Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 685-689
Received: 15-11-2017
Accepted: 16-12-2017

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Physico-chemical and antinutritional studies of chickpea

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Abstract
The physico-chemical composition, antinutritional factors and protein quality of sixteen chickpea genotypes were studied. Proximate composition varied significantly (p<0.05) among different types of chickpea genotypes. The moisture content, 100 seed weight and germination per cent varied from 8.14 to 9.50%, 13.61 to 24.70g and 79.32to 94.84%, respectively among different chickpea genotypes. Among antinutritional factors phytic acid content and polyphenols content in chickpea were ranged from 8.44 to 12.89 mg/g and 72.33 to 108.48mg/100g, respectively. Free fatty acid content in chickpea varied from 2.37 to 2.65 mg KOH/g. In terms of protein quality, the methionine content in chickpea was ranged from 1.17 to 1.54 g/16g N and tryptophan content in chickpea was ranged from 1.08 to 1.45 g/100 g protein. The protein quality and antinutritional quality of chickpea genotypes provide useful information for breeding program and establishes chickpea as component of a balanced diet of underprivileged people.

Keywords: Chickpea, antinutritional factor, methionine and tryptophan

Introduction
Pulses are the second most important group of crops after cereals. Globally, more than two dozen pulse crops are grown. India is the largest producer of pulses in the world with 25 per cent share in the global production. Pulse crops play an important role in vegetarian diet of our country. Legumes are widely grown throughout the world and are an utmost essential part of the human diet since the early ages. In the developing countries legume is an important source of protein for human health. Pulses are rich sources of protein, carbohydrates, vitamins and minerals. Being a rich source of protein, minerals vitamins and crude fiber, pulses are considered as healthy food and offer nutritional security to millions of population suffering with protein malnutrition. Pulses have shown plentiful health benefits, e.g. lower glycemic index for people with diabetes (Chillo *et al*., 2008) [6]. Due to the presence of dietary fiber content in legume it prevents cancer, cardiovascular and many other diseases. The presence of antinutritional components restricts its use by interfering with digestion of carbohydrates and proteins. Chickpea seed have good quality of amino acid composition, high protein bioavailability and relatively low levels of anti-nutritional factors. On the basis of this quality it's considered a suitable source of dietary proteins. The pulse proteins are mainly deficient in sulphur containing amino acids and tryptophan (Shad *et al*., 2009) [21]. Pulses are a good source of the essential amino acid, lysine, but are deficient in sulphur containing amino acids, methionine, cystine and tryptophan (Singh and Jambunathan 1982) [22]. On the other hand, cereals contain lower amounts of protein and are deficient in lysine, but have adequate amounts of the sulphur containing amino acids (*viz*. methionine and cysteine) (Eggum and Beames 1983) [10].
Phytate has a strong binding capacity to form complexes with divalent minerals. At physiological pH most of the phytate-mineral complexes are insoluble and make the minerals like calcium, zinc, magnesium and iron biologically unavailable. Phytic acid is decreases zinc uptake in animals and humans (Davies and Olpin, 1979, Tumlund *et al*., 1984) [9, 25]. Polyphenols are major antioxidants in the human diet and common constituents of foods from plant. Phenolic compound having different biological properties which act as an antioxidant, antiaging, anticarcinogenic, anti-inflammatory activities, cardiovascular protection and improvement of endothelial function. Chickpeas are good sources of protein and carbohydrate. Its protein quality is better than other legumes such as pigeon pea, black gram and green gram (Kaur and Singh, 2005) [13]. According to the size, shape and colour of the seeds, two types of chickpea are usually acknowledged. Kabuli chickpea is large seeded with salmon white testa, is grown mainly in the Mediterranean area, central Asia and America and desi chickpea is

small seeded with a light brown testa, is cultivated mostly in India and east Africa (Rincon *et al*., 1998) [19]. The present experimental study has determined the antinutritional composition and protein quality of the sixteen chickpea genotypes.

Material and methods

The chickpea seed materials were collected from different areas, namely, Indian Institute of Pulses Research, Kalyanpur, Kanpur (U. P.), Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur (M. P.) and Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad (U.P.). The Biochemical analyses were carried out in the Biochemistry and Biotechnology Laboratory, Department of Crop Sciences, Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (M.P.).

Moisture content: The moisture content was determined by the method of AOAC (1999).

