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Changes in phytic acid, polyphenol, free fatty acid content and calorific value in chickpea varieties during storage

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Abstract

The effect of storage periods on quality of chickpea seeds was studied in Biochemistry and Biotechnology Laboratory, Department of Crop Sciences, Faculty of Agriculture, MGCGVV Chitrakoot, Satna (M.P.). The experiment was laid out in completely randomized design (CRD) with three replications for each variety. The data presented here represent pooled mean of two years. All the sixteen chickpea genotypes were stored at room temperature in different storage materials *viz.* aluminium container box, polythene bag and cotton bag. The observations were recorded at 0 month or before storage, 6 month and 12 month storage periods. During storage study it was recorded that phytic acid, polyphenol and calorific value decreased with increasing storage time while free fatty acid content increased with increasing storage time. The maximum and the minimum loss of both phytic acid and polyphenol content were recorded in cotton bag and aluminium box, respectively during storage of chickpea seeds. The maximum increase of free fatty acid was recorded in aluminium box and minimum increase was observed in cotton bag during storage. The maximum reduction of calorific value was recorded in polythene bag and minimum reduction was observed in aluminium box during storage of chickpea. Antinutritional factors *viz.* phytate and polyphenols were found within permissible limit in all the tested chickpea varieties.

Keywords: Antinutritional factor, chickpea, storage material and storage time

Introduction

The chickpea (*Cicer arietinum* L.) is commonly known as gram, chana, garbanzo bean, Indian pea, Ceci bean and Bengal gram. Chickpea belongs to the family *Fabaceae* and sub family *Papilionaceae* or *Faboideae*. Chickpea are mainly two types Desi and Kabuli. Desi chickpea has small, darker seed and a rough coat and is cultivated in India, Ethiopia, Mexico and Iran. Kabuli has lighter colour, bolder seeds and a smooth seed coat, mainly grown in southern Europe, Northern Africa, Afghanistan, Pakistan and Chile. Chickpea is used as animal feed in many countries in the form of green husk, green or stems straw and leaves are used for livestock feed. Chickpeas, having 21% starch helpful in textile industries for providing light finish to silk, wool and cotton clothes. Among various grain legumes, it is one of the ancient domesticated popular pulse crops of India and has versatile form of uses in both food and feed. Chickpea is considered to have medicinal properties, and it is used for blood purification. Chickpea contains 21.1 per cent protein, 61.5 per cent carbohydrate and 4.5 per cent fat. It is also rich in calcium, iron and niacin (Singh, *et al.*, 2003) [29]. Besides these, chickpea is a leguminous crop; it has added beneficial improvement in soil fertility status and contributing to enhance the yield and protein content of the succeeding cereal crop in the rotation. Chickpea is an important *Rabi* pulse grown in India and the mature seed may be consumed as whole or split into 'dhal', vegetable and its flour used for various preparations. The presence of certain antinutritional factors such as tannins, phytates and trypsin inhibitors showed poor nutritive value. It has been also reported by some authors (Siddhuraju *et al.*, 2000) [28]. Trypsin inhibitors and tannins create hindrance and inhibit the digestibility of protein and starch. The release of essential amino acids particularly, methionine is hampered by the presence of inhibitors. They are heat labile, whereas, Phytic acid reduces the bioavailability of some essential minerals *viz.* iron and zinc *etc.* (Rehman and Shah, 2001) [23]. Storability of seeds mainly depends on several factors such as genetic, initial seed quality, seed size, provenance, storage environment, pest and diseases *etc.*

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It has been also hypothesized that well developed seeds with ample initial capital of food reserves store for longer period than undersized seeds with less food reserves (Oxley, 1948)^[22]. On the contrary, small seeds have also been proved to store for longer period than equal to big and medium seeds (Mohanrao, 1993)^[18]. As greater variations are evident with respect to seed size both in Kabuli and Desi varieties, the research on storage potential of different sized seeds is felt necessary. Most pulse seed should only be stored for 12 months, although longer storage periods are possible with high quality seed provided both grain moisture and temperature within the silo can be controlled. Rapid deterioration of grain quality occurs under conditions of high temperature/moisture and poor seed quality including weathered, cracked and diseased seed. Ideally chickpea needs to be stored at 13% moisture content and at temperatures below 30°C.

