Legume Research, 39 (3) 2016 : 359-369
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.legumeresearch.in

Physiochemical and nutritional attributes of raw and soaked seeds of chickpea (*Cicer arietinum* L.) genotypes

Satvir Kaur*, Sarbjit Kaur, Anil K Gupta and Jagmeet Kaur

Punjab Agricultural University,
Ludhiana-141 004, India.

Received: 06-06-2015 Accepted: 25-11-2015 DOI:10.18805/lr.v0iOF.9434

ABSTRACT

The present investigation aims to analyze nutrient profile, antinutritional traits and physiochemical characteristics in raw (dry seeds) and water soaked seeds of eighteen chickpea genotypes comprising 16 cold stress tolerant and 2 susceptible. Raw seeds of tolerant genotypes were found to have higher total sugars and protein content and lower amount of trypsin inhibitors. Higher content of raffinose family oligosaccharides in tolerant genotypes is an additional characteristic for their tolerant behaviour and it does not interfere with the digestion as with soaking these oligosaccharides are reduced by 87%. The enhanced mineral content with soaking in tolerant chickpea genotypes might be attributed to the better physiochemical characteristics in terms of higher hydration capacity and swelling index which leads to greater permeable and softer cotyledons that might help in leaching out of mineral ligands. The higher nutritional value along with better physiochemical characteristics observed in tolerant genotypes render them useful at consumer level as well as from breeding point of view.

Key words: Antinutritional factors, *Cicer arietinum*, Cold stress, Minerals, Nutritional quality, Physiochemical properties, Soaking.

INTRODUCTION

Food legumes including chickpea provide economical source of high proportion of proteins, fat, carbohydrates, dietary fibres, B-group vitamins (thiamin, riboflavin, niacin) and minerals all over the world and ranked 2nd after cereals with respect to their consumption order (Vasistha and Srivastava, 2011 ; Wang *et al.*, 2010). Leguminous seeds constitute one of the richest and cheapest source of 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).

The nutritional utilization of legumes is negatively affected by the presence of non- nutritive or antinutritional factors such as trypsin inhibitor, phytic acid, phenolic compounds, raffinose series oligosaccharides (RFOs), tannins and saponin in their seeds (Wang *et al* 2010). Although mineral and protein content of legumes is generally high but the bioavilability is poor due to the presence of phytic acid which is the main inhibitor of iron and zinc absorption and also complexes with proteins (Ramakrishna *et al* 2006). Protease inhibitors impair protein digestion by inhibiting serine proteases -trypsin and chymotrypsin (Guillamon *et al* 2008). RFOs are generally undesirable due to flatus factors as human alimentary tract is deprived of α-galactosidase capable of hydrolyzing the α-1-6 galactoside linkage, therefore these oligosaccharides accumulate in large intestine and undergo anaerobic fermentation by bacteria (Sat and Keles 2002). Phenols bind to positively charged proteins, amino acids or multivalent cations and minerals such as iron, zinc and calcium in foods and decrease their bioavailability (Gilani *et al* 2005). Tannins also affect the nutritive value of legumes as it bind to enzyme and non enzyme proteins to form tannin – protein complex and results in inactivation of digestive enzymes and reduce protein digestion and also form more complex bonds with starch, cellulose and minerals and reduce their digestion (Khandelwal *et al* 2010; Katoch 2013). Saponins are naturally occurring surface active glycosides that reduce absorption of nutrients either directly by binding with or by inactivating enzymes involved in digestion process and cause systemic toxicity. Saponins causes lysis of RBCs and also disturb the fluidity of biomembranes as it causes leakage of cells by creating holes (Jain *et al* 2009).

Legumes are generally consumed after soaking, cooking and heating as these processing methods improve the nutritional quality of food legumes and grains to various extents (Vasudeva and Vishwanathan 2010). However, heat treatment destroys some of the amino acids and vitamins as

*Corresponding author's e-mail: satvir_pau@yahoo.co.in.

well (Jain *et al* 2009). The most effective treatments are fermentation and germination but their application remains limited because of the additional workload they imply on the particular organoleptic properties they induce. Previous studies have shown that a long soaking period before fermentation, germination and cooking leads to a reduction in phytate content and to an enhancement of mineral bioavailability (Hagir *et al* 2007). Ramakrishna (2008) also reported that soaking of bean seeds reduced trypsin inhibitor activity, phytates, tannins and total phenols to the extent of 51%, 15%, 35% and 43% respectively. The reduction of antinutritional factors after soaking may be due to leaching out of these compounds into soaking water (Ramakrishna et al 2006). Soaking is the preferred method for processing chickpea at domestic level. The effect of soaking may provide useful information for optimization of use of chickpea seeds as the food products since soaking have proved beneficial for the nutritional quality for common seeds.

Currently, the focus is on developing high yielding varieties to fill the gap between demand and supply of food legumes with little emphasis on their nutritional quality. 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. Therefore, there is a need to screen the varieties for their nutritional evaluation before releasing them (Suneja *et al.,* 2011). There is no detailed study of the physical quality parameters of cold stress tolerant and susceptible chickpea genotypes. Secondly the consumers preferably use the chickpea after overnight soaking. Therefore we assessed the biochemical and physiochemical characteristics of tolerant and susceptible genotypes before and after overnight soaking.

MATERIALS AND METHODS

The present investigations were carried out on 16 cold stress tolerant and 2 cold stress susceptible chickpea genotypes obtained from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The seeds of chickpea (in triplicates) were crushed to fine powder in pestle and mortar and the contents were passed through 80µm sieve to have uniform powder which was stored for extraction and assay of various components in triplicates. All the parameters were studied in raw and 24 h soaked chickpea genotypes.

Biochemical Characteristics

Extraction and estimation of nutritional traits: Total soluble sugars were extracted from 200 mg powdered chickpea seeds by crushing with 80% ethanol followed by 70% ethanol (Kaur *et al.,* 2000). Total bound fructose was determined after destroying the free fructose with 30% NaOH by resorcinol HCl procedure (Kaur *et al.,* 2000). The sugar free residue was dried at 60°C and used for the estimation of starch. 100 mg of powdered chickpea seeds were kept overnight in 25 ml of 0.1 N NaOH to extract total proteins from them. The supernatant after centrifugation at (5000´g) was used for the estimation of total proteins (Lowry *et al.,* 1951). Zinc, iron and copper were estimated by atomic absorption spectrophotometry (Varian Spectra AA 240) after diacid digestion of 200 mg powdered chickpea seeds in nitric acid: perchloric acid:: 4:1 at 200^0C for 100 min and final volume was made with glass distilled water. Calibration of mineral measurements was performed using mineral standards along with appropriate acid blanks.

Extraction and estimation of antinutritional traits: Antinutritional factors such as phenolic compounds, tannins, saponins, phytic acid and trypsin inhibitors were extracted and estimated as described previously by Kaur *et al.,* (2014). Phenolic compounds were extracted by refluxing seed powder with 80% aqueous methanol. The refluxed material after filtration was used to estimate total phenols. Tannins were estimated using Folin-Denis reagent along with tannic acid (10-100µg) as a standard. Saponins were extracted with acetone and later with methanol from the seed powder and estimated using saponin (0-40µg) as a standard. The phytic acid was extracted from the powered seeds with 1.2% HCl and precipitated with ferric chloride and organic phosphorous was estimated. The trypsin inhibitor was estimated by using N α-benzoyl-DL-arginine p-nitroanilide (BAPNA) as a substrate.

