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Effect of traditional methods of processing on the nutrient contents and some antinutritional factors in newly developed cultivars of green gram [*Vigna radiata* (L.) Wilezek] and black gram [*Vigna mungo* (L.) Hepper] of Assam, India

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Abstract: The aim of this study was to compare the effects of various processing treatments (soaking, pressure cooking and germination) on two newly developed cultivars of each green gram (Vigna *radiata* (L.) Wilezek) and black gram (*Vigna mungo* (L.) Hepper). The present study revealed higher nutrient content in black gram than in green gram. The antinutritional factors were found to be higher in black gram than in green gram. Significant reductions in antinutritional factors were observed due to the processing treatments. Germination increased the protein content to the highest level in both the cultivars (24.04-25.35% in green gram and 28.57-28.82% in black gram) and the highest vitamin C content in SBC 47 (5.55 mg/100g) as compared to the other treatments. Starch content was found to be lowest in germination in SGC 20 (32.52%) and SBC 40 (37.92%) followed by soaking in SGC 16 (34.80%) and SBC 47 (38.18%). Amongst the various processing treatments, pressure cooking was found to be most effective in the retention of the nutrients in both the gram cultivars.

Keywords: Traditional processing, nutrients, antinutritional factors, green gram, black gram, Assam (India)

Introduction

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. Legumes not only add variety to diet but also serve as an economical source of supplementary proteins for a large human population. In India they provide the only high protein component of the average diet and over 10 million tones are consumed annually (Bishnoi, 1992; Sood *et al*., 2002). Grain legumes are being cultivated in India since time immemorial. They have high total protein content (20-26%) and can be considered as a natural supplement to cereals. After fish (dry) which provides 335g protein per kg, grain legumes provide 220-250 g protein per kg. Hence legumes are considered as a “poor man’s meat”. The Green revolution has not increased the productivity of pulses; instead its emphasis on cereals has often led to decreased legume production. Therefore in the present context of our economic development exploitation of legumes in the diet in combination with cereals to make it nutritionally balanced appears to be the only feasible approach to eliminate “protein calorie” malnutrition in the near future.

Green gram (*Vigna radiata*) and black gram (*Vigna mungo)* are two of the most important food legumes grown and consumed in India. But till today only a few aspects of their nutritional composition have been studied (Grewal and Jood 2006; Khattak *et al.* 2007; Khattak *et al.,* 2008). Legumes unfortunately contain greater varieties of toxic constituents than any other plant family. The toxic compounds consist of some flavonoids, alkaloids, tannins, cyanogenic compounds, phytate and trypsin inhibitors. For human consumption the legumes are processed by various methods which include soaking, boiling, sprouting, pressure cooking and fermentation depending upon tradition and taste preferences. These processing treatments are also effective in eliminating the antinutritional factors. Effect of the various processing treatments on the nutritional and anti nutritional composition of legumes especially green and black gram have also been studied (Chang *et al.,* 2008; Khattab *et al.,* 2009).

Assam and the North-Eastern region of India

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being a rich reservoir of different germplasms of pulses, various locally developed cultivars of legumes are grown here. The present investigation was undertaken to study the chemical compositions of two newly developed cultivars each of green gram (*Vigna radiata*) and black gram (*Vigna mungo)* grown in different agro climatic conditions of Assam and the effect of various processing treatments with reference to nutritional and antinutritional factors.

Materials and Methods

Sample collection and preparation

Two newly developed mature seed materials each of green gram (SGC 16 and SGC 20) and black gram (SBC 40 and SBC 47) were collected from Regional Agricultural Research Station, Assam Agricultural University, Nagaon, Assam, India. Seeds were cleaned for wholesomeness after discarding broken hull seeds, shriveled seeds, seeds having off color, taste and foreign material. The seeds were washed and sun dried and kept in good quality glass containers at room temperature (27±1^{0}C) for about 4 weeks.

Processing

Traditional domestic processing techniques like soaking, cooking and germination of seeds were employed to observe the nutritional variation as affected by these treatments. The results were compared with the raw (grinding) seeds. The grinding was done using pestle and mortar. Soaking of seeds were done by soaking with distilled water for 24 h. For pressure cooking seeds were initially soaked with distilled water for 24 h and then cooked in a pressure cooker (105±2^{0}C) for 15 min at 1 kg/cm^2 steam pressure. For germination, seeds were kept on a moist filter paper in a covered petriplate with 29^{0}C for 48-72 h. After every treatment the seeds were grounded finely and kept in air tight container till analysis was completed.