Germination per cent and 100 seed weight: Germination tests were conducted according to the procedure described by Vimala and Pushpamma (1983) [26].

Phytic acid: Phytic acid was determined by the following procedure given by Wheeler and Ferrel (1971) [27]. Weighed 0.1 g defatted sample and transferred into a centrifuge tube and mixed with 5% TCA and vortexes the mixture for 30 minutes. The tubes were incubated on water bath at 65^{O}C for 10 minute and centrifuged at 5000 rpm for 15 min. The residue washed three times and transferred into a 25 ml of volumetric flask and volume was made up to 25 ml. In one ml of supernatant 0.5 ml of ferric chloride solution was added. Tubes were heated in a boiling water bath for 45 minutes. The tubes were cooled and volume was made up to 7.5 ml with water. The available ferric ion after the reaction was determined by reaction with potassium- thiocynate which developed a blood-red color compound. The absorbance was recorded at 485 nm against a reagent blank.

Polyphenol: Polyphenol content in chickpea seed was determined as procedure laid down by Malik and Singh (1980) [14]. Weighed 0.5 g of the chickpea seed sample and ground with a pestle and mortar in 10 ml 80% ethanol. Centrifuged the homogenate at 10,000 rpm for 20 minutes and the supernatant was collected. Again re-extract the seed sample with 5 ml ethanol and collected the supernatant and pooled together. The supernatant was evaporated up to dryness on a water bath. Dissolve the residue in 5 ml of distilled water. Take 1.0 ml of aliquots into test tubes and make up the volume 3 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added in each tube. The contents were mixed well and kept in a water bath for 1 minute. Absorbance was recorded at 650 nm against a reagent blank.

Free Fatty acid: Free fatty acid content in chickpea seeds was determined by following the procedure of Cox and Pearson (1962) [7]. Extracted fat was collected in 250 ml of conical flask and to it 25 ml of neutral solvent was mixed along with, few drops of phenolphthalein indicator. The contents were titrated against 0.1 N Potassium hydroxide solutions. Free fatty acids were calculated as the acid value (mg KOH / g).

$$\text{Acid value (mg KOH / g)} = \frac{\text{Titre value x Normality of KOH x 56.1}}{\text{Weight of sample in (g)}}$$

Methionine: Methionine content was determined through procedure given by the Horn *et al*., (1946) [12]. Weighed 0.5 g of defatted powdered sample of chickpea, transferred into a 50 ml conical flask. Then 6 ml of 2N HCl was added into it and autoclaved at 15 lb psi for one hour. A pinch of activated charcoal was added to autoclaved hydrolysate sample and heated to boil. The charcoal was washed with hot water. The filtrate was neutralized with 10 N NaOH to pH 6.5. The volume was made up 50 ml with distilled water after cooling to ambient temperature. The 25 ml solution was transferred in 100 ml conical flask. 3 ml of 10% NaOH was added along with by 0.15 ml Sodium nitroprusside. After 10 minute 1 ml of glycine solution was added. After another 10 minute 2 ml orthophosphoric acid was added and shaked vigorously. The intensity of red color was recorded at 520 nm by spectrophotometer.

$$\text{Methionine content (g/16 g N)} = \frac{\text{Methionine content from graph} \times 6.4}{\text{Percentage of N in sample}}$$

Tryptophan: The tryptophan was determined by the procedure given by Spice and Chambers (1949) [23]. 100mg air-dried powered of the defatted chickpea sample was transferred to a 50 ml conical flask. 30 mg p-Dimethylaminobenzaldehyde and 10 ml of 19N H_2SO_4 solutions was added and shaked well. The test mixture contents were kept for 12 hours at room temperature in the dark for incubation. After incubation the mixtures was centrifuged for 15 min at 5000 rpm and supernatant wascollected. Then 0.1 ml of 0.45% $NaNO_2$ solution was added and mixed well. After 30 min measured the color intensity (blue colour) at 545 nm by Spectrophotometer.

$$\text{Tryptophan content (g/100g protein)} = \frac{\text{Tryptophan \% in sample}}{\text{Protein \% in sample}} \times 100$$