Soaking of seeds and physiochemical characteristics: Three 100 seed sample of each cultivar were taken. Then volume and weight of dry seeds were measured. The seeds were first surface sterilized by treating with 0.1% mercuric chloride. The sterilized seeds were rinsed and soaked in 250 ml distilled water for a period of 24 h at room temperature (28 ± 2^0 C). The soaked seeds were rinsed with distilled water and then used for extraction and estimation of physiochemical, nutritional and antinutritional factors. Hydration capacity, swelling index, swelling capacity, seed density and 100-seed weight were evaluated by the methods of William *et al.,* (1983).

Statistical analysis: Mean and standard deviation were calculated. Mean values were subjected to Tukey's test using SPSS version 16.1. Multivariate Principal Component Analysis was also applied to genotypes.

RESULTS AND DISCUSSION

Nutritional components in raw and soaked chickpea seeds: The nutritive value of grain legumes depend primarily on their nutrient contents and presence or absence of

antinutritional factors and toxic factors. Average content of total sugars was found to be 61.77 mg g^{-1} in chickpea genotypes and ICC88505 was found to contain the highest amount of total sugars being 86.68 mg g^{-1} (Table 1). Ferial and Esmat (2011) also reported 64.6 g kg^{-1} total sugars in chickpea seeds. Tolerant genotypes had higher average content of total sugars (62.85 mg g^{-1}) than susceptible (53.13 mg g^{-1}) genotypes (Table 1). Sugars act as osmolytes as well as signaling molecules and are important constituents for stress tolerance. Higher total sugars observed in tolerant genotypes might be responsible for their stress tolerance behaviour. The average reduction in total sugar content with soaking was found to be 10.55% in chickpea seeds (Table 1). However, the reduction in total sugar content with soaking was found to be more in susceptible (33.66%) as compared to tolerant genotypes (8.16%). The reduction of sugars is mainly due to their solubility in water.

Total protein content in chickpea genotypes varied from 13.17% to 27.13% with a mean value of 23.86% (Table1). Higher protein content (23-27%) was observed in all the tolerant genotypes except for IPC 97-72, ICCV 88506 and ICCV 88503 which recorded 13.17%, 20.28% and 22.76% protein content respectively. However, PBG-1 and GPF-2, the susceptible genotypes had protein content between 20.19-21.62%. Our results are in agreement to those reported by Wang *et al.,* (2010); Ferial and Esmat (2011) and Aguilera *et al.,* (2009) who reported 22.94 %, 24.63% and 22.39 % protein content respectively in chickpea genotypes. The reduction in protein content after soaking was found to be 18.69% in chickpea genotypes. Huma *et al.,* (2008) reported that reduction in protein content (14.78 – 21.83 %) after cooking the presoaked legumes might be attributed to partial removal of essential as well as non - essential amino acids with other nitrogenous compounds formed as a result of chemical degradation of protein into water soluble amino acids.

Starch content of chickpea genotypes varied from 29.25% in GL28028 to 46.18% in IPC 94-94 with a mean value of 39.21% (Table 1). Starch content was found to be higher in susceptible genotypes. Wang *et al.,* (2010) had also reported 382.3 g kg^{-1} starch in chickpea seeds. There was 56.85% reduction observed in starch content with 24 h soaking. The decrease in starch content after soaking might be due to the fact that legume starch is composed of insoluble portion and soluble portion and soluble part might have leached out from seeds during soaking resulting in reduced starch content (Aguilera *et al.,* 2009). Zinc content varied from 2.88 in IPC 2004-55 to 8.15 mg/100g in GL 28028 with a mean value of 5.64 mg/100g (Table 1). Wang *et al.,* (2010) reported 4.07 mg/100g zinc content in chickpea seeds. Average content of iron and copper in genotypes were found to be 3.45 mg/100g and 0.1 mg/100g respectively (Table 1). Zinc content was found to be higher than iron followed by copper. Hemalatha *et al.,* (2007) also reported higher content of zinc than iron in food grains and lower amount of copper content. It was observed that the mineral content increased after 24 h soaking in chickpea genotypes and the increase in iron and zinc was found to be more in tolerant genotypes. The reason of increased mineral content after 24 h soaking might be due to the loss of water soluble phytic acid which complexes with iron, zinc and copper thereby reducing their availability. Soaking of legumes offers a practical household method to reduce inhibitors of mineral absorption, especially phytic acid and tannin thereby contributing to enhanced zinc and iron absorption (Hemalatha *et al.,* 2007). Our results indicated that tannin and phytic acid are reduced with soaking (Table 2). Chopra and Sankhala (2004) observed significant increase in iron availability after soaking as well as after germination of horse gram and moth bean seeds. Germination of legumes significantly increased zinc content by 128.8% in white beans, 200% in black gram and 37.7% in pigeon pea, as phytic acid is hydrolysed during germination which resulted in more bioavailability of minerals for human body (Sangronis and Machado, 2007).

Antinutritional components in raw and soaked chickpea seeds: Trypsin inhibitor is a to widespread antinutritional substance which block trypsin activity thereby reducing digestibility of proteins (Sharma *et al.,* 2013). The trypsin inhibitor was found to be in the range of 209.85 -283.35 $TIUg^{-1}$ with an average value of 249.22 $TIUg^{-1}$. The tolerant genotypes had lower average content of trypsin inhibitor (248.28 $TIUg^{-1}$) than susceptible genotypes (256.72 $TIUg^{1}$). There was 20.92 % reduction in trypsin inhibitor after 24 h soaking of chickpea seeds and this reduction was found to be more in tolerant genotypes (Table 2) indicating their better nutritional profile. The average amount of phytic acid was 19.42 mg g^{-1} in chickpea seeds and it was found to be higher in tolerant than susceptible genotypes (Table 2). An average of 32.23 % reduction was observed in phytic acid content in all the chickpea genotypes after 24 h soaking. Soaking triggered the phytase synthesis and phytase activity was found to be maximum after soaking which results in leaching out of phytic acid leading to thereby reducing its content (Tajoddin *et al.,* 2011).