Chemical analysis

Protein was estimated by Lowry *et al.* (1951). 2% of Sodium Carbonate and 0.5% of Copper Sulphate was prepared. The moisture content of the sample was analyzed by the AOAC (1984) method. Crude fiber content was analyzed by the method developed by Sadasivam and Manickam (1992). Reducing sugar was estimated by the dinitrosalicylic acid (DNS) method (Miller, 1972). Non-reducing sugar was estimated by the method developed by Malhotra and Sarkar (1979). Starch content was analyzed by the method developed by Hedge (1962). Vitamin C content was estimated by the method developed by Sadasivam and Balasubraminan (1987). Trypsin Inhibitor Activity was determined as per the method described by Thimmaiah (1999). Phytic acid was determined by the method developed by Sadasivam and Manickam (1992). Tannin content was determined by Folin-Denis method (Sadasivam and Manickam, 1992). Data were analyzed following standard statistical procedure of CRD (Completely Randomized Design) experimental design as per procedure laid down by Panse and Sukhatme (1978) and using the software STATISTICA.

Results and Discussion

Effect of various processing methods on nutrients

Moisture content in both the cultivars of green gram and black gram with different methods of processing varied significantly (Table 1). In both the cultivars of green gram and black gram highest moisture content was obtained in pressure cooking followed by soaking, germination and control. Similar trends have been reported by several workers in legumes and pulses (Chakraborty 1993; Bhagya *et al.,* 2007; Ghavidel *et al.,* 2007; Khattack *et al.,* 2007)

Total ash was recorded highest in control over the other processing treatments in both the cultivars of green gram and black gram (Table 1). There was little variation in ash content of matured seeds in the cultivars of both the legumes. The decrease in ash content might be due to the loss of minerals during the process of treatments employed. Many workers (Barman 1998; Kaur and Kapoor, 1991; Osman, 2007; Meinirs *et al.,* 1976; Bhagya *et al.,* 2007) also supported similar results which can be correlated with our present findings. Crude fiber in raw seeds of both the cultivars of green gram (4.12% and 4.07% in SGC 16 and SGC 20 respectively) and black gram (4.90% and 4.58% in SBC 40 and SBC 47 respectively) registered highest as compared to the various processing treatments. Lowest levels of crude fiber in green gram (2.60% and 2.73% in SGC 16 and SGC 20 respectively) and black gram (3.18% in SBC 40) was found in pressure cooked samples for 15 minutes and in case of SBC 47 the lowest was with germination. Chetia (1991) reported that the decrease in the crude fiber content can be attributed to the dilution effect on nutrients in processed and cooked samples with the increase in the moisture content. Similar results was also reported by Rajaram and Janardan (1990) in *Vigna* spp and Bhagya *et al.* (2007) in a wild mangrove cultivar.

Protein content in green gram and black gram

Table1. Effect of various processing treatments on chemical composition of newly developed green gram and black gram cultivars of Assam, India

