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Saponins and Lectins of Indian Chickpeas (*Cicer arietinum*) and Lentils (*Lens culinaris*)

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Varying genotypes of chickpeas and lentils were evaluated for saponins and lectins. Chickpeas and lentils contain two types of sapogenols - sapogenol A and sapogenol B. Morphologically different genotypes of chickpea *viz*., BG 256, JG 74, KWR 108, DCP 92-3, KAK 2, JKG 1, BG 1053, L 550 and Sadabahar were differing in their sapogenol A and B contents. Sapogenol A in grain of chickpea genotypes varied in the range of 211.9±1.8 mg/100g to 352.2±1.2 mg/100g and sapogenol B from 413.5±0.5 to 568.8±0.3 mg/100g. Total sapogenol of chickpea grain ranged between 651.0±0.1 mg/100g and 860.2±0.3 mg/100g. Highest sapogenol B and total sapogenols were present in JKG 1 and KAK 2 genotypes of chickpeas. The lectins in chickpeas were present in the range of 1160±3.6 to 1375 ±4.3 HU/g grain. Varying types of lentil genotypes *viz*., DPL 15, DPL 58, DPL 62, PI 4, PL 406, PL 639, JL 1, VL 1, K 75 and Ranjan also differed in their sapogenols and lectins significantly. Sapogenol A and B in lentils were present in the range of 198.1±0.1 to 332.8±0.8 mg/100g and 361.0±0.3 to 452.9±0.5 mg/100 g, respectively whereas total sapogenol was found in the range of 569.6±0.1 to 700.5±0.5 mg/100 g grains. Lentils contain almost half of the lectins than chickpeas i.e. in the range of 513±1.5 to 617±1.5 HU/g.

Key words: Chickpeas, lentils, sapogenol A and B, total sapogenols, lectins

In recent years, consumption of food legumes such as chickpeas, lentils, peas and beans has been gaining popularity not only in Asia but also in western countries, including the USA, because of their health advantages. Chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) are important food legumes of the world (1). Both of these are important food crops of India as well as of many tropical countries. Major chickpea and lentil producing countries are India, Turkey, Pakistan, Canada, US and Syria. Chickpeas and lentils are consumed in the form of decorticated split seed (*dahl)* alongwith cereals such as rice, wheat and maize, and also in the preparation of snacks and sweets. These are excellent source of protein, carbohydrate, dietary fibre, minerals and vitamins. However, several anti-nutritional compounds are found in biologically significant amount in the raw seed (2). Common anti-nutrients present in food legumes are enzyme inhibitors, phytates, flatulence factors and lectins (2-5). Processing such as soaking, dehusking and cooking reduces these anti-nutrients and enhances the digestibility and availability of nutrients (6,7). Soaking helps in removing major anti-nutrients and enhances the digestibility of grain. Cooking makes the grain edible by making them tender and also aids in the flavour development. The degree of removal or reduction of toxic constituents depends on duration of treatment and stability of compounds.

Among the naturally occurring compounds of food legumes, saponins are attracting considerable interest as a result of their diverse properties both deleterious and beneficial (8,9). Saponins are responsible for haemolytic, membranolytic, and fungitoxic activities. Certain beneficial effect of saponins such as lowering of plasma cholesterol levels in humans (10,11), anticarcinogenic activity (12,13), inhibitory effect on infectivity of HIV *in vitro* (14), antioxidant activity of saponins (15), and protective effect on liver injury (16). Considerable quantity of soyasaponins are reported in soybean and beans (17-19). Soyasaponins are classified into two major groups, soyasaponin A and B (20). Group A acetylated saponins present in soybean are mostly responsible for undesirable bitter and astringent taste (21,22), whereas Group B saponins possess several health benefits (23). Recent *in vitro* studies have established that the health benefits such as hypocholesterolemic (cholesterol lowering) effect, anti-carcinogenic, anti-oxidative, anti-tumor, anti-virus, anti-

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hepatic, anti-diabetic and hepato-protective properties of food legumes are due to presence of group B saponins (24). Dietary saponins of soybeans are advantageous in preventing hypercholesterolemia and aortic atherosclerosis in rats (25). Group A saponins are naturally occurring form, and Shiraiwa *et al* (26) identified six different saponins, designated as Aa, Ab, Ac, Ad, Ae and Af, according to their elution order from high performance liquid chromatography (HPLC). However acid hydrolysis of all six saponin A compounds yielded the common aglycone, soyasapogenol A (27). The other soyasaponins, which have been isolated as soyasaponins I, II, III and IV and V contain soyasapogenol B as the common aglycone (20, 28). The total soyasaponin content is approximately twice the total soyasapogenol content (29). Soyasaponin I and VI of chickpeas and lentils were reported by Ruiz *et al.* (30) Saponin and sapogenol contents of seed of different varieties of legumes were reported by Price *et al.* (31) and Shi *et al.* (18) reported degradation of saponin B during cooking and autoclaving of navy beans. Rupasinghe *et al*. (27) developed a simple HPLC method for quantitation of sapogenol A and sapogenol B in soyabeans. Taking advantage of this technique, chickpea and lentil genotypes were studied for their sapogenol A and B contents.