Saponin content varied from 15.30 (ICCV 96029) to 40.92 mg g^{-1} (IPC 98-51) in chickpea genotypes (Table 2). Soaking in water for 24 h led to 44.94 % reduction in saponin content. The stability of saponin in legume seeds undergoing

Table 1: Distribution of nutritional factors and minerals (iron, zinc and copper) in raw and soaked seeds of chickpea genotypes

Tolerant genotypes	Total sugars* (Raw)	Total sugars* (Soaked)	Total Protein** (Raw)	Total Protein** (Soaked)	Starch** (Raw)	Starch** (Soaked)	Zn*** (Raw)	Zn*** (Soaked)	Fe*** (Raw)	Fe*** (Soake)	Cu*** (Raw)	Cu*** (Soaked)
ICCV88503	59.59^{cdef}±5.51	50.60^{jk}±1.88	22.76^{bc}±8.66	20.37^{bc}±0.87	34.68^{h}±8.27	11.78^{h}±3.75	3.17	8.00	3.42	15.30	0.30	0.86
ICCV88506	59.59^{cdef}±7.25	50.72^{kl}±2.05	20.28^{e}±7.34	19.85^{e}±6.48	42.09^{bcd}±15.32	16.98^{cde}±10.83	5.84	5.32	5.84	9.70	0.21	0.55
ICCV92944	62.09^{cd}±3.69	51.44^{l}±0.45	23.11^{d}±22.16	18.23^{d}±2.47	38.37^{fg}±16.98	16.13^{def}±0.64	6.73	8.93	8.73	14.00	0.32	0.59
ICCV96029	47.51^{ef}±4.81	58.99^{fg}±0.90	23.47^{d}±29.56	18.17^{d}±8.36	38.92^{efg}±20.24	18.16^{bcd}±0.94	5.67	7.15	5.31	7.60	0.37	0.55
ICCV96030	51.67^{def}±1.92	51.36^{ijk}±1.28	24.27^{abc}±22.24	20.78^{abc}±6.99	40.50^{odef}±16.04	16.56^{de}±1.60	6.22	7.73	5.50	8.73	0.19	0.57
IPC98-51	58.34^{cdef}±0.00	59.87^{fg}±1.23	26.86^{a}±1.34	22.03^{a}±8.87	40.47^{edef}±8.82	17.30^{bcde}±0.98	7.05	5.02	5.93	6.90	0.09	0.17
IPC94-94	60.42^{cde}±1.59	55.62^{ghi}±2.81	25.59^{abc}±9.17	20.84^{abc}±2.80	46.18^{a}±6.25	21.73^{a}±11.11	6.58	9.35	4.21	11.18	0.15	0.43
IPC97-72	65.01^{bcd}±3.04	62.25^{ef}±2.87	13.17^{f}±9.037	11.73^{f}±13.10	42.58^{bcd}±0.57	19.45^{b}±24.29	5.05	2.08	1.61	8.38	0.34	0.27
IPC2004-55	65.84^{bcd}±1.66	53.45^{hij}±1.45	26.83^{ab}±0.81	21.71^{ab}±2.82	34.05^{h}±8.01	18.86^{bcd}±1.16	2.88	7.64	1.03	7.44	0.09	0.33
GL28008	52.92^{def}±3.43	50.24^{a}±0.25	26.71^{a}±2.10	22.07^{a}±2.60	30.97^{i}±10.17	14.23^{fg}±1.28	4.90	8.56	0.62	7.12	0.16	0.23
GL28019	59.59^{cdef}±4.16	58.21^{fg}±2.62	25.74^{abc}±5.70	20.82^{abc}±2.92	36.01^{gh}±10.54	19.28^{bc}±0.66	4.10	3.62	0.72	3.34	0.17	0.24
GL28028	70.01^{bc}±7.20	67.00^{d}±0.02	26.55^{c}±8.96	20.05^{c}±5.29	29.25^{i}±15.96	12.14^{gh}±0.87	8.15	9.83	2.20	4.79	0.01	0.49
ICCV2004-53	76.68^{ab}±3.60	71.72^{b}±0.10	26.37^{abc}±8.99	21.09^{abc}±4.41	41.95^{cd}±8.34	17.37^{bcde}±1.59	6.69	4.95	1.50	2.06	0.01	0.03
ICCV88505	86.68^{a}±13.54	74.63^{c}±1.86	24.80^{c}±8.33	20.00^{c}±3.79	44.98^{ab}±11.09	16.44^{de}±0.77	5.02	8.86	1.80	3.22	0.01	0.29
PDG84-16	60.84^{cd}e±3.96	50.74^{de}±1.06	24.03^{d}±4.39	17.92^{d}±4.87	41.45^{cde}±2.87	17.75^{bcde}±0.88	4.26	8.00	2.90	5.05	0.018	0.26
PG96006	68.76^{bc}±3.69	56.79^{gh}±2.99	27.13^{abc}±0.67	21.01^{abc}±2.49	40.05^{def}±6.90	15.58^{ef}±0.35	7.94	7.59	0.20	3.09	0.024	0.17
Mean	62.85	57.72	24.23	19.79	38.91	16.86	5.64	7.03	2.30	7.36	00.18	0.37
Susceptible genotypes												
PBG-1	60.42^{cde}±8.86	34.14^{m}±2.46	20.19d^{e}±2.78	16.84^{de}±3.45	40.17^{odef}±13.82	17.79^{bcd}±19.90	2.90	4.54	3.90	02.77	00.09	00.38
GPF-2	45.84^{f}±4.41	36.70^{m}±0.97	21.62^{e}±3.35	15.68^{e}±6.76	43.11^{bc}±2.11	16.90^{cde}±0.80	7.19	7.77	3.00	02.87	00.11	00.05
Mean	53.13	35.42	20.90	16.26	41.64	17.35	5.04	6.15	3.45	2.82	0.10	0.21
Total mean	61.77	55.25	23.86	19.40	39.21	16.91	5.04	6.94	3.45	2.52	0.10	0.2

* Data is expressed as mg g-1; ** Data are expressed as % ; *** Data are expressed as mg /100g .

Values with different letters in the same column are significantly different ($p<0.05$)

Table 2: Distribution of antinutritional factors (trypsin inhibitor**, phytic acid*, saponin*, tannin*, total phenols* and bound fructose of raffinose series oligosaccharides) in raw and soaked seeds of chickpea genotypes

