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Domestic processing effects on physicochemical, nutritional and anti-nutritional attributes in soybean (*Glycine max* L. Merill)

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Abstract

Effects of soaking and cooking methods on physicochemical characteristics, nutrients and antinutrients in twenty soybean genotypes were studied. Batches of seeds were soaked for 18 h in distilled water, 1% citric acid and 2% sodium bicarbonate solutions at room temperature and then boiled in water. Raw soybean genotypes exhibited 36.5-43.2% protein, 20.7-22.2% oil, 2.5-8.3% total soluble sugars, 1.1-10.4% sucrose, 11.1-18.8 mg/g tannins, 14-36.2 mg/g phenols, 5.1-24.5 mg/g phytate, 30-102.5 mg/g trypsin inhibitor activity and 9.3-27 mg/g saponins. Soaking in distilled water and/or different solutions followed by cooking resulted in significant reductions in the levels of protein, oil and antinutrients and enhanced the carbohydrates in soybean seeds. Cooking of soaked seeds resulted in higher losses of antinutrients in comparison to unsoaked seeds. Among the various treatments, soaking in 1% citric acid solution followed by cooking for 30 min resulted in maximum reduction in most of the antinutrients studied.

Introduction

Food legumes are an important part of average human diet as they are one of the important sources of protein, carbohydrate, dietary fibers, vitamins and minerals. Soybean contains approx 40-45% protein and 18-22% oil (Goyal *et al*., 2012) and is a rich source of vitamins and minerals. Raw soybean contains a number of antinutritional factors such as trypsin inhibitors, phytic acid, saponins and phenols etc which decrease nutritive value of grain legumes and cause health problems to both human and the animals when taken in large amounts (Mikic *et al*., 2009; Sharma *et al*., 2011). Trypsin inhibitors can block either trypsin or chymotrypsin, reduce the hydrolysis of dietary protein, decrease amino acid absorption and thereby reduce digestibility (Roy *et al*., 2010). Phytic acid chelates mineral nutrients such as copper, zinc, manganese, iron and calcium thus reducing their availability (Ramakrishna *et al*., 2006). Phenols and tannins or their oxidized products can form complex with amino acid, protein, enzymes and also adversely affect their digestibility (Khandelwal *et al*., 2010). These antinutrients should be removed to improve the nutritional quality and organoleptic acceptability of legumes so that they can be effectively used as potential human food.

The antinutrients present in the raw seed are partly removed during domestic processing resulting in improving its nutritional quality. Different processing techniques such as soaking, cooking and germination etc are employed which causes number of physicochemical, biochemical, nutritional and sensory changes in legumes. These processing methods can enhance the nutritional value of soybean by increasing the availability of amino acid, vitamins and also protein digestibility (Prodanov *et al*., 2004). Soaking reduces the antinutritional factors such as protease enzyme inhibitor, phytic acid, certain minerals and α-galactosides due to their partial or total solubilization and removal with discarding solution (Pordanov *et al*., 2004). Soaking is also employed prior to number of other processing treatments such as germination, cooking and fermentation. During cooking, seeds are presoaked prior to boiling in water until they become tender but can also cause loss of certain essential nutrients for eg. water soluble vitamins can leach into the cooking medium during boiling (El-Adawy, 2002). Boiling generally decreases naturally occurring heat sensitive antinutritive factors and volatile compounds (Akande and Fabiyi, 2010).

In North-west India especially Punjab, there is alarming situation since last few years as water tables are depleting due to continuous adaptation of wheat-rice cropping system by the farmers. There is urgent need for crop diversification and soybean is considered one of the important leguminous crop as it can replace rice in wheat-rice cropping cycle, has less water requirements and can fix nitrogen symbiotically. Previous work has been done in our laboratory where germplasm available in Institute

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has been screened for nutrients, antinutrients and bioactive compounds (Goyal *et al*., 2012). Stability of the selected genotypes on the basis of their high nutritional quality and antinutrients has been studied under agroclimatic conditions of Punjab. In the present investigation, the effect of different processing treatments on physicochemical, nutritive and antinutritive properties of twenty selected soybean genotypes has been carried out.

Materials and Methods

Seeds

Seeds of twenty soybean genotypes were procured from Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. The seeds were hand cleaned, freed from broken and damaged seeds, dust and other external materials followed by different soaking treatments prior cooking.

Soaking Treatment

Hundred seeds of each genotype were separately soaked in distilled water, sodium bicarbonate solution (2% w/v) and citric acid (1% w/v) and were allowed to imbibe in respective solutions overnight with seed to solution ratio of 1 : 3 (1 part of seed and 3 parts of solution) at 25°C. The soaking solution was drained off and rinsed twice with distilled water followed by hot air oven drying at 50-60°C for 24 h.