Phytate has a strong binding capacity to form complexes with divalent minerals. Most of the phytate-mineral complexes are insoluble at physiological pH and make the minerals like calcium, zinc, magnesium and iron biologically unavailable. There exists a general consensus that phytic acid will decrease zinc uptake in animals and humans (Davies and Olpin, 1979; Tumlund *et al.*, 1984)^[4, 31]. There is strong support for the prevailing opinion that phytates inhibited iron absorption in man (Hallberg *et al.*, 1989; Sandberg and Svanberg, 1991)^[10, 24]. Polyphenols are common constituents of foods of plant origin and are the major antioxidants in the human diet. These compounds possess various biological properties which provide a number of benefits, including antioxidant, apoptotic, antiaging, anticarcinogenic and anti-inflammatory activities, cardiovascular protection and improvement of endothelial function. Food legumes being economical sources of protein, calories, certain vitamin and minerals are an essential component in the diet of 700 million people around the world (Khan 1987)^[14]. However, consumption of legumes is restricted due to the scarcity caused by their present low yields and consequent higher cost and due to certain defects in their food use qualities (Elias and Bressani 1974, Khan and Ghafoor 1978)^[6, 13]. Food energy is potential energy derives from food constituents through process of cellular respiration i.e. the quantity of heat released by a unit weight or unit volume of a substance during complete combustion. Keeping all above facts in view, the present study was carried out to know the changes in phytic acid, polyphenol, free fatty acid content and calorific value in chickpea varieties during storage in different storage materials.

Materials and Methods

The seed materials were collected from different region / areas namely, Indian Institute of Pulses Research, Kalyanpur, Kanpur (U. P.), Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M. P.) and Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) for the purpose of analyzing in the laboratory. The procured seeds were stored at room temperature in three different packaging materials viz. cotton bag, polythene bag and aluminium box. The experiment was laid out in completely randomized design (CRD) with three replications for each variety. The data presented in Table-1, 2, 3 &4 represent pooled mean of two years. The biochemical analysis of procured seed materials were done at zero months (before storage) and the same material analysed at six months interval of storage up to one year i.e. the observations were recorded at 0 month or before storage, 6 month and 12 month storage periods. The 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.).

Phytic acid: Phytic acid was determined by the following procedure given by Wheeler and Ferrel (1971)^[32]. Weighed 0.1 g defatted sample and transferred into a centrifuge tube and mixed with 5% TCA and vortexed the mixture for 30 minutes. The tubes were incubated on water bath at 65°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- thiocyanate which developed a blood-red colour 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)^[16]. 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-extracted the pellets/sediments with 5 ml ethanol and collected the supernatant and pooled together. The supernatant was evaporated up to dryness on a water bath. The residue was dissolved in 5 ml of distilled water. One millilitre (1.0 ml) of aliquots was taken into test tubes and volume was made up to 3 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent was added. After 3 minutes, 2 ml of 20% Na₂CO₃ 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)^[3]. Extracted fat was collected in 250 ml of conical flask and to it 25 ml of neutral solvent was added and 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).

Calorific Value: Calorific value of chickpea seed samples were determined with the help of the Bomb Calorimeter supplied by Macro Scientific Instrument Company (MAC), New Delhi. Weighed 1 g of benzoic acid as standard / sample and a pellet of it were made by the pellet machine. Tied the pellet of benzoic acid with pre weighed 10 cm length cotton thread then tied the pre weighed 15 cm length nichrome wire in both the grooves of bomb. The pellets were hanged on the nichrome wire, and then air tight the lid of bomb. Bomb was connected to the oxygen cylinder through gas pipe. Pressure of bomb was kept below 400 PSI. Knob of oxygen cylinder was closed and bomb was removed from gas pipe connection. Bomb was kept in bomb calorimeter which was filled with two liter distilled water. The electrode of bomb was attached with digital bomb calorimeter. Sensor and stirrer was kept inside the bomb calorimeter. Switched on the digital bomb calorimeter. When the temperature was stable then pressed rise button and temperature was set to zero with the help of

digital knob. Fire button was pressed and note down the maximum rise in temperature from digital bomb calorimeter.