Tolerant genotypes	Trypsin Inhibitor (Raw)	Trypsin Inhibitor (Soaked)	Phytic acid (Raw)	Phytic acid (Soaked)	Saponin (Raw)	Saponin (Soaked)	Tannin (Raw)	Tannin (Soaked)	Total phenol (Raw)	Total phenol (Soaked)	Bound fructose (Raw)	Bound fructose (Soaked)
ICCV88503	S.No.	186.90^{d}±18.91	21.81^{cd}±1.57	14.32^{bc}±0.84	36.42^{ab}±0.71	22.26^{a}±0.39	5.49^{gh}±0.27	2.25^{cdefg}±0.02	0.91^{efgh}±0.02	0.52^{de}±0.00	10.40^{cde}±0.02	0.57^{j}±0.01
ICCV88506	239.85^{fg}±6.54	219.70^{bcd}±22.58	16.70^{fghij}±0.91	10.57^{efg}±0.69	32.64^{bc}±0.46	15.54^{bcdef}±0.50	5.65^{gh}±0.09	2.55^{abcd}±0.07	0.78^{gh}±0.02	0.47^{e}±0.00	9.41^{defg}±0.07	0.43^{k}±0.01
ICCV92944	240.45^{efg}±1.72	220.35^{bcd}±12.94	28.97^{a}±1.96	19.27^{a}±1.05	28.86^{cdefg}±0.22	10.38^{ghi}±0.22	7.40^{bcd}±0.82	2.16^{defg}±0.22	0.98^{defg}±0.05	0.82^{a}±0.03	11.90^{b}±0.69	2.01^{d}±0.06
ICCV96029	226.35^{ghi}±2.41	213.40^{bcd}±1.57	22.15^{c}±0.65	14.19^{bc}±0.55	15.30^{h}±0.22	9.36^{i}±0.20	5.71^{fgh}±0.01	1.88^{g}±0.21	0.80^{fgh}±0.05	0.62^{c}±0.00	10.39^{cde}±0.14	2.75^{a}±0.06
ICCV96030	251.25^{cdef}±9.61	144.75^{e}±14.16	16.93^{efghi}±0.91	11.58^{def}±0.56	26.22^{defg}±0.18	13.38^{defgh}±0.23	5.25^{h}±0.55	1.93^{fg}±0.21	1.13^{cde}±0.02	0.54^{d}±0.05	11.78^{b}±0.41	1.24^{g}±0.08
IPC98-51	274.95^{ab}±3.44	225.30^{bc}±18.72	16.13^{ghij}±0.26	11.51^{def}±0.94	40.92^{a}±0.39	23.22^{a}±0.22	6.68^{def}±0.36	2.94^{a}±0.28	1.15^{cd}±0.08	0.60^{c}±0.00	09.22^{efg}±0.77	1.22^{g}±0.05
IPC94-94	259.35^{bcde}±7.12	216.68^{bcd}±17.81	19.40^{de}±1.41	12.85^{bcde}±0.77	17.16^{h}±0.08	09.90^{hi}±0.21	7.00^{cde}±0.00	2.61^{abc}±0.00	1.26^{bc}±0.15	0.74^{b}±0.00	09.14^{fg}±0.24	1.49^{f}±0.02
IPC97-72	264.55^{abc}±17.32	197.70^{cd}±5.57	26.13^{b}±1.31	19.57^{a}±0.79	25.08^{fg}±0.39	11.76^{fghi}±0.22	6.21^{efgh}±0.18	2.09^{efg}±0.03	0.94^{defgh}±0.10	0.60^{d}±0.02	10.48^{cd}±0.19	1.28^{g}±0.03
IPC2004-55	261.60^{bcd}±3.49	243.15^{ab}±2.87	27.84^{ab}±0.65	19.77^{a}±0.51	24.72^{fg}±0.31	17.28^{bcd}±0.10	7.72^{ab}±0.82	2.41^{bcde}±0.04	0.83^{fgh}±0.12	0.20^{h}±0.00	09.26^{efg}±0.20	1.18^{g}±0.04
GL28008	217.65^{hi}±7.99	117.83^{ef}±14.74	17.49^{efgh}±0.26	12.36^{cdef}±1.29	30.48^{cdef}±0.16	18.30^{b}±0.24	8.43^{a}±0.36	2.23^{cdefg}±0.43	0.93^{efgh}±0.06	0.19^{h}±0.00	09.22^{efg}±0.92	2.20^{c}±0.08
GL28019	209.85^{i}±6.12	87.75^{f}±17.70	14.77^{ij}±1.31	09.12^{g}±0.75	24.24^{g}±0.59	12.64^{efghi}±0.24	6.13^{efgh}±0.27	2.24^{cdefg}±0.05	0.83^{fgh}±0.05	0.38^{f}±0.00	13.39^{a}±0.64	0.73^{j}±0.03
GL28028	234.90^{fgh}±3.44	220.10^{ab}±9.27	18.63^{efg}±0.52	11.63^{def}±0.57	29.16^{cdefg}±0.16	13.74^{cdefgh}±0.28	5.81^{fgh}±0.09	2.24^{bcde}±0.12	1.06^{cde}±0.19	0.64^{c}±0.01	10.28^{cdef}±0.22	0.62^{ij}±0.01
ICCV2004-53	283.35^{a}±1.02	124.80^{e}±20.83	17.61^{efgh}±0.65	11.27^{defg}±0.41	29.70^{cdefg}±0.63	16.02^{bcde}±0.08	7.06^{bcd}e±0.06	2.89^{a}±0.05	1.08^{cde}±0.02	0.30^{g}±0.01	10.92^{bc}±0.24	2.41^{b}±0.05
ICCV88505	244.05^{defg}±2.74	195.30^{cd}±2.42	15.90^{hij}±0.00	10.47^{fg}±0.59	31.02^{bcde}±0.26	17.58^{bc}±0.15	6.05^{efgh}±0.36	2.07^{efg}±0.00	0.74^{h}±0.02	0.37^{f}±0.00	10.89^{bc}±0.37	1.00^{h}±0.04
PDG84-16	240.15^{efg}±4.44	216.00^{bcd}±10.75	17.04^{efghi}±1.31	11.13^{defg}±1.49	31.80^{bcd}±0.315	16.08^{bcde}±0.13	6.37^{defg}±0.3	2.34^{bcdef}±0.15	1.02^{def}±0.02	0.64^{c}±0.01	11.14^{bc}±0.43	0.62^{ij}±0.03
PG96006	262.35^{bcd}±3.80	226.80^{bc}±4.43	19.31^{def}±1.31	14.81^{b}±0.84	29.04^{cdefg}±0.15	14.82^{bcdef}±0.28	5.33^{h}±0.09	2.22^{cdefg}±0.04	1.26^{bc}±0.07	0.41^{f}±0.00	09.03^{g}±0.50	1.65^{e}±0.04
Mean	248.28	191.03	19.80	13.40	28.29	15.14	6.39	2.31	0.98	0.50	10.43	1.34
Susceptible genotypes												
PBG-1	235.80^{fgh}±3.13	225.40^{a}±7.31	18.63^{efg}±0.52	13.50^{bcd}±1.81	25.20^{efg}±0.36	14.22^{cdefg}±0.07	8.03^{abc}±0.44	2.55^{abcd}±0.19	1.44^{ab}±0.03	0.21^{h}±0.03	7.64^{h}±0.67	0.70^{ij}±0.02
GPF-2	277.65^{ab}±4.33	265.17^{a}±7.23	14.08^{ij}±0.52	08.96^{g}±0.60	33.48^{bc}±0.32	25.14^{a}±0.27	5.81^{fgh}±0.64	2.73^{ab}±0.06	1.54^{a}±0.08	0.23^{h}±0.00	8.26^{h}±0.16	0.27^{j}±0.02
Mean	256.72	245.28	16.36	11.23	29.34	19.68	6.92	2.64	1.49	0.22	7.95	0.49
Total mean	249.22	197.06	19.42	13.16	28.41	15.64	6.45	2.36	1.04	0.47	10.15	1.24

* Data are expressed as mg g-1 ; ** Data are expressed as IU g-1

Values with different letters in the same column are significantly different ($p<0.05$)

processing directly influences the quality and nutritional value of the food and it is saponin B that make up the majority of the saponins in legumes (Hubert *et al.*,, 2005). Saponins have the ability to foam in aqueous solution. Saponin B is more soluble in water due to its sugar chain structure composition. Long time soaking allow the seeds to continuously absorb water to breakdown oligosaccharides attached to aglycone and further soften the tissue matrix to complete hydration (Shimelis and Rakshit 2007). This water absorption resulted in leaching of saponin.