Lectins are proteinaceous toxic factors that interact with glycoprotein on the surface of red blood cells and causing them to agglutinate. Food legumes have long been known to contain protein compounds which agglutinate the red blood cells (32,33). Haemagglutinating properties of several tropical seeds have been studied earlier. Complete reduction of haemagglutinating activity has been reported, when heated at 100°C under moist conditions (33).

Materials and Methods

***Seed samples*:** Nine genotypes of chickpea *viz*., BG 256, JG 74, KWR 108, DCP 92-3, KAK 2, JKG 1, BG 1053, L 550 and Sadabahar 112, and ten genotypes of lentils *viz.,* DPL 15, DPL 58, DPL 62, PI 4, PL 406, PL 639, JL 1, VL 1, K 75 and Ranjan were selected for this study. The seeds of these genotypes were collected in triplicate from the crop grown at Indian Institute of Pulses Research, Kanpur, India. The seeds of all the genotypes were dried at 70 ºC in an oven and powdered to a uniform particle size in a seed grinder Perten model 3303 and analysed for saponins and lectins.

***Determination of saponins and lectins*:** The saponins were analysed by hydrolysing them to sapogenols using technique as described by Rupasinghe *et al.* (27). These sapogenols (A and B) were quantified by HPLC as per method of Shi *et al.* (18) using C-18 reverse phase column of 150 mm x 4.6 mm i.d. x 5 µm particle size and UV detector (205 nm). The quantification of sapogenols A and B was done using standard curve of sapogenol A and B obtained from ChromaDex (USA). The Shimadzu HPLC model 10Avp fitted with quaternary pump, autosampler, controller, oven and UV detector was run isocratically at a flow of 0.9ml/min acetonitrile: water in the proportion of 66:34. The sapogenol A and B were calculated in samples of chickpea and lentil genotypes and reported as mg/100 g grain on dry weight basis. Lectins were quantified by haemagglutinating test as per method of Grant *et al.* (32) and reported as haemagglutinating units (HU) per gram grain on dry weight basis. The results are shown in table 1 and 2 for sapogenols and lectins of chickpea and lentils respectively.

***Statistical analysis* :** The Statistical Package for the Social Sciences (SPSS, version 13) was used for all data analysis. The data were analysed by one- way analysis of variance (ANOVA). Significance of the differences was defined as $P<0.05$.

Results and Discussion

Chickpeas: The saponins are the glycosides of sapogenol A and B. Wide variability exists in sapogenol A and B contents of grain of different genotypes of chickpeas (Table 1). The sapogenol A content was found in the range of 211.9 to 352.2 mg/100 g in seed. Highest sapogenol A was found in KAK 2 and BG 256 genotypes of chickpeas. The sapogenol B content was found in the range of 413.5 to 568.8 mg/100g in the grain of chickpea genotypes. Highest sapogenol B was found in JKG 1 genotype of chickpeas, whereas KWR 108 and

JG 74 had the least sapogenol B. Total sapogenol content of seed in different genotypes was found in the range of 651.0 to 860.2 mg/100 g grain (Table 1). Highest total sapogenol was present in JKG 1 and KAK 2 genotypes, whereas BG 1053 and KWR 108 had the least total sapogenol. The saponins earlier were considered as anti-nutrients, are now being considered beneficial due to their cholesterol lowering effect (10,11) and cancer preventive (12,13) properties. It also helps in protection of liver (16) and infectivity against HIV (14). Wide variability exists in different types of chickpeas grown in different countries. Devi and Kurup (34) have reported 0.2 – 0.3 % saponins in chickpea and black gram on dry weight basis. Price *et al.* (35) have reported 0.23% saponin content in chickpea. Ireland and Dziedzic (28) have reported presence of two saponins in chickpeas. Savage and Deo (35) have reported 0.4% saponins in chickpea. Tava *et al.* (36) have reported 1.77 and 2.19g/100g of sapogenol B in *desi* and *kabuli* types, respectively. Kerem *et al.* (37) have reported 2.5% saponin in chickpea on dry weight basis.

