biochemical similarity. The cultivars were found to cluster in major four groups. The three cultivars JKG 1(S No 27), DCP 923(S No 4) and L 550(S No 28) formed a single cluster. This cluster has one desi (DCP 923) and two kabuli (JKG 1 and L 550) cultivars. DCP 923 had higher content (270.5 mg/g) among all the cultivars and the protein content in JKG 1 and L 550 were found to be in similar range (189.0 mg/g-193.5 mg/g).The cultivars in this cluster had lower amount of tannins.The second cluster has two desi cultivars namely Avrodhi (S.No 1) and JG 315(S.No 14) and two kabuli cultivars Virat (S.No 29) and BG 1053(S.No 30). The cultivars in this group had fairly good amount of total sugars, starch and protein content. Phytic acid in this cluster showed lot of variation as JG 315 and BG 1053 had higher phytic acid content and Avrodhi and Virat had lower phytic acid content. This information about the cultivars could be useful for the plant breeders to develop varieties with fairly good protein and low phytic acid contents. Five desi cultivars KWR 108 (S No 15), GNG 469 (S No 7), BG 256 (S No 2), Radhay (S No 17) and Vijay (S No 21) grouped in one cluster. All the cultivars in this cluster had fairly good amount of total sugars , protein and starch content except for Radhay which had the lowest protein content (169.0 mg/g).The fourth cluster has both desi and kabuli cultivars. The two desi cultivars Dahood yellow (S No 6) and JG 74 (S No 13) were found to appear distinctly alone from all the cultivars . They had fairly good amount of nutritional constituents and higher amount of total phenols, tannin, phytic acid and trypsin inhibitors. This observed variation in cultivars could be useful in breeding programme.

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