The average amount of tannin was found to be 6.45 mg g^{-1} in chickpea genotypes (Table 2). The highest amount of tannin was observed in GL 28008 (8.43 mg g^{-1}) and lowest in ICCV 96030 (5.25 mg g^{-1}). Ferial and Esmat (2011) reported 4.63 mg g^{-1} tannin content in chickpea seeds. Tannins bind with the proteins of saliva and mucosal membrane of the mouth during food mastication (Akinyede *et al.*, 2005) and have been reported to decrease the digestibility of proteins and carbohydrates, thereby causing growth depression (Vadivel and Janardhanan 2005). Tannins are resistant to degradation, so are likely to remain in the digestive tract and not being absorbed and transported to other tissues. Total phenol content varied from 0.74- 1.54 mg g^{1} with an average value of 1.04 mg g^{-1} (Table 2). The average content of total phenols in tolerant and susceptible genotypes was found to be 0.98 mg g^{-1} and 1.49 mg g^{-1} respectively. Phenolics and tannins are known to inhibit the activities of digestive enzymes and hence the presence of even low levels of these are not desirable from nutritional point of view. In the present investigation, the content of total phenols in tolerant genotypes was found to be lower than susceptible genotypes indicating their better nutritional quality. Total phenols and tannins were reduced by 54.36% and 63.36% respectively after soaking (Table 2). Our results are in confirmation with Hagir *et al.*, (2007) who also reported reduction in total phenols and tannins after soaking. Soaking of soybean and peanut seeds for 6 h soaking followed by 48 h germination on wet muslin cloth significantly reduced the tannin and phenolic content (Rusydi and Azrina, 2012). The loss in phenolic contents in faba beans with soaking, boiling and autoclaving was due to leaching of active compounds into the soaking and cooking medium (Siah *et al.*,, 2013). Shimelis and Rashit (2007) observed that reduction in tannin content after germination was due to the formation of hydrophobic association of tannin with seed protein and also their leaching into water while soaking. Khandelwal *et al.*, (2010) observed that during soaking polyphenol oxidase get activated and results in dehydration and loss of polyphenols.

Bound fructose of raffinose series oligosaccharides in chickpea seeds showed great variation in tolerant genotypes (Table 2). Average content of bound fructose was higher in tolerant genotypes (10.43 mg g^{-1}) as compared to susceptible genotypes (7.95 mg g^{-1}). Bound fructose of raffinose series oligosaccharides is important factor in desiccation stress tolerance but these RFOs also interfere with the digestion. The maximum reduction (87.72%) after soaking was observed

Table 3: Physiochemical characteristics of chickpea genotypes

Tolerant genotypes	Weight of 100 dry seeds(g)	Weight of 100 soaked seeds(g)	Volume of 100 dry seeds (ml)	Volume of 100 soaked seeds (ml)	Hydration capacity ($gseed^{-1}$)	Swelling capcity ($mlseed^{-1}$)	Swelling index	Seed density (gml^{-1}) (dry)	seed density (soaked)
ICCV88503	18.54	38.07	30.00	070.00	0.195	0.400	0.013	0.618	0.546
ICCV88506	18.48	36.31	32.50	080.00	0.178	0.475	0.014	0.609	0.487
ICCV92944	15.05	31.43	27.50	067.50	0.163	0.400	0.014	0.554	0.465
ICCV96029	23.29	46.63	37.50	100.00	0.233	0.625	0.016	0.624	0.466
ICCV96030	21.38	44.67	35.00	105.00	0.232	0.700	0.020	0.610	0.428
IPC98-51	18.88	39.76	30.00	85.00	0.208	0.550	0.018	0.645	0.469
IPC94-94	16.38	34.51	27.50	62.50	0.181	0.350	0.012	0.601	0.234
IPC97-72	18.15	37.94	27.50	77.50	0.197	0.500	0.018	0.661	0.489
IPC2004-55	17.51	37.89	27.50	72.50	0.193	0.450	0.016	0.640	0.522
GL28008	23.97	48.69	37.50	97.50	0.247	0.600	0.016	0.641	0.500
GL28019	22.70	47.36	35.00	97.50	0.246	0.625	0.017	0.648	0.485
GL28028	15.61	33.03	27.50	57.50	0.174	0.300	0.010	0.569	0.574
ICCV2004-53	20.32	40.92	32.50	87.50	0.205	0.550	0.017	0.628	0.467
ICCV88505	19.91	40.44	32.50	75.00	0.205	0.425	0.013	0.615	0.539
PDG84-16	15.36	31.81	25.00	62.50	0.164	0.375	0.015	0.614	0.509
PG96006	25.36	51.41	42.50	105.00	0.260	0.625	0.014	0.598	0.489
Mean	19.43	40.05	31.71	81.40	0.200	0.496	0.015	0.617	0.479
Susceptible genotypes									
PBG-1	11.51	24.13	20.00	45.00	0.126	0.250	0.012	0.575	0.536
GPF-2	12.69	26.14	25.00	50.00	0.134	0.250	0.010	0.507	0.522
Mean	12.10	25.13	22.50	47.50	0.130	0.250	0.011	0.541	0.529
Total Mean	18.61	38.39	30.69	77.63	0.197	0.469	0.015	0.609	0.484

in bound fructose content in chickpea genotypes as compared to other nutritional and antinutritional factors. This implies that accumulation of RFOs in seeds of tolerant genotypes during development may possibly contribute for their tolerance behaviour. This mean higher content of RFOs in tolerant genotypes is an additional characteristic to protect them as it does not interfere with the digestion.

Physiochemical characteristic of seeds: There was a large variation in the 100 seed wt, 100 seed volume and hydration capacity in all the chickpea genotypes (Table 3). It was observed that seed weight, seed volume, hydration capacity, swelling index and seed density was found to be more in tolerant genotypes as compared to susceptible genotypes. Seed size of tolerant genotypes (mean- 19.43g) is more as compared to susceptible genotypes (12.10g). Average value of 100 seed wt., 100 seed volume and hydration capacity were 18.616g, 38.397 ml and 0.197 g seed^{-1} respectively. As physiochemical parameters are found to higher in tolerant genotypes. So, they might be better as compared to the susceptible genotypes in terms of cooking quality. Ozer *et al.*, (2010) also observed that physical properties of chickpea such as seed weight, seed density, moisture content and ash content can influence cooking quality.

Swelling capacity varied from 0.25 – 0.7 ml seed^{-1} with mean value of 0.469 ml seed^{-1} and swelling index varied from 0.01 (GPF-2) – 0.02 (ICCV 96030). Seeds with high hydration and swelling capacity may have coats with greater permeability and softer cotyledons (Ozer *et al.*, 2010). Seed density among chickpea genotypes varied significantly with highest in IPC 97- 72 i.e. 0.661 g ml^{-1} and lowest in GPF-2 i.e. 0.507 g ml^{-1}. In the present investigation the cold tolerant genotypes having higher hydration capacity and swelling index might be important from cooking point of view.

