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Changes in sapogenols in lentils (*Lens culinaris*) during soaking and cooking

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Lentils (*Lens culinaris* Medik.) are one of the oldest known crops. People in the Near East produced them for the first time more than 8000 years ago. Like most legumes, lentils have a high nutritious value, consisting of about 25% protein, 60% carbohydrate and 1.0% fat. In particular, they are a good source of essential amino acids, such as lysine and arginine. Furthermore, lentils contain dietary fiber, iron, folate, magnesium and zinc (Ruiz *et al*. 1996, Popelka *et al*. 2004). Lentils are a rich, inexpensive source of protein, both for human and animal nutrition, which can complement cereal protein for several essential amino acids. Besides these lentils also contain saponins, high levels of saponins reduce micronutrient bioavailability, but beneficial effects also have been reported (Thompson 1993). By virtue of their purported intestinal action, saponins could provide a non-systemic alternative to the commonly used HMGCoA reductase inhibitors (statins). Because of their complementary mechanism of action, saponins could be particularly well suited for combination therapy with statins or other systemic hypolipidemic agents (Morehouse *et al.* 1999). Saponins have also been reported to provide resistance against insect in legumes (Applebaum *et al.* 1990).

Certain beneficial effect of saponins such as lowering of plasma cholesterol levels in humans (Lee *et al.* 2005), anticarcinogenic activity (Nishino *et al.* 1986), inhibitory effect on infectivity of HIV *in vitro* (Nakashima *et al.* 1989), antioxidant activity of saponins (Yoshiki and Okuda 1995), and protective effect on liver injury (Kuzuhara *et al.* 2000). Considerable quantity of saponins are present in peas and beans (Ireland and Dziedzic 1987, Shi *et al*. 2009). Soyasaponins are classified into two major groups, soyasaponin A and B (Gurfinkel and Rao 2002). Group A acetylated saponins present in soybean are mostly responsible for undesirable bitter and astringent taste, whereas Group B saponins possess several health benefits (Rao and Gurfinkel 2000). Recent *in vitro* studies have established that the health benefits such as hypocholesterolemic (cholesterol lowering) effect, anti-carcinogenic, anti-oxidative, anti-virus and hepato-protective properties of food legumes are due to presence of group B saponins (Fournier *et al.* 1998). Group A saponins are naturally occurring form, and Shiraiwa *et al.* (1991) 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 (Rupasighe *et al.* 2003). The other soyasaponins, which have been isolated as soyasaponins I, II, III and IV and V contain soyasapogenol B as the common aglycone (Berhow *et al.* 2002). The total soyasaponin content is approximately twice the total soyasapogenol content (Gestetner *et al.* 1966).

Lentil saponins are triterpene glycosides, mainly group A and group B saponins. The hydrolysis of saponins releases two sapogenols, viz. sapogenol A and sapogenol B. These sapogenols can be quantified by HPLC using ELSD (Rupasinghe *et al.* 2003) or UV detector (Shi *et al.* 2009). Owing to the nutraceutical role of saponins in human health, this study was carried out to work out composition of sapogenols in varying genotypes of lentils and their changes during soaking and cooking.

Three groups of lentil genotypes, viz. small, medium and bold seeded were selected for this study. The seeds of small seed genotypes (PL 4, PL 639 and VL 1), medium seed genotypes (DPL 15, K 75, PL 406, JL 1 and Ranjan) and bold seed genotypes (DPL 58 and DPL 62) were collected in triplicate from the crop grown at Indian Institute of Pulses Research, Kanpur, India during 2005-06. One smaller lot of the seed (50g) was dried at 70 °C and powdered to a uniform particle size in a seed grinder Perten model 3303, and the remaining bigger lot of seeds (450 g) was used for processing techniques such as soaking and cooking. The seeds of all the genotypes were soaked in water (1:7) for 10-12 hr and the