Result and Discussion

The physico-chemical characters *viz.* moisture content, 100 seed weight, germination per cent and antinutritional factor *viz*. phytic acid, polyphenol and free fatty acid and protein quality *viz*. methionine and tryptophan are depicted in Table-1. The colour and their shape was depicted in Table-2. The moisture content in sixteen chickpea genotypes ranged from 8.14% to 9.50%. The chickpea genotype Vishal recorded significantly maximum moisture content 9.50%, followed by RSG-888, RSG-963 and Annigiri-1 with moisture percentage of 9.22%, 9.19% and 9.18%, respectively, whereas the minimum moisture content was recorded in JG-11 (8.14%). The results are fairly comparable with that of Bibi *et al*., 2007 and Sastry *et al*., 2007. 100 Seed weight of sixteen chickpea genotypes varied significantly. 100 Seed weight, among sixteen chickpea genotypes were ranged from 13.61g to 24.70g. JG-130 (J) chickpea genotype was recorded the maximum 100 seed weight 24.70g which was followed by JG-130 (K) 24.41g and Vishal (24.33g), however, minimum 100 seed weight (13.61g) was recorded with the chickpea genotype RSG-888. These findings were in good agreement with the findings of Munirathnam and Sangita (2009) [15]. The germination per cent in sixteen varieties of chickpea were ranged from 79.32% to 94.84%. Among sixteen varieties of chickpea the JG-130 (K) showed maximum germination per cent (94.84%) followed by KPG-59 (94.05%) and Annigiri-1

(94.00%). The minimum germination per cent was recorded in PG-043 (79.32%). This result is also in accordance with the finding of Noura *et al.*, (2014) [16] who reported germination per cent in two chickpea genotypes in the range of 89 to 97 per cent. The phytic acid content ranged from 8.44mg/g to 12.89mg/g in the sixteen chickpea genotypes, showing significant differences between them. KPG-59 contained the highest amount of phytic acid (12.89mg/g) followed by JG-130(K) and CSJ-595 (12.72 and 12.29 mg/g), respectively and RSG-888 was found to contained the lowest amount of phytic acid (8.44mg/g). The phytic acid content ranged from 2.97 to 19.07 mg/g (Oberoi *et al*., 2010) [17]. The polyphenol content in sixteen different genotypes of chickpea were ranged from 72.33 to 108.48 mg/100g. The maximum polyphenol content was recorded in Vijay (108.48mg/100g) followed by Annigiri-1 and JG-63 (104.27 & 102.72mg/100g). The minimum was recorded in DCP-92-3 (72.33mg/100g). The finding is also similar with Srivastava and Srivastava (2004) [24] for polyphenol in chickpea who obtained the range for polyphenols from 0.07 to 0.11 per cent. The free fatty acid content of chickpea genotypes varied significantly. Among the sixteen varieties of chickpea, JG-63 (2.69 mg KOH/g) was found superior genotype than the other. The minimum free fatty acid content was recorded in JG-16 (2.37 mg KOH/g). This results are in accordance with Shad *et al*., (2009) [21] who reported that acid value of chickpea ranged from 2.40 to 2.50 mg KOH/g and Zia-Ul-Haq *et al*., 2007 [28] have reported acid value ranged from 2.55 to 2.73 mg KOH/g in chickpea.

The methionine content in chickpea was ranged from 1.17 to 1.54g/16 g N. Among the sixteen varieties of chickpeas, CSJ-595 (1.54 g/16 g N) was found superior genotypes in respect of methionine content. The findings are fairly similar with EL- Adawy (2002) [11], Alajaji and EL- Adawy (2006) [2], Daur *et al*., (2008) [8], and Abu-Salem and Abou-Arab (2011) [1], reported that methionine content was ranged from 1.54 to 1.6 g/16 g N in chickpea seeds. The tryptophan content in chickpea was ranged from 1.08 to 1.45g/100g protein. Among the sixteen genotype of chickpea JG-130, Kanpur (1.45g/100g protein) showed maximum tryptophan content and the minimum tryptophan content was recorded in RSG-963 (1.08 g/100g protein). These results are found in accordance with Bala *et al*., (1994) [4] who reported that tryptophan content was ranged from 0.41 to 1.45 mg/g in chickpea. The perusal of data (Table 2) revealed that among the sixteen varieties of chickpea, five varieties of chickpea showed yellowish brown color, four varieties showed light brown color, three varieties showed greyish brown color, two varieties showed brown color and two varieties showed reddish brown color. Out of sixteen genotypes ten varieties showed angular shape, five varieties showed the smooth shape and one variety showed smooth and pea shaped. It might be due to variability of the varieties/genotypes to reflect the identity of the each distinct character of the variety. The present finding is similar to Pundir *et al*., 1985 [18].