Cooking/Boiling Treatment

Pre soaked soybean were kept in one litre beaker containing water and were boiled on a hot plate until they became tender. After boiling the excess water was drained off and the soybean seeds were further dried in hot air oven at 55°C for 24 h. Later, seeds were crushed into fine powder by Cemotec 1090 sample mill and then respective chemical analysis was carried out on these samples.

Physicochemical characteristics and nutrients

Physical seed characteristics viz seed weight (g), seed volume (ml), seed density, water absorption (%), volume expansion (%), swelling capacity (g/seed) and hydration capacity (ml/seed) and their indices were determined by the methods of Santhan and Shivshankar (1978). The contents of protein and lipid in seed powder were determined by NIR Method using Infratec TM 1241 Grain analyzer from Foss (North America). Soluble sugars were extracted from the soybean seed powder with 80% ethanol followed by 70% ethanol. Total soluble sugar levels in the pooled extract were determined with the phenol-sulfuric acid reagent (Dubois *et al.*, 1956) using glucose as standard. Sucrose content was determined after destroying the free fructose with 30% KOH by resorcinol-HCl procedure (Roe, 1934).

Antinutrients

Trypsin inhibitor (TI) activity was determined as described by Kakade *et al.* (1974). One unit of trypsin inhibitor activity is defined as the quantity of inhibitor which reduces the activity of trypsin by one unit at 37°C. Phytic acid (Vaintraub and Lapteva 1988), total phenolic compounds (Swain and Hillis, 1959), tannins (Sadasivam and Manickam, 1992) and saponins (Fenwick and Oakenfull, 1983) in the powdered seeds was determined.

Data analysis

All results in this study are reported as means of three replicates. Means and standard deviation for different physicochemical characteristics, nutrients and antinutrients were calculated using software Statgraphics Centurion Version XV: II (Statpoint, Inc.). One way analysis of variance (ANOVA) was carried out to determine the significant differences between means among the different treatments at $P < 0.05$.

Results and Discussion

The hundred seed weight and volume of soybean genotypes ranged from 8.2-12.3 g and 12.5-20.5 ml respectively (Table 1). PK 1026 and R-11 had the largest and smallest seed weight respectively, while SL 568 and R-5 had the largest seed volume and R-11 had the least. Soybean contained protein with a range of 36.5-43.2%, with an average value of 40%. Genotype PK 1042 showed the highest protein content of 0.432 mg/g seeds. Oil content was in the range of 20.7 (R 5 & PK 1042) to 22.2% (SL 773). Total soluble sugars and sucrose content ranged from 2.5-8.3% and 1.1-10.4% respectively. Genotype SL 791 showed the highest total soluble sugars and sucrose content. These genotypes can be preferred in terms of their nutritional attributes. Soybean genotypes were found to have a significant variation in the phytic acid content as it varied from 5.1 (SL 791) to 24.5 mg/g seeds (DS 98-2). SL 989 had the lowest trypsin inhibitor activity of 30 mg/g seeds and Sel-P had the highest activity of 102.5 mg/g seeds. Phenolic content of the genotypes varied from 14.0 to 36.2 mg/g with a mean value of 26.7 mg/g seeds. SL 793 was found to have the lowest content of total phenols. Tannin content was in the range of 11.1 (R-11) to 18.8 mg/g seeds (SL 790) whereas saponin content varied from 9.3 to 27.0 mg/g seeds with mean value of 15.2 mg/g seeds.

Table 1. Genotypic variability in 100 seed weight and volume, nutrients and ntinutrients in soybean genotypes