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water was discarded. The soaked seeds were crushed in a mortar and pestle and analysed for saponins. Moisture content was worked out in soaked seeds. The soaked seeds were also cooked in a pressure cooker. The cooked seeds were then crushed and used for analysis of saponins. Moisture content was also worked out in the cooked seed so as to convert data of analysis on dry weight basis. The saponins were analysed by hydrolysing them to sapogenols using technique as described by Rupasinghe *et al.* (2003). These sapogenols (A and B) were quantified by HPLC as per method described by Vasishtha and Srivastava (2011) using C-18 reverse phase column of 150 mm × 4.6 mm i.d. × 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 0.9 ml/min acetonitrile:water in the proportion of 66:34. The sapogenol A and B were calculated in unprocessed, soaked and cooked samples of lentil genotypes and reported as mg/100 g grain on dry weight basis. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 13 and Duncan´s new multiple range test was used to compare means of the treatments. The data are presented as means ± standard deviation and the significance of the differences were defined as $P<0.05$. The results of sapogenol A, B and total in unprocessed, soaked and cooked grains in different genotypes of chickpeas during soaking and cooking are reported in Table 1, 2 and 3.

Table 1 Effect of soaking and cooking on sapogenol A, sapogenol B and total sapogenol of lentils

Processing technique	Sapogenol A	Sapogenol B	Total sapogenol
Raw (unprocessed)	267.4±47.0[a]	377.5±27.1[a]	644.9±40.0[a]
Soaked	243.5±47.0 [a]	319.8±35.0[b]	563.3±48.9[b]
Cooked	245.8±44.0 [a]	0±0.0[c]	245.8±45.9[c]
SEm	37.8	20.8	36.4
CD (P=0.05)	77.5	42.7	74.6

Values are Mean ± standard deviation

All means bearing different superscripts in columns are significantly different on application of Duncan´s new multiple range test (P< 0.05)

Saponins undergo changes during soaking and cooking of grain of lentils. On soaking, the sapogenol A reduced by 8.9%, but the reduction was not significant (Table 1). Average content of sapogenol A on soaking was 243.5 mg/100g. Highest sapogenol A on soaking was in K 75 (315.4 mg/100g) and lowest in DPL 62 (183.7 mg/100g) (Table 2). On cooking, average content of sapogenol A in seed was 245.8 mg/100g. High sapogenol A was observed in cooked grain of JL 1, K 75, PL 406 and low sapogenol A in DPL 62 and Ranjan varieties. The sapogenol A was not affected during pressure cooking of soaked seed (Table 1).

Soaking as well as cooking influenced sapogenol B content of the grain, significantly. All the varieties responded in the same manner except magnitude of variation in sapogenol content was differing. On soaking, average sapogenol B for all the varieties was 319.8 mg/100g. The range of sapogenol B for different varieties, on soaking was 295.7 mg/100g to 410.6 mg/100g (Table 2). During soaking, sapogenol B reduced significantly by 15.3% (Table 1). The sapogenol B was completely lost during cooking in all the varieties of lentil. Similar results were reported for navy beans by Shi *et al.* (2009). Vasishtha and Srivastava (2011) have reported a reduction of 93.3% in sapogenol B content during cooking of dehusked pigeonpea. The sapogenol B of

Table 2 Sapogenol A and B in unprocessed, soaked and cooked seed of lentil genotypes

Genotypes	Sapogenol A			Sapogenol B		
	Unprocessed	Soaked	Cooked	Unprocessed	Soaked	Cooked
DPL 15	248.7±1.73	202.5±0.60	215.4±0.36	379.6±0.15	312.6±0.65	ND
DPL 58	307.2±0.25	275.4±0.39	258.7±0.17	368.8±0.05	345.7±0.09	ND
DPL 62	205.4±0.45	183.7±0.09	196.1±0.12	452.9±0.47	410.6±0.10	ND
PL 4	289.6±0.58	258.7±0.19	278.9±0.98	369.7±0.81	310.7±0.10	ND
PL 406	291.1±1.15	276.9±0.09	295.7±0.68	362.9±1.08	302.7±0.05	ND
PL 639	257.0±0.05	243.6±0.14	236.9±0.27	375.7±0.78	315.7±0.18	ND
JL 1	317.6±0.15	291.3±0.27	302.6±0.61	361.0±0.30	303.2±0.14	ND
VL 1	226.1±1.10	202.4±0.36	210.6±0.15	365.5±0.57	298.9±0.19	ND
K 75	332.8±0.85	315.4±0.36	284.4±0.36	367.6±0.25	302.3±0.32	ND
Ranjan	198.1±0.10	185.4±0.36	178.9±0.91	371.5±0.57	295.7±0.18	ND
Mean	267.4	243.5	245.8	377.5	319.8	ND
SEm	0.68	0.26	0.45	0.48	0.21	
CD (P=0.05)	1.41	0.55	0.93	1.00	0.44	