In respect of protein quality aspects CSJ-595 and JG-130 variety were found best due to fulfill the complete nutrition profile of amino acid and it contain the highest amount of methionine and tryptophan among sixteen varieties and can be include further for hybridization programme to improve protein quality through developing cultivars rich in methionine contents along with high protein content by the process of hybridization in these genotypes. Antinutritional factors in all the varieties were found within permissible limit. Moreover, for human consumption the legumes are processed by various methods which includes soaking, boiling, sprouting, pressure cooking and fermentation process depending upon custom, tradition, choice and taste preferences of the consumers. These processes are helpful to treat and also effective to eliminate and minimize the antinutritional factors.

Table 1: Physico-Chemical and antinutritional factor in chickpea

S. No	Genotype	Moisture %	100 seed Weight (g)	Germination %	Phytic acid mg/g	Total polyphenol mg/100g	Free Fatty acid mg KOH/g	Methionine g/16 gN	Tryptophan g/100 g protein
1	DCP-92-3	8.56	16.14	90.50	11.41	72.33	2.57	1.17	1.08
2	JG-130	8.72	24.41	94.84	12.72	84.57	2.48	1.52	1.45
3	RSG-963	9.19	18.41	90.50	11.16	76.43	2.40	1.44	1.08
4	JG-16	8.47	19.54	92.00	11.63	96.72	2.37	1.41	1.23
5	Annigiri-1	9.18	13.88	94.00	10.31	104.27	2.45	1.34	1.44
6	RSG-888	9.22	13.61	91.50	8.44	88.66	2.67	1.26	1.14
7	JG-130	8.69	24.70	90.50	11.54	92.14	2.46	1.17	1.32
8	JG-63	8.80	16.07	93.00	10.35	102.72	2.69	1.45	1.39
9	VIJAY	8.36	17.62	87.00	11.36	108.48	2.44	1.39	1.34
10	VISHAL	9.50	24.33	89.50	12.09	102.26	2.63	1.50	1.35
11	JG-11	8.14	20.64	91.00	11.31	97.82	2.45	1.53	1.40
12	PG-043	8.58	19.42	79.32	11.90	82.66	2.38	1.17	1.17
13	KPG-59	9.00	17.71	94.05	12.89	86.93	2.41	1.47	1.31
14	NDG8-202	8.69	18.34	88.00	10.53	81.66	2.61	1.25	1.19
15	RKG-137	8.69	21.44	90.50	11.79	82.67	2.63	1.32	1.31
16	CSJ-595	8.51	21.01	91.00	12.29	87.37	2.65	1.54	1.10
G. Mean		8.77	19.20	90.45	11.36	90.48	2.52	1.37	1.27
C.D. at 5%		0.18	0.82	0.13	1.51	14.74	0.16	0.07	0.72
Sem ±		0.62	0.29	0.45	0.41	4.01	0.04	0.26	0.25

Table 2: Colour and Shape of Chickpea

S. No	Genotype	Colour	Shape
1	DCP-92-3	Yellowish Brown	Angular
2	JG-130	Light Brown	Smooth and Pea Shaped
3	RSG-963	Greyish Brown	Angular
4	JG-16	Light Brown	Angular
5	Annigiri-1	yellowish Brown	Smooth
6	RSG-888	Greyish Brown	Angular
7	JG-130	Brown	Smooth
8	JG-63	yellowish Brown	Angular
9	VIJAY	Yellowish Brown	Angular
10	VISHAL	Light Brown	Smooth
11	JG-11	Yellowish Brown	Smooth
12	PG-043	Reddish Brown	Angular
13	KPG-59	Greyish Brown	Angular
14	NDG8-202	Reddish Brown	Angular
15	RKG-137	Light Brown	Angular
16	CSJ-595	Brown	Smooth

Acknowledgements
Authors extended sincere thanks to the Director of IIPR, Kanpur, V.Cs, JNKVV, Jabalpur and NDUA&T, Kumarganj, Faizabad for providing seed material to carry out research work.

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