Correlation between biochemical and physiochemical characteristics: Statistical correlation of all physiochemical and biochemical characteristics among chickpea genotypes has been carried out (Table 4). Value of correlation coefficient varied from -0.772 (phytic acid and hydration capacity) to 0.98 (between 100- seed weight and hydration capacity). All the physiochemical parameters were found to be positively and significantly correlated with each other and total sugars. Proteins were found to have significant correlation with hydration capacity. Total phenols were found to be negatively and significantly correlated with seed weight, hydration capacity, swelling index, seed density and bound fructose of raffinose series oligosaccharides. Ozer *et al.*, (2010) also reported significant positive correlation between seed weight, hydration capacity, swelling capacity and swelling index It was observed that tolerant genotypes had lower total phenol

Table 4: Correlation matrix of biochemical and physiochemical characteristics in chickpea genotypes

	Weight of 100-seed	Volume seeds	Hydration capcity	Swelling capacity	Swelling index	Seed density	Total sugars	Protein	Starch	Trypsin inhibitor	Phytic acid	Saponin	Tannin	Total phenols	Bound fructose of RFOs	Zinc	Iron	Copper
`Weight of 100-seed		0.964***	0.980***	0.905***	0.585**	0.598**	0.491**	0.363	-0.176	-0.221	-0.111	-0.132	0.246	-0.463*	0.334	0.121	-0.338	0.273
Volume seeds			0.921***	0.846***	0.440*	0.382	0.008	0.394	-0.142	-0.199	-0.158	-0.094	-0.343	-0.367	0.273	0.290	-0.244	0.279
Hydration capacity				0.913***	0.638***	0.635***	0.045	0.400*	-0.224	-0.215	-0.772***	-0.147	-0.219	-0.434*	0.384	0.112	-0.355	0.241
Swelling capcity					0.846***	0.641***	-0.102	0.241	-0.089	-0.158	-0.048	-0.136	-0.207	-0.409*	0.436*	0.097	-0.113	0.306
Swelling index						0.755***	-0.106	0.009	0.00	0.046	0.133	-0.115	0.003	-0.394	0.479**	-0.141	0.028	0.266
Seed density							0.193	0.035	-0.106	-0.128	0.113	-0.097	0.106	-0.625***	0.308	-0.395	-0.319	0.151
Total sugars								0.161	0.121	0.169	0.069	0.129	0.042	-0.284	0.171	0.025	-0.363	-0.372
Protein									-0.341	-0.074	-0.268	0.086	0.132	-0.018	0.087	0.236	-0.186	-0.485**
Starch										0.438*	-0.204	-0.097	-0.200	0.264	-0.102	0.153	0.242	0.086
Trypsin inhibitor											0.077	0.345	-0.137	0.429*	-0.343	0.297	0.008	-0.201
Phytic acid												-0.312	0.270	-0.280	0.059	-0.209	0.205	0.493**
Saponin													-0.100	0.084	0.112	0.109	0.046	-0.262
Tannin														0.074	-0.281	-0.352	-0.055	-0.294
Total phenols															-0.551**	0.338	0.053	-0.315
RFOs																-0.039	0.062	0.245
Zinc																	0.197	-0.128
Iron																		0.330
Copper																		

Values with *, ***and *** are significant at 0.1, 0.05 and 0.01 level of significance respectively

content and higher bound fructose of raffinose series oligosaccharides. Bound fructose of raffinose series oligosaccharides were significantly positively correlated with swelling index and swelling capacity. It was observed that soaking significantly reduced the bound fructose of raffinose family oligosaccharides. Phytic acid was found to be negatively correlated with the hydration capacity (Table 2). Copper was found to be significantly positively correlated with phytic acid and negatively with the proteins.

Grouping of genotypes based on biochemical and physiochemical parameters: The dendrogram was constructed by using UPGMA (unweighed pair group of arithmetic means) based on biochemical and physiochemical study in raw seeds of chickpea genotypes which separated 18 genotypes into two major clusters- cluster I and II (Fig 1a). The five genotypes were clustered in cluster I, all were found to have high protein and medium starch content which were further classified into two subclusters- A and B. Subcluster A comprised of 3 genotypes out of which two genotypes – ICCV 88503 and IPC 2004-55 also showed great similarity. GL 28008 and GL 28028 were found to be more close as they had high protein, medium starch and four of the antinutritional factors in similar amount in subcluster B. The cluster II grouped 12 genotypes which were further classified into two subclusters – C and D. The subcluster C grouped 7 genotypes which had low to medium level of protein (20-24%), high starch and mineral content. ICCV 96030 and PDG 84-16 were found to be highly related to each other as they had similar content of protein, starch and two antinutritional factors. The susceptible cultivar GPF-2 remained alone in the cluster where PBG-1 clustered with ICCV88506**.** The subcluster D grouped 5 genotypes which had high protein (more than 24%), starch (more than 40%), mineral content and medium phytic acid content. Two of the genotypes – IPC 98-51 and PG 96006 in subcluster D were closely related as they had similar protein and starch content. IPC 97-72 remained unclustered as it had minimum amount of protein (13%) and higher amount of phytic acid.

The genotypes were clustered in into two clusters; – I and II in dendrogram by using UPGMA based on biochemical and physiochemical study in soaked seeds (Fig 1b). The cluster-I was the major cluster which comprised of 14 genotypes out of which 13 were further classified into two subclusters- A and B. Subcluster A is further splitted into five sub subclusters - A_1, A_2, A_3, A_4 and A_5 consisting of 11 genotypes. ICCV88503 and GL 28028 were grouped in A_1 which had high protein, mineral and low hydration capacity, swelling capacity, swelling index and high seed density. A_2 comprised of 4 genotypes having low protein, sugar and high mineral and trypsin inhibitor. A_4 had 3 genotypes, which had high content of protein, starch, trypsin inhibitor and tannins. The sub subclusters A_3 consisted of only one genotype – ICCV 88505 which had high sugar, protein, zinc and low level of five of the antinutritional factors. IPC 94-94 was present in A_5. The susceptible genotypes PBG 1 and GPF 2 were closely grouped in subcluster B. These genotypes showed similarity in having low amount of nutritional and physiochemical characteristics. IPC97-72 remained separate in cluster-I as it had minimum amount of protein content. The four genotypes ICCV 96030, GL 28008, ICCV 2004-53 and GL 28019 were clustered in cluster-II as they all had high protein content, physiochemical characteristics and low trypsin inhibitor activity. Two genotypes IPC98-51, PG96006 were found to be closely related in dendrogram of both the raw and soaked seeds (Figs 1a 1nd 1b). ICCV 96029 and PDG84-16 were found in the same cluster in raw seeds while in soaked seeds, they were found to be closely related. ICCV 96030 cluster differently in raw and soaked seeds. IPC97-72 remained unclustered in both raw and soaked seeds. Susceptible genotypes PBG-1 and GPF-2 grouped together in same cluster in raw seeds whereas in soaked seeds they are closely related.