Crop	Genotype	Treatments	Moisture (%)	Ash (% dry wt.)	Crude fiber (% dry wt.)	Protein (% dry wt.)	Fat (% dry wt.)	Starch (% dry wt.)	Reducing sugar (mg/100g)	Nonreducing sugar (mg/100g)	Ascorbic acid (mg/100g)
Green gram	SGC 16	Control	9.16	3.27	4.12	23.96	1.60	56.87	724.97	7.10	3.79
		Soaking	39.37	2.85	3.74	22.90	1.27	34.80	741.95	7.99	3.02
		Cooking	42.05	3.01	2.60	21.75	1.32	44.86	793.88	8.58	4.68
		Germination	35.63	2.98	3.56	25.35	1.14	37.55	749.32	8.19	5.15
		S.Em±	0.33	0.21	0.04	0.03	0.07	0.18	1.94	0.14	0.76
		C.D. at 5%	0.73	0.45	0.09	0.06	0.15	0.39	4.22	0.31	1.66
	SGC 20	Control	9.26	3.22	4.07	22.96	1.67	57.23	729.23	7.11	4.77
		Soaking	40.26	2.90	3.79	23.87	1.29	35.23	748.28	7.85	4.76
		Cooking	41.49	2.94	2.73	19.75	1.27	43.42	765.30	8.65	4.59
		Germination	34.98	2.88	3.22	24.04	0.99	32.52	738.75	8.14	5.09
		S.Em±	0.53	0.17	0.06	0.03	0.10	0.31	2.02	0.10	0.11
		C.D. at 5%	1.15	0.38	0.13	0.07	0.22	0.68	4.39	0.21	0.24
Black gram	SBC 40	Control	8.70	3.67	4.90	27.87	1.54	41.72	742.45	7.81	3.84
		Soaking	36.98	3.35	3.86	26.83	1.19	39.31	751.05	8.17	4.78
		Cooking	39.33	3.01	3.18	25.20	1.28	38.45	788.85	8.49	3.95
		Germination	32.10	2.84	3.57	28.82	1.15	37.92	748.55	8.18	5.27
		S.Em±	0.34	0.12	0.09	0.09	0.05	0.50	3.39	0.06	0.35
		C.D. at 5%	0.74	0.26	0.20	0.19	0.09	1.08	7.39	0.14	0.77
	SBC 47	Control	8.59	3.18	4.58	27.15	1.26	41.42	731.03	3.84	4.51
		Soaking	35.76	3.09	4.23	27.30	1.27	38.18	751.28	4.78	4.67
		Cooking	41.73	2.95	3.90	26.27	0.99	39.04	801.03	3.95	5.32
		Germination	35.20	2.20	3.68	28.57	1.13	38.22	739.63	5.27	5.55
		S.Em±	0.59	0.09	0.03	0.57	0.06	0.21	5.69	0.35	0.20
		C.D. at 5%	1.28	0.19	0.06	1.23	0.12	0.45	12.39	0.77	0.43

cultivars in different processing treatments varied significantly (Table 1). Pressure cooking showed highest decrease in protein content in both the green gram (21.75% and 19.75% in SGC 16 and SGC 20 respectively) and black gram cultivars (25.20% and 26.27% in SBC 40 and SBC 47 respectively). Zia-ur-Rahman *et al.* (2005) reported the reduction in protein content during cooking of some food legumes. The highest protein content was observed in germinated seeds in both the green gram (25.35% and 24.04% in SGC 16 and SGC 20 respectively) and black gram cultivars (28.82% and 28.57% in SBC 40 and SBC 47 respectively). Several other workers also observed increased protein content after germination in legumes (Ghavidel *et al.,* 2007; Osman 2007).

Untreated seeds (control) exhibited the highest crude fat content which under different processing methods showed gradual decrease and the lowest value was observed in germinated seeds in green gram (1.14% and 0.99% in SGC 16 and SGC 20 respectively). While in black gram cultivars lowest fat content was observed with germination in SBC 40 (1.15%) and cooking registered the lowest value in SBC 47 (0.99%). Ghavidel *et al.* (2007) also reported significant reduction of fat content on germination in some lentils, green gram, cow pea and chick pea. The decrease in fat content in cooking and soaking as compared to untreated seeds can be corroborated with the findings of other workers (Osman, 2007; Murthy and Uns, 1979; Ramakrishna *et al.,* 2006).

Highest level of starch was recorded in untreated seeds of green gram (56.87% and 57.23% in SGC 16 and SGC 20 respectively) and black gram cultivars (41.72% and 41.42% in SBC 40 and SBC 47 respectively) as compared to soaking. Comparison among three different processing methods, the loss of starch over the control was highest during germination. The present results are in accordance with several workers (Jood *et al.,* 1988; Zia-ur-Rahman *et al.,* 2005; Ramakrishna *et al.,* 2006).

There was significant variation in reducing sugar content of green gram and black gram cultivars in response to different processing treatments. Pressure cooking evinced highest reducing sugar content in both green gram (793.88 mg/100g and 765.30 mg/100g in SGC 16 and SGC 20 respectively) and black gram cultivars (788.85 mg/100g and 801.03 mg/100g in SBC 40 and SBC 47 respectively). Cooking and germination also caused significant increase in the reducing sugar content. The reducing sugar observed by Chakraborty (1993) in the five cultivars of green gram ranged from 641.61 mg/100g to 794.50 mg/100g. Jood *et al.* (1988) also reported that cooking, autoclaving and germination increased the level of total reducing sugars in chick pea and black gram which supports the present results.

Pressure cooking of seeds exhibited the highest levels of non reducing sugars in green gram (8.58 mg/100g and 8.65 mg/100g in SGC 16 and SGC 20 respectively) and in SBC 40 of black gram cultivars (8.49 mg/100g). The observed pattern of changes in green gram and black gram (Table 1) are in consistent with those of several other workers (Jood *et al.,* 1988; Rao and Belavody, 1978).