Results and Discussion

The phytic acid, polyphenol, free fatty acid and calorific value are depicted in Table- 1, 2, 3 & 4. The phytic acid content among sixteen varieties of chickpea was ranged from 8.44 to 12.89 mg/g at before storage (0 month). After six month of storage the phytic acid content decreased and ranged from 7.22 to 11.04 mg/g in cotton bag, 7.52 to 11.45 mg/g in polythene bag and 7.66 to 11.83 mg/g in aluminium box. The phytic acid content further decreased and ranged from 5.95 to 9.09 mg/g in cotton bag, 6.60 to 10.23 mg/g in polythene bag and 7.26 to 11.05 mg/g in aluminium box after twelve month of storage. The result regarding phytic acid content of chickpea seed varied significantly. The result indicated a total decrease of phytic acid in stored genotypes of chickpea as compare to fresh sample i.e. before storage of chickpea. Among the sixteen genotypes of chickpea, KPG-59 was found to contain highest amount of phytic acid at six and twelve-month storage during both the year. The maximum loss of phytic acid content was recorded in cotton bag followed by polythene bag and minimum loss of phytic acid was recorded in aluminium box at six and twelve-month storage during both the years. This finding is in conformity with Kaur *et al.* (2014) [12] and Oberoi *et al.* (2010) [20] who have reported that phytic acid content ranged from 4.74 to 20.40 mg/g and 2.97 to 19.07 mg/g in chickpea, respectively in their independent studies. These results were also comparable to those investigated by earlier workers, who reported that phytic acid content decreased during storage in chickpea (Zia-Ul-Haq *et al.*, 2007 [33], Gulati and Sood 1998 [9], El-Adawy 2002 [5], Moreno *et al.*, 2000 [19], Chitra and Singh, 1998) [2]. Jones and Boulter (1983) [11] reported that during storage phytase hydrolyses phytin to release bound calcium and magnesium. It is evidenced from the Table- 2 that the polyphenol content among the sixteen genotypes of chickpea varied significantly. Among the sixteen genotypes of chickpea, Vijay (108.48 mg/100g) was found to contain maximum amount of polyphenol during both the year at 0 month or before storage. The present findings were in good agreement with the findings of Sharma *et al.*, (2013) [26] who reported that polyphenol content in chickpea ranged from 101-178 mg/100g. The result found to be relatively low as compared to already reported literature (Sis *et al.*, 2008) [30]. This variation in polyphenol content might be primarily due to differences in genetical constituents of genotypes. The result indicated a total decrease of polyphenol content in stored samples of chickpea as compared to fresh sample i.e. before storage of chickpea seeds. The maximum reduction of polyphenol content was observed in cotton bag and minimum reduction was observed in aluminium box. The decrease in phenolic constituents with increases storage temperature may be due to oxidative degradation. The result is also similar with Granito

et al., (2008) [8]. The decreases of total phenol content during storage are probably due to oxidation by poly phenol oxidase (PPO) (Altunkaya and Gokman, 2008) [11]. The result presented in Table-3 among the sixteen varieties of chickpea, JG-63 (2.69 mg KOH/ g) was found to contain highest amount of free fatty acid at 0 month or before storage. This results are in accordance with Shad *et al.*, (2009) [25] and Zia-Ul-Haq *et al.*, (2007) [33] who reported that acid value of chickpea ranged from 2.40 to 2.50 mg KOH/ g and 2.55 to 2.73 mg KOH/ g, respectively. The result indicated total increases of free fatty acid in the stored samples of chickpeas as compared to fresh samples i.e. before storage of chickpea. The maximum increase of free fatty acid was recorded in aluminium box and minimum increase was observed in cotton bag. The results are in line with the finding of Gopinath *et al.* (2011) [7], reported that free fatty acid content increased during storage in red gram and green gram. Increases free fatty acid contents were observed during the storage with infestation of other legumes such as cowpea (Ojimelukwe *et al.*, 1999) [21]. The storage enhanced the role of lipolytic activity of grains, which resulted in a decrease of total lipid and triglycerides and in an increase in phospholipids, free fatty acids and peroxide value. The calorific values of all sixteen varieties of chickpea collected from three different locations varied significantly among them are depicted in Table-4. Out of sixteen genotypes of chickpea, Vijay (382.90 kcal/ 100g) was found the superior genotype for calorific value at 0 month or before storage. The findings are much in favour with Shad *et al.* (2009) [25], reported that calorific value ranged from 368-373 kcal /100 g in chickpea. The result is also similar with Khan *et al.* (1995) [15], reported that the calorific value ranged from 324-369 kcal/ 100 g in chickpea. This variation might be attributed due to climatic changes and varietal differences. Total calorific value decreased with the increased storage period. It was evident that total carbohydrate, crude protein and fat content of chickpea seeds decreased during storage, therefore it is concluded that calorific value must also decreased because it is dependent on the content of these three macro molecules. The results are similar with Modgil (2003) [17] who reported that calorific value decreased with storage time increased in green gram. This might be due to consumption of endosperm part rich in carbohydrates, which contributes as major portion in the legumes. The maximum loss of calorific value was recorded in polythene bag and minimum loss was observed in aluminium box. Antinutritional factors *viz.* phytate and polyphenols were found within permissible limit in all the tested chickpea varieties. 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: Phytic acid content (mg/g) in chickpea varieties during storage in different packaging materials