The Principal Component Analysis of all the traits studied in raw and soaked seeds was done to find relationship between them (Fig-2a). The values of means for all traits in raw seeds comprising of the minimum and maximum values along with the values of standard deviation were calculated. The maximum standard deviation was for starch (45.98) followed by protein (34.35) content showing maximum variation to the data recorded. This also indicated that the genotypes used were of diverse origin.The variation present among chickpea genotypes for protein and starch content depicted scope of variation for the improvement in nutritional quality of the genotypes. The eigen values were calculated to decide the number of factors. Three components /factors were extracted having higher eigen values. These factors contributed to total of 58.38% to variability amongst genotypes studied. PC-I, PC-11 and PC-III explained 31.66%, 14.52% and 12.20% variability. The factor loadings were computed by correlation of the respective principal components with traits under study in raw seeds. The major contributor to PC-I was total phenol followed by trypsin inhibitor, tannin, saponin, zinc, starch, iron and other factors contributed negatively to the PC-I. This means that genotypes with inferior physiological characterstics tended to increase total phenol, saponin, tannin and starch content. In PC-II, protein contributed maximum. The factor loading revealed that minerals followed by starch contributed highly to PC-III.

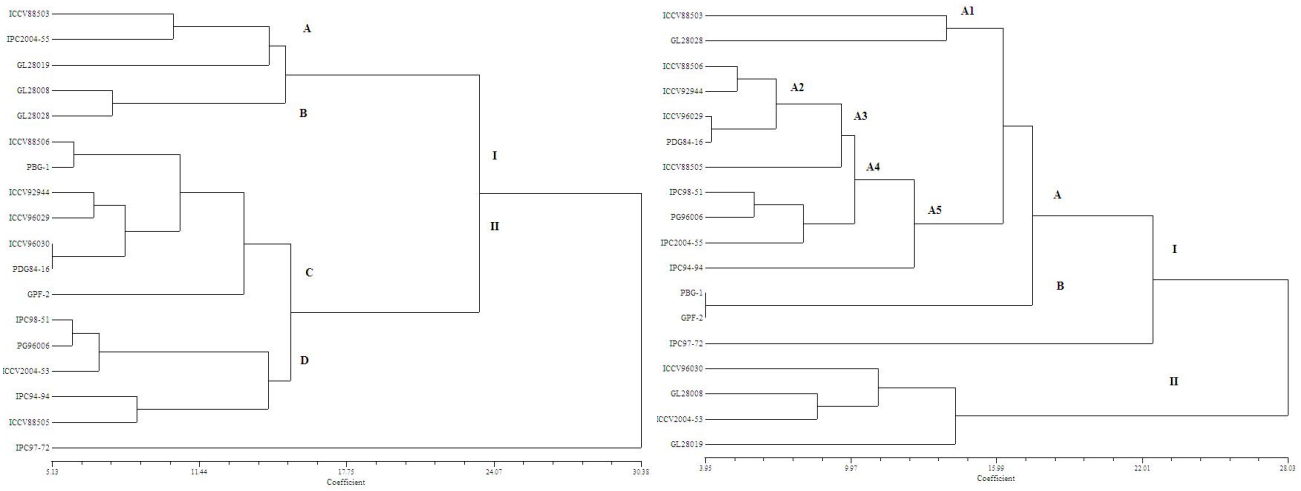

Fig 1: Dendrogram based on physiochemical and biochemical studies in raw (a) and soaked seeds (b) of chickpea genotypes

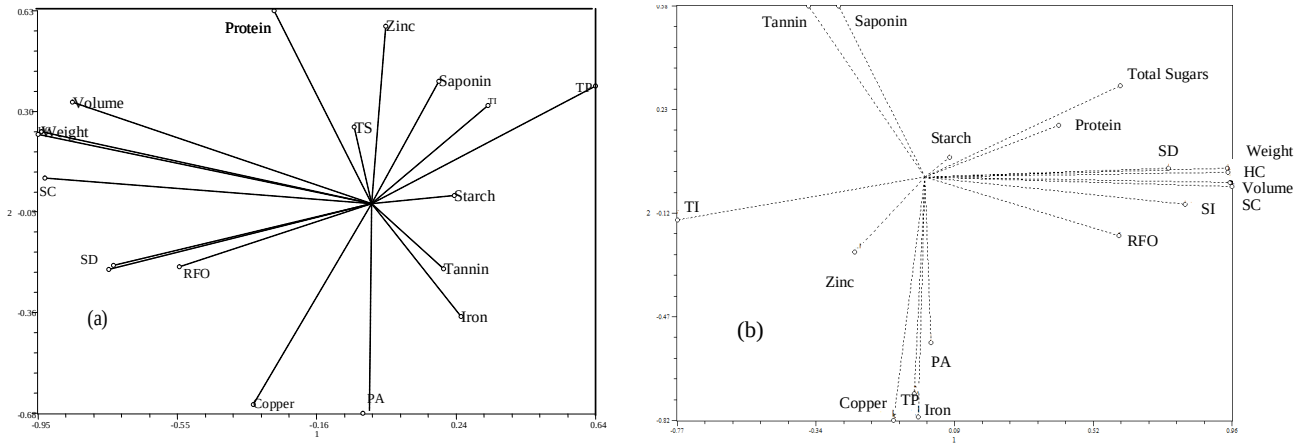

Fig 2: Loading plot of first two components from PCA analysis of different traits of raw (a) and soaked seeds (b) of chickpea genotypes

In PCA of all traits studied in soaked seeds of chickpea genotypes (Fig 2b), The maximum standard deviation was calculated for trypsin inhibitor (50.53) and minimum for swelling index (0.02). PC-I, PC-11 and PC-III contributed 36.56%, 17.33% and 13.21% variability to the total of 67.12% variability. Physiochemical factors, total sugars and raffinose family oligosaccharides contributed highly to the PC-I. Tannins contributed maximum to the PC-11.The major contributor to PC-III was zinc followed by protein, copper and total sugars.

CONCLUSIONS

The present investigation imparts the detailed nutritional attributes of raw and soaked cold tolerant and susceptible chickpea genotypes which would be useful at the consumption level as well as for plant breeders for development of high yielding tolerant genotypes of high nutritional quality. There was significant difference observed in all the biochemical and physiochemical characteristics studied in 16 cold tolerant and 2 susceptible chickpea genotypes. Three of the tolerant genotypes ICCV 88506, GL 28019 and ICCV 88505 had good amount of nutritional components and also contained lower content of antinutritional traits and these can be further used to develop tolerant genotypes of good nutritional quality. 24 h soaking in distilled water reduced all the antinutritional factors. The tolerant genotypes were better in physiochemical characteristics than susceptible genotypes. Thus the cold tolerant genotypes are not only better from nutritional point of view but might be better for cooking quality.

ACKNOWLEDGEMENTS

Thanks are due to University Grants Commission (UGC) New Delhi for funding the present research work.