Vitamin C content of green gram and black gram cultivars showed a significant variation among the various processing methods and germination evinced highest content (5.15 mg/100g and 5.09mg/100g in SGC 16 and SGC 20 of blackgram and 5.27 mg/100g and 5.55 mg/100g in SBC 40 and SBC 47 of green gram). Similar results were also reported (Bhagya *et al.,* 2007).

Effect of various processing methods on antinutritional factors

Effect of different processing treatments on trypsin inhibitor activity (TIA) of green gram and black gram cultivars has been presented in Table 2. The variation in TIA content in the seeds of different cultivars used in the present study may be attributed to their genetical variation causing the synthesis and deposition of this antitrypsic protein to different extent. Green gram cultivars, *viz.,* SGC 16 and SGC 20 showed significant difference in TIA in response to various processing treatments employed. Unprocessed raw seeds (control) of green gram recorded the highest level of TIA (2566.63 TIU/g and 2509.98TIU/g in SGC 16 and SGC 20 respectively) which was followed by germination of seeds. However, the data presented in Table 2, clearly showed that germination of seeds was more effective than soaking, in removing TIA. Since trypsin inhibitors are low molecular weight proteins, their extraction from seeds to the soaking medium is quite possible. A decrease in TIA during germination may perhaps be because of mobilization and breakdown of chemical constituents including trypsin inhibitor. Lowest TIA level was recorded against pressure cooking of seeds. This may be due to heat labile nature of trypsin inhibitor. Cooking generally inactivates heat sensitive factors such as trypsin inhibitors as a result of denaturation of these heat-liable proteins. Therefore, pressure cooking for 15 min. was found to be most effective treatment in reducing TIA level, in green gram. Between two cultivars of green gram, SGC 16 was found to have more amount of TIA than SGC 20. Black gram cultivars possessed a lower TIA than green gram cultivars as presented

Table 2. Effect of various processing treatments on antinutritional factors of newly developed green gram and black gram cultivars of Assam, India

Crop	Genotype	Treatments	Trypsin inhibitor content (TIU/100g)	Phytic acid (mg/100g)	Tannin (mg/100g)
Green gram	SGC 16	Control	2566.63	664.76	509.25
		Soaking	1145.36	550.80	329.25
		Cooking	591.95	430.62	418.25
		Germination	1341.84	452.80	307.00
		S.Em±	24.32	2.61	4.13
		C.D. at 5%	52.98	5.68	9.00
	SGC 20	Control	2509.98	692.40	534.25
		Soaking	1251.61	545.47	382.50
		Cooking	596.63	489.35	383.75
		Germination	1271.38	460.99	284.25
		S.Em±	53.73	20.62	53.18
		C.D. at 5%	117.09	44.94	115.87
Black gram	SBC 40	Control	2463.25	1147.03	859.29
		Soaking	1077.01	744.60	664.28
		Cooking	652.50	546.78	546.56
		Germination	1071.05	718.08	526.37
		S.Em±	26.34	5.43	7.71
		C.D. at 5%	57.40	11.82	16.79
	SBC 47	Control	2442.38	1124.22	868.16
		Soaking	1281.41	734.19	676.66
		Cooking	570.89	616.78	571.28
		Germination	490.39	734.78	514.63
		S.Em±	14.45	5.97	3.46
		C.D. at 5%	31.49	13.01	7.54

in Table 2. Like green gram cultivars, processing methods significantly lowered TIA in black gram also. Highest TIA level was recorded against the control (2463.25TIU/g and 2442.38TIU/g in SBC 40 and SBC 47 respectively). Pressure cooking was found to be most effective in reducing TIA in SBC 40 and in SBC 47, but germination caused highest decrease in TIA values. Ramakrishna *et al.* (2006) reported that the raw dry Indian bean (*Dolichos lablab L.*) having a very high trypsin inhibitory activity which progressively decrease by 51% during the 12 h soaking period and further reached to 17% at 32 h germination period. Grewal *et al.* (2006) reported that soaking (12 h) reduced the TIA by 7 and 6%, respectively, in both the cultivars of green gram. Khattab *et al.* (2009) reported that soaking had reduced the TIA by 10.22–19.85% in cowpea, pea and kidney bean. Osman (2007) also reported that soaking of the *Dolicos lablab* bean overnight reduced TIA by 6.3% and cooking of soaked beans caused further reduction in TIA content (66.7%).