Genotype	100 seed weight (g)	100 seed volume (ml)	Protein (g/100g dry wt)	Oil (g/100g dry wt)	Sucrose (g/100g dry wt)	Soluble Sugars (g/100g dry wt)	Total Phenols (mg/g)	Tannins (mg/g)	Phytate (mg/g)	TIA (mg/g)	Saponins (mg/g)
SL 989	10.2	15	40.7	21.8	3.0	4.1	24.2	18.4	13.9	30.0	19.6
SL 992	10.0	14	39.2	21.5	5.5	4.0	33.5	12.1	18.0	50.1	20.8
PK 1026	12.3	18	41.5	20.8	6.3	4.7	36.2	16.6	18.7	56.5	19.8
DS 2604	10.1	15	38.1	21.6	1.1	3.8	23.3	18.2	21.9	30.5	19.6
Sel-P	9.1	14	40.2	22.1	7.0	3.5	30.6	16.9	19.3	102.5	17.9
DS 98-2	9.9	15	38.3	21.0	2.9	3.5	21.0	18.1	24.5	40.5	21.1
SL 313	11.0	20	36.5	21.5	4.3	3.1	25.9	18.5	11.5	87.5	20.9
SL 799	9.7	15	38.5	21.6	7.3	3.6	27.1	17.7	17.3	58.5	20.7
SL 790	8.9	14	38.4	21.7	4.4	2.5	34.5	18.8	18.9	78.5	20.4
SL 793	9.6	16.5	41.3	21.2	7.2	3.1	14.0	12.2	6.1	40.2	10.0
SL791	9.7	15	42.4	21.4	10.4	8.3	14.9	16.7	5.1	56.5	9.3
SL 773	9.6	15.5	39.7	22.2	6.4	5.6	27.1	12.5	13.5	31.5	13.8
R-11	8.2	12.5	41.5	21.6	4.5	3.5	21.9	11.1	7.3	92.5	16.9
R-5	11.6	20.5	41.5	20.7	7.3	6.7	22.2	13.2	18.5	30.2	10.7
YMV-35	10.1	15.2	40.2	21.4	2.8	3.8	35.4	17.5	15.5	85.4	19.8
PK 1042	10.7	15.5	43.2	20.7	2.4	3.7	31.8	18.6	12.6	54.5	22.6
SL-568	11.2	20.5	37.5	21.7	1.4	6.4	16.5	18.3	15.1	60.2	19.0
SL-592	9.9	15.5	39.1	21.6	2.5	3.0	34.6	15.8	14.4	32.5	27.0
NRC-05-154	9.0	18.5	40.5	21.5	2.0	5.3	26.6	11.2	5.6	30.5	16.9
SL-903	8.7	19.5	42.5	21.8	3.9	4.0	32.2	15.7	18.9	30.2	17.5
Range	8.2-12.3	12.5-20.5	36.5-43.2	20.7-22.2	1.1-10.4	2.5-8.3	14.0-36.2	11.1-18.8	5.1-24.5	30.0-102.5	9.3-27.0
Mean±SD	9.97±1.0	17.2±2.4	40.0±1.82	21.5±0.41	4.63±2.5	4.31±1.5	26.7±6.91	15.9±2.76	14.8±5.5	53.9±23.8	15.2 ±4.4
CD($P \leq 0.05$)	0.93	1.92	1.11	1.11	1.45	0.85	1.25	1.45	2.22	15.65	2.25

Table 2. Physiochemical characteristics of soybean seeds soaked in water, citric acid and sodium bicarbonate solution and cooking

Parameters	Distilled water soaking	1% Citric acid soaking	2% sodium bicarbonate soaking	After boiling in distilled H_2O	CD ($P < 0.05$)
100 seed weight (g)	19.4±1.59	24.4±2.17	23.6±1.94	25.9±2.1	1.23
100 seed volume (ml)	22.1±2.72	27.7±2.88	26.6±2.77	29.3±2.5	1.71
Seed density (g/ml)	0.62±0.07	0.66±0.10	0.65±0.09	0.60±0.1	NS
Water Absorption (%)	95.3±20.1	150.7±10.3	139.2±6.69	156.6±18.6	9.47
Volume Expansion (%)	40.5±17.1	82.7±30.6	74.3±21.4	82.4±22.4	14.7
Swelling Capacity (g/seed)	0.06±0.02	0.13±0.017	0.11±0.02	0.13±0.0	.011
Swelling Index	0.40±0.17	0.85±0.23	0.67±0.20	0.80±0.2	0.13
Hydration Capacity (ml/seed)	0.09±0.01	0.15±0.02	0.14±0.01	0.16±0.0	0.01
Hydration Index	0.89±0.28	1.51±0.10	1.39±0.06	1.60±0.2	0.11

Values are mean ±SE, (n = 20).

To study the effect of soaking and cooking on physicochemical, nutritional and antinutritional factors in soybean, batches of seeds were soaked overnight (18 h) in distilled water, 1% citric acid and 2% sodium bicarbonate solution and then boiled in water for 30 min respectively. Raw seeds were also boiled without soaking. The minimum cooking time for the overnight soaked soybean seeds were 65, 55 and 20 min for distilled water, 1% citric acid and 2% sodium bicarbonate soaking treatments respectively. Overnight soaking of raw seeds in distilled water, 1% citric acid and 2% sodium bicarbonate solution resulted in significant increase in seed weight and volume by 48.6, 59.1 & 57.8 and 22.2, 37.9 & 35.3% respectively (Table 2). The maximum increase in 100 seed weight, volume, seed density, volume expansion, swelling capacity swelling index values was observed in citric acid treatment. Boiling for 30 min of presoaked soybean seeds in distilled water further resulted in increased seed weight, volume, water absorption, hydration capacity and index values. Among different genotypes, R-11 had maximum increase in 100 seed weight and volume, water absorption, volume expansion, swelling capacity and index as well as hydration index values after overnight distilled water soaking. R-11 also showed maximum increase in volume expansion and swelling capacity in acid and alkali treatments or boiling for 30 min after distilled water soaking treatment as compared to other genotypes. Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting (Singh,