REFERENCES

Aguilera, Y., Martin-Cabrejas, M. A., Benitez, V., Molla, E., Lopez-Andreu, F. J. and Esteban, R. M. (2009). Changes in carbohydrate fraction during dehydration process of common legumes. *J Food Compost Anal*., **22**: 678-683.

Akinyede, A. I., Amoo, I. A. and Eleyinmi, A. F. (2005). Chemical and functional properties full fat and defatted Dioclea reflexa seed flours. *J Food Agric Environ*., **3**:112"115.

Boye, J., Zare, F. and Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Res Int*., **43**: 414"431.

Chopra, S. and Sankhala, A. (2004). Effect of soaking and sprouting on tannin, phytate and in vitro iron in under – utilized legumes – horse gram (*Dolichos biflorus*) and moth bean (*Phaseolus aconitifolius*). *J Food Sci Technol*., **41**: 547 – 550.

Ferial, M. A. and Esmat, A. A., (2011). Physico – chemical properties of tempeh produced from chickpea seeds. *J Am Sci*., **7**: 107 – 118.

Fenwick, D. E., Fenwick, D. E. and Oakenfull, D. (1983). Saponin content of food plants and some prepared foods. *J Sci Food Agric*., **34**: 186-191.

Gilani, G. S., Cockell, K.A. and Sepehra, E. (2005). Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *J Assoc Anal Chem Int*., 88: 967-987.

Guillamon, E., Pedrosa, M. M., Brubano, C., Cuadrado, C., de Cortes Sanchez, M. and Muzquiz, M. (2008). The trypsin inhibitors present in seeds of different grain legume species and cultivar. *Food Chem.* **107**: 68-74.

Hagir, B. E M., Samia, M. A,R., Wisal, H. I., Amro, B. H., Elfadil, E. B. and Abdullahi, H. E. T. (2007). Content of antinutritional factors and HCl – extractability of minerals from white bean (*Phaseolus vulgaris*) genotypes: Influence of soaking and / or cooking. *Food Chem*., **100** : 362-368.

Hemalatha, S., Patel, K. and Srinivasan, K. (2007). Influence of heat processing on bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J Trace Elem Med Biol*., **21**: 1-7.

Hubert, J., Berger, M. and Dayde, J. (2005). Use of simplified HPLC-UV analysis for soyasaponins B determination: study of saponin and isoflavone variability in soybean genotypes and soy – based health food products. *J Agric Food Chem*., **53**: 3923 – 3930.

Huma, N., Anjum, F.A., Sehar, S., Khan, M. I. and Hussain, S. (2008). Effect of soaking and cooking on nutritional quality and safety of legumes. *Nutr Food Sci*., **38**: 570 -577.

Jain, K. A., Kumar, S. and Panwar, J. D. S. (2009). Antinutritional factors and their detoxification in pulses : A Review. *Agric Rev.*, **30**: 64-70.

Katoch, R. (2013). Nutritional potentialof rice bean (*Vigna Umbellata*): An underutilized legume. *J Food Sci*., **78**: 8-16.

Kaur, S., Gupta, A. K. and Kaur, N. (2000). Effect of GA_3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. *Plant Growth Regul.*, **30**: 61"70.

Kaur, S., Kaur S., Gupta, A. K. and Kaur, N. and Javed M (2014). Biochemical and nutritional characterization of chickpea (*Cicer arietinum*) genotypes. *Indian J Agric Sci.*, **84:** 479–486

Khandelwal,S., Udipi,S.A. and Ghurge,P.(2010). Polyphenols and tannins in indian pulses: Effect of soaking , germination and pressure cooking, *Food Res* Int., **43**:526-530.

Lowry, O. H., Rosebrough, N. T., Farr, A. L. and Randall, R. J. (1951). Protein measurement with folin phenol reagent. *J Biol Chem.*, **193**: 265-275.

Ozer, S., Karakoy, T., Baloch, F. S., Killan, B. and Ozkan, H. (2010). Nutritional and physiochemical variation in Turkish kabuli chickpea (*Cicer arietinum* L.) landraces. *Euphyt.*, **175**: 237-249.

Ramakrishna, V. (2008). Changes in antinutritional factors in Indian bean (*Dolichos lablab* L.) seeds during germination and their behaviour during cooking. *Nutr Food Sci*.,**38**: 6 – 14.

Ramakrishna, V., Rani, J. P. and Rao, P. R. (2006). Antinutritional factors during germination in Indian bean (*Dolichos lablab* L.) seeds. *World J Dairy Food Sci.*, **1**: 6-11.

Rusydi, M. R. and Azrina, A. (2012). Effect of germination on total phenolic, tannin and phytic acid contents in soybean and peanut. *Int Food Res J.*, **19**: 673-677.

Sangronis, E. and Machado, C. J. (2007). Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. *Swiss Soc Food Sci Technol*., **40**:116 – 120.

Sat, I. G. and Keles, F. (2002). The effect of soaking and cooking on the oligosaccharide content of seker a dry bean variety (*P. vulgaris* L.) grown in Turkey. *Pakistan J Nutr*, **1**: 206 – 208.

Siah, S., Wood, J. A., Agboola, S., Konczak, I. and Blanchard, C. L. (2013). Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba* L.) differing in seed coat colours. *Food Chem.,* **142**:461–468

Sharma, S., Yadav, N., Singh, A. and Kumar, R. (2013). Nutritional and antinutritional profile of newly developed chickpea (*Cicer arietinum* L.) varieties. *Int Food Res J.,* **20:** 805-810.

Shimelis, E. A. and Rakshit, S. K. (2007). Effect of processing on antinutrients and *in vitro* protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chem*., **103:** 161 – 172.

Suneja, Y., Kaur, S., Gupta, A.K. and Kaur, N. (2011). Levels of nutritional constituents and antinutritional factors in black gram (*Vigna mungo* L. Hepper). *Food Res Int*., **41:** 621-628.

Tajoddin, M. D., Shinde, M. and Lalitha, J. (2011). In vivo reduction the phytic acid content of mung bean (Phaseolus aureus L) genotypes during germination. *American-Eurasian J Agric Environ Sci*., **10:** 127 – 32.

Vadivel, V. and Janardhanan, K. (2005). Nutritional and antinutritional characteristics of seven indian wild legumes. *Plant Foods Hum Nutri*, **60:** 69"75.

Vasistha, H. and Srivastava, R. P. (2011). Effect of soaking and cooking on dietary fibre components of different type of chickpea genotypes. *J Food Sci Technol.,* **50**: 579-84.

Vasudeva, S. and Vishwanathan, K.H. (2010). Hydration behavior of food grains and modeling their moisture pick up as per Perleg's equation: PartII. Legumes. *J Food Sci Technol* ., **47:** 42-46.

Wang, N., Hatcher, D.W., Tyler, R.T. and, Toews R., Gawalko,E.J. (2010). Effect of cooking on composition of bean (*Phaseolus vulgaris L*.) and chickpeas (*Cicer arietinum L*.). *Food Res Int*, **43:** 589-594.

William, P. C., Singh, K. B. and Nakkoul, H. (1983). Relation of some physical aspects of kabuli type chickpeas to cooking time. *J Sci Food Agric*., **34:** 492 – 496.