Experimental findings on phytic acid content of green gram and black gram cultivars, under different processing treatments along with the control revealed (Table 2) that phytic acid content of raw black gram was much higher than those present in the raw green gram seeds. Between the two cultivars of green gram, SGC 20 showed higher phytic acid content than SGC 16. The variation that had been found among the different cultivars was due to the differential accumulation depending on genetic control of their synthesis that influences deposition. The observed value of phytic acid in green gram conforms well to the values reported by Kataria *et al.* (1989) in green gram. There was highly significant variations in phytic acid content of green gram cultivars due to various processing treatments. Highest level of phytic acid was found in control seeds which subsequently decreased due to various treatments. In SGC 16, highest reduction of phytic acid was achieved during pressure cooking, and in SGC 20, it was during germination. Pressure cooking caused 35.2% and germination caused 33.4% decrease of phytic acid content over the control (unprocessed) values. The decrease might be attributed to leaching of the antinutrients into soaking water under the influence of concentration gradient, which governs the rate of diffusion. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. Similar losses of phytic acid during soaking and germination have been reported by Grewal *et al.* (2006). Khattab *et al.* (2009) reported that soaking caused a 42.82 – 48.91% reduction in phytic acid content. This could be due to the fact that phytic acid in dried legumes exists wholly as a water-soluble salt presumably as potassium phytate (Crean and Haisman, 1963). Effect of various processing treatments in lowering the phytate content of black gram was also found to be significant. Raw unprocessed seeds registered highest phytate level which gradually came down when seeds were subjected to soaking, pressure cooking and germination. Pressure cooking of seeds recorded the lowest phytate level (546.78 mg/100g and 616.78 mg/100g in SBC 40 and SBC 47 respectively). Therefore, this treatment was proved to be the most effective in lowering phytic acid level in black gram. The observed reduction in phytic acid content of legume seeds during heat treatments may be partly due to the heat labile nature of phytic acid and the formation of insoluble complexes between phytate and other components (Udensi *et al*., 2007). Crean and Haisman (1963) also reported that in cooking, phytic acid combines with the calcium and magnesium of the seeds to form insoluble calcium and magnesium phytates. The reduced phytate value observed during different processing treatments might be attributed to the heat effect and changed permeability of seed coat.

Data on tannin content of green gram and black gram cultivars as affected by various processing treatments, depicted higher level (Table 2) of tannin in black gram cultivars than the green gram cultivars. The wide variation of tannin content recorded in the present study, in two different legumes may be due to the variation of pigment in the seed coat of green gram and black gram. Tannin content of green gram cultivars varied significantly due to various processing treatments. Tannin content present in raw seeds of green gram showed a sequential decline with pressure cooking, soaking and germination. Germination of green gram seeds reduced tannin content to a greater extent compared to other treatments (307.00 mg/100g and 284.25 mg/100g in SGC 16 and SGC 20 respectively). So, this treatment was found to be most effective in destroying tannin content in green gram seeds. Khattab *et al.* (2009) reported highest reduction of tannins content with boiling followed by autoclaving and microwave cooking in some legumes such as cowpea, pea and kidney bean. The reduction of tannins after soaking, boiling, microwave cooking and autoclaving is mainly due to the fact that those compounds, in addition to their predominance in seed coats are water soluble and consequently leach into the liquid medium (Reddy and Pierson, 1994; Kumar *et al*., 1979). This decrease could also be related to the fact that these compounds are heat labile and degrade upon heat treatment (Rakic *et al.,* 2007). Tannin content of black gram cultivars also showed significant variation due to different processing treatments. Highest amount of tannin was present in unprocessed (control) seeds (859.29 mg/100g and 868.16 mg/100g in SBC 40 and SBC 47 respectively) which significantly decreased with subsequent soaking, cooking and germination. Germination was found to be most efficient in reducing the tannin content (526.37 mg/100g and 514.63 mg/100g in SBC 40 and SBC 47 respectively) in black gram seeds. These results are in line with those of Rehman and Shah (2005) who stated that tannin content of black grams, red kidney bean and white kidney bean significantly reduced after ordinary cooking and pressure cooking at 121^0C for 20 min, respectively.

Conclusion

The results obtained in the present study showed that amongst the various processing treatments, pressure cooking was found to be most effective in retention of the nutrients in cultivars of both the legumes. While for the removal of the antinutritional factors, both pressure cooking and germination were found to be most effective among all the processing treatments.

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