Mean values of three determinations ± standard deviation

Table 3 Total sapogenol in unprocessed, soaked and cooked seed of lentil genotypes

Genotypes	Total sapogenols		
	Unprocessed	Soaked	Cooked
DPL 15	628.6±0.58	515.2±0.16	215.4±0.36
DPL 58	676.1±0.50	621.0±0.05	258.7±0.17
DPL 62	658.4±0.42	594.3±0.34	196.1±0.12
PL 4	659.3±0.83	569.4±0.38	279.0±0.98
PL 406	654.0±0.05	579.6±1.75	295.7±0.68
PL 639	632.7±0.73	559.3±0.32	236.9±0.27
JL 1	678.6±0.64	594.5±0.51	302.6±0.61
VL 1	591.6±0.15	501.2±0.05	210.6±0.15
K 75	700.5±0.50	617.7±0.30	284.4±0.36
Ranjan	569.6±0.08	481.0±0.04	178.9±0.91
Mean	644.9	563.3	245.8
SEm	0.42	0.50	0.45
CD (P=0.05)	0.88	1.05	0.93

Mean values of three determinations ± standard deviation

lentil was much lower than pigeonpea.

Total sapogenol in soaked seed of different varieties of lentil was in the range of 501.2 to 621.0 mg/100g (Table 3). The average of total sapogenol in soaked seed of all the genotypes was 563.3g/100g. Applebaum *et al.* (1969) have reported very high saponins (6.6%) in lentil on fat free basis, which consisted of sapogenol and sugar moiety. Gestetner *et al.* (1966) reported that the sapogenol:sugar ratio was constant (1:1) in saponins of soybeans so that the saponin content could be calculated from the yield of sapogenols. Total sapogenol reduced significantly by 12.6% during soaking. Highest total sapogenol was observed in soaked seed of DPL 58 (621.0 mg/100g) and lowest in VL 1 variety (501.2 mg/100g). The average total sapogenol in cooked grain of lentil was 245.8 mg/100g. Total sapogenol in different genotypes was present in the range of 178.9 to 302.6 mg/100g. Soaking and cooking of lentil leads to a significant reduction of 61.9% in total sapogenol of grain (Table 1). Duhan *et al.* (2001) reported a reduction of 28 to 38 % in total saponin content of pigeonpea during cooking of dehusked grain. Vasishtha and Srivastava (2011) reported a reduction of 64.0% in total sapogenol content of dehusked pigeonpea during cooking.

The left over sapogenols A, B and total in grain after cooking will be responsible to provide health benefits of saponins to human beings. The larger proportion of saponins in cooked grain is therefore considered a positive trait of the food legumes from health point of view.

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SUMMARY

Ten varying genotypes of lentil, viz. DPL 15, DPL 58, DPL 62, PL 4, PL 406, PL 639, VL 1, JL 1, K 75 and Ranjan were subjected to soaking and cooking. Soaking and cooking had significant effect on the sapogenols of lentils. Sapogenols are triterpenoid of saponins and produced on hydrolysis of saponins, which are responsible for protection against cancer and tumor, hypocholesterolemic and hepato-protective benefits of health. Sapogenol A was not affected significantly during soaking as well as cooking of lentils. A reduction of 15.3% in sapogenol B was observed during soaking and complete loss of sapogenol B was noticed during pressure cooking of soaked grain. Total sapogenol decreased by 12.6% and 61.9% during soaking and pressure cooking of grain, respectively. There was a wide variability in sapogenol A, B and total in different genotypes in unprocessed, soaked and pressure cooked grain of lentils. The unprocessed grains of lentils had 198.1 to 332.8 and 362.9 to 452.9 mg/100g sapogenol A and sapogenol B, respectively.

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