Lectin content in chickpea grain was relatively high and present in the range of 1160 to 1375 HU/g grain, and the average lectin in the grain of chickpea was 1255 HU/g grain (Table 1). The genotypes differed significantly for their haemagglutinating activity. However, highest activity was shown by BG 256 genotype of chickpeas. The lectins are responsible for degeneration of red blood corpuscles, hence required to be destroyed before consumption of pulses by processing techniques (32,33).

Lentils: Lentil is having relatively low sapogenols than chickpeas. Sapogenol A in raw seed of different genotypes of lentil was found in the range of 198.1 to 332.8 mg/100g (Table 2). Average sapogenol A in grain of lentil was 267.4 mg/100g. Highest sapogenol A in lentil was found in K 75 (332.8 mg/100g) and lowest in Ranjan variety (198.1 mg/100g). Sapogenol B in raw grain of different genotypes of lentil was found in the range of 361.0 to 452.9 mg/100g. Average sapogenol B in lentil genotypes was 377.5 mg/100g. Highest sapogenol B was present in DPL 62 (452.9 mg/100g) and lowest in JL 1 (361.0 mg/100g). Total sapogenol in raw grain of lentils was present in the range of 569.6Hu/g to 700.5mg/100g. Average total sapogenol in lentil genotypes was 644.9 mg/100g. Highest total sapogenol was in K 75 (700.5 mg/100g) and lowest in Ranjan (569.6 mg100g) genotypes. Ruiz *et al*. (38) have reported two saponins in lentil in the range of 0.09 to 0.11%. They have reported that the saponin content of seeds with a brown testa was significantly lower than those with a beige or green testa. Sagratini *et al.* (39) have reported wide variability in two saponins of 32 samples of lentil. Savage and Deo (35) have reported that the saponin content in lentils was in the range of 0.37 to 0.46%.

Table 1: Sapogenols and lectins in chickpea genotypes

Genotypes	Sapogenol A	Sapogenol B	Total Sapogenol	Lectins
BG 256	322.8±1.2	460.0±1.0	782.8±0.7	1375±4.3
JG 74	242.2±1.2	425.2±0.2	667.4±0.4	1246±1.0
KWR 108	241.0±1.0	413.5±0.5	654.5±0.5	1360±3.6
DCP 92-3	249.0±1.0	435.6±0.3	684.3±0.3	1209±2.6
KAK 2	352.2±1.2	490.8±0.8	843.0±0.5	1215±2.0
JKG 1	291.5±0.5	568.8±0.3	860.2±0.3	1225±2.6
BG 1053	211.9±1.8	439.3±0.3	651.0±0.1	1278±2.0
L-550	293.3±0.5	444.3±0.3	737.5±0.5	1160±3.6
Sadabahar	262.3±1.2	478.8±0.8	744.0±0.5	1226±1.0
Mean	274.0	461.81	736.07	1255
SEm	0.92	0.47	0.37	2.2
CD 0.05	1.93	0.98	0.77	4.5

S Em - Standard error of mean; C D - Critical difference at 5%

Table 2: Sapogenols and lectins in lentil genotypes

Genotypes	Sapogenol A	Sapogenol B	Total Sapogenol	Lectins
DPL 15	248.7±1.7	379.6±0.1	628.6±0.6	614±1.0
DPL 58	307.2±0.2	368.8±0.0	676.1±0.5	609±2.0
DPL 62	205.4±0.4	452.9±0.5	658.4±0.4	600±3.0
PL 4	289.6±0.6	369.7±0.8	659.3±0.8	580±0.5
PL 406	291.1±1.1	362.9±1.1	654.0±0.0	517±0.5
PL 639	257.0±0.1	375.7±0.8	632.7±0.7	513±1.5
JL 1	317.6±0.1	361.0±0.3	678.6±0.6	572±0.5
VL 1	226.1±1.1	365.5±0.6	591.6±0.1	617±1.5
K 75	332.8±0.8	367.6±0.2	700.5±0.5	612±0.5
Ranjan	198.1±0.1	371.5±0.6	569.6±0.1	617±2.0
Mean	267.4	377.5	644.9	585
SEm	0.7	0.5	0.4	1.2
CD 0.05	1.4	1.0	0.9	2.6

S Em - Standard error of mean; C D - Critical difference at 5%

The lentils have relatively low lectins, hence causes lower haemagglutinating activity (Table 2). The range of lectins in raw grain of different genotypes of lentils was 513 to 617 HU/g. Average lectin in lentil genotypes was 585 HU/g. High lectins were observed in VL 1, Ranjan, DPL 15, DPL 58 and K 75 genotypes and low lectins were present in PL 639 and PL 406. The lectins are heat labile and degrades with moist heat i.e. during cooking or pressure cooking. The pressure cooking has been suggested for complete destruction of lectins in food legumes (33).

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