S. No.	Genotype /Varieties	Before storage	After 6 Month			After 12 Month		
			Cotton	Polythene	Aluminium	Cotton	Polythene	Aluminium
			Mean	Mean	Mean	Mean	Mean	Mean
1	DCP-92-3 (C)	11.41	9.87	10.14	10.42	8.20	9.09	9.88
2	JG-130 (K)	12.72	11.04	11.33	11.60	9.09	10.08	11.02
3	RSG-963	11.16	9.57	9.99	10.15	7.73	8.57	9.68
4	JG-16	11.63	9.97	10.43	10.78	8.34	9.23	10.05
5	Annigiri-1	10.31	8.75	9.10	9.40	7.18	7.94	8.84
6	RSG-888	8.44	7.22	7.52	7.66	5.95	6.60	7.26

7	JG-130 (J)	11.54	9.90	10.23	10.51	8.11	9.18	9.87
8	JG-63	10.35	8.86	9.25	9.41	7.47	8.52	9.04
9	VIJAY	11.36	9.60	9.94	10.33	7.81	8.77	9.89
10	VISHAL	12.09	10.50	10.61	10.94	8.29	9.88	10.41
11	JG-11	11.31	9.83	10.12	10.37	8.14	9.15	9.86
12	PG-043	11.90	10.14	10.39	10.78	8.28	9.23	10.25
13	KPG-59	12.89	10.90	11.45	11.83	8.91	10.23	11.05
14	NDG8-202	10.53	9.08	9.45	9.65	7.38	8.29	9.15
15	RKG-137	11.79	10.16	10.41	10.70	8.14	9.34	10.21
16	CSJ-595	12.29	10.47	10.83	11.16	8.67	9.94	10.67
G. Mean		11.36	9.74	10.08	10.36	7.98	9.00	9.82
SEm±		0.40	0.35	0.35	0.37	0.28	0.34	0.36

Table 2: Polyphenol content (mg/100g) in chickpea varieties during storage in different packaging materials

S. No.	Genotypes/Varieties	Before storage	After 6 Month			After 12 Month		
			Cotton	Polythene	Aluminium	Cotton	Polythene	Aluminium
		Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	DCP-92-3 (C)	72.33	52.78	60.71	65.09	16.38	27.34	37.02
2	JG-130 (K)	84.57	63.52	71.04	77.00	17.92	30.99	41.16
3	RSG-963	76.43	55.04	61.89	67.71	17.85	27.89	33.34
4	JG-16	96.72	69.69	78.34	84.10	21.34	36.47	44.48
5	Annigiri-1	104.27	75.36	85.51	92.62	24.48	44.31	52.09
6	RSG-888	88.66	63.57	73.58	78.87	21.40	38.63	46.33
7	JG-130 (J)	92.14	64.51	76.34	81.39	24.29	37.55	44.91
8	JG-63	102.72	81.27	86.68	94.61	22.59	34.60	41.17
9	VIJAY	108.48	73.65	84.78	95.27	28.57	41.16	49.59
10	VISHAL	102.26	81.28	86.29	91.60	26.38	40.68	48.79
11	JG-11	97.82	74.63	82.31	86.44	22.31	36.88	43.56
12	PG-043	82.66	58.98	66.55	73.72	23.75	33.93	40.34
13	KPG-59	86.93	61.56	68.35	74.84	17.87	29.63	41.65
14	NDG8-202	81.66	61.22	68.28	72.54	19.18	29.19	39.61
15	RKG-137	82.67	57.84	65.91	71.27	18.53	28.89	39.21
16	CSJ-595	87.37	66.32	71.29	78.20	19.19	28.33	37.91
G. Mean		90.48	66.33	74.24	80.33	21.38	34.16	42.57
SEm±		3.92	3.27	3.28	3.56	1.29	2.01	1.87

Table 3: Free Fatty Acid content (mg KOH/g) in chickpea varieties during storage in different packaging materials

S. No.	Genotype/Varieties	Before storage	After 6 Month			After 12 Month		
			Cotton	Polythene	Aluminium	Cotton	Polythene	Aluminium
		Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	DCP-92-3(C)	2.57	2.73	2.86	2.95	3.24	3.50	4.29
2	JG-130 (K)	2.48	2.63	2.78	2.92	3.20	3.46	4.06
3	RSG-963	2.40	2.52	2.67	2.81	2.96	3.35	3.88
4	JG-16	2.37	2.50	2.64	2.73	2.83	3.10	3.79
5	Annigiri-1	2.45	2.66	2.81	2.94	2.98	3.48	4.11
6	RSG-888	2.67	2.91	3.03	3.16	3.29	3.47	4.48
7	JG-130 (J)	2.46	2.62	2.71	2.83	2.88	3.27	4.13
8	JG-63	2.69	2.82	2.95	3.15	3.18	3.54	4.59
9	VIJAY	2.44	2.61	2.76	2.95	3.11	3.29	4.04
10	VISHAL	2.63	2.83	2.89	3.14	3.22	3.63	4.21
11	JG-11	2.45	2.61	2.74	2.83	2.95	3.48	3.94
12	PG-043	2.38	2.52	2.64	2.81	2.83	3.32	3.58
13	KPG-59	2.41	2.56	2.66	2.81	2.85	3.42	3.57
14	NDG8-202	2.61	2.77	2.95	3.23	3.12	3.69	4.11
15	RKG-137	2.63	2.84	2.96	3.15	3.29	3.74	4.44
16	CSJ-595	2.65	2.80	2.90	3.02	3.40	3.67	3.83
G. Mean		2.52	2.68	2.81	2.96	3.08	3.46	4.07
SEm±		0.05	0.06	0.06	0.07	0.08	0.07	0.12

Table 4: Calorific Value (kcal/100g) in chickpea varieties during storage in different packaging materials

S. No.	Genotype/Varieties	Before Storage	6 Month			12 Month		
			Cotton bag	Polythene bag	Aluminium box	Cotton bag	Polythene bag	Aluminium box
		Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	DCP-92-3 (C)	348.23	325.60	303.46	340.89	296.62	281.56	311.88
2	JG-130 (K)	340.42	324.69	299.66	333.17	284.62	277.42	308.92
3	RSG-963	358.00	337.35	326.70	340.26	311.34	302.28	325.70
4	JG-16	348.43	331.50	310.09	339.25	294.38	283.59	316.94

5	Annigiri-1	338.77	316.89	293.86	329.28	282.82	272.66	307.79
6	RSG-888	355.97	315.57	307.53	344.45	295.69	287.41	303.40
7	JG-130 (J)	334.63	305.73	294.15	319.26	280.37	264.75	301.60
8	JG-63	354.66	333.13	319.21	344.32	308.68	292.06	330.98
9	VIJAY	382.90	323.97	307.83	346.97	307.36	291.29	325.98
10	VISHAL	367.99	329.75	315.46	347.15	301.82	295.29	326.01
11	JG-11	350.91	320.44	310.40	329.35	294.96	284.03	308.61
12	PG-043	378.54	321.15	302.60	345.67	285.98	271.69	314.47
13	KPG-59	368.83	309.52	296.65	331.10	285.84	265.75	310.02
14	NDG8-202	343.66	323.15	294.56	337.02	285.81	268.04	311.13
15	RKG-137	357.12	325.00	307.17	348.33	289.94	276.18	320.14
16	CSJ-595	365.46	326.18	306.61	341.45	289.55	273.00	313.18
G. Mean		355.91	323.10	305.99	338.62	293.49	280.44	314.80
SEM±		5.28	3.14	3.47	3.45	3.58	4.12	3.23

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