Table 3. Effect of processing treatments on nutritional factors in soybean genoytpes

	Nutrients (g/100 g dry weight)s			
	Protein	Oil	Sucrose	Total Soluble Sugars
Raw seeds	40.0±1.82	21.5±0.41	4.63±2.5	4.31±1.5
Soaking in distilled water	38.7±2.20	21.2±0.45	4.89±2.46	4.55±1.45
Soaking in 2% sodium bicarbonate	37.3±2.70	20.6±0.69	5.09±2.38	4.85±1.44
Soaking in 1% citric acid	34.1±1.95	19.5±0.84	5.56±2.30	5.39±1.57
Cooking of raw seeds	38.4±3.11	20.1±0.84	5.34±2.36	5.1±1.48
Cooking after distilled water soaking	32.2±2.37	20.8±0.45	5.49±2.37	5.16±1.49
Cooking after 2% sodium carbonate soaking	30.6±2.88	20.3±0.76	5.53±2.44	5.32±1.42
Cooking after 1% citric acid soaking	28.0±2.28	19.1±0.84	5.89±2.31	5.67±1.55
Critical difference (P < 0.05)	1.67	0.43	NS	NS

Values are mean ±SE, (n = 20).

Table 4. Effect of processing treatments on antinutritional factors in soybean genoytpes

	Antinutrients (mg/g)				
Treatments	Total Phenol	Tannins	Phytate	Saponins	TIA
Raw seeds	26.7±6.91	15.9±2.76	14.8±5.5	18.2±4.39	53.9±23.8
Soaking in distilled water	18.44±4.79 (30.1)	13.56±2.56 (14.7)	12.59±5.0 (14.9)	12.9±3.50 (29.1)	49.6±23.9 (7.98)
Soaking in 2% sodium carbonate	12.32±5.36 (53.9)	12.3±2.54 (22.6)	9.87±4.66 (33.3)	8.37±2.51 (54.0)	39.19±22.9 (27.3)
Soaking in citric acid	10.01±3.09 (62.5)	11.55±2.62 (27.4)	7.06±3.58 (52.3)	7.54±2.33 (58.6)	31.69±21.7 (41.2)
Cooking of raw seeds	7.37±2.56 (72.4)	11.05±2.56 (30.5)	6.34±3.14 (57.2)	6.16±2.51 (66.2)	13.07±7.92 (75.7)
Cooking after dist water soaking	15.2±4.54 (43.1)	9.14±2.30 (42.5)	8.12±3.74 (45.1)	8.10±2.07 (55.5)	39.7±22.9 (26.3)
Cooking after 2% sodium carbonate soaking	9.10±2.89 (65.9)	7.96±2.39 (50.0)	5.83±3.06 (60.6)	5.37±2.33 (70.5)	18.87±9.8 (64.9)
Cooking after citric acid soaking	6.08±2.28 (77.2)	6.3±1.88 (60.4)	3.94±2.16 (73.4)	4.14±1.68 (77.3)	13.54±9.1 (74.9)
CD (P ≤ 0.05)	2.55	1.54	2.51	1.75	11.95

Values in paranthesis indicate percent change in antinutrients's contents after various treatments.
Values are mean ± SE, (n = 20).

2001). Studies indicated that soaking in acid/alkaline solution had increased the rate of water absorption possibly due to the enhanced permeability of the seed coat and softening of cotyledons. Soaking in $NaHCO_3$ has been reported to stimulate rapid water absorption in peas (Bongirwar and Srinivasan, 1977) and urdbean (Singh *et al.*, 2000).

Mean protein and oil contents of 20 genotypes decreased significantly after soaking in acid/alkali solution (Table 3). Maximum decrease in protein content was observed in citric acid treatment. Boiling of raw seeds showed significant decrease in oil content in soybean. Soaking for 18 h in distilled water, acid or alkali further followed by boiling for 30 min resulted in significant decrease in protein content of soybean genotypes whereas oil content decreased non-significantly. SL 773 showed maximum decrease in protein content after soaking in distilled water and 1% citric acid followed by boiling for 30 min treatments whereas maximum decrease in protein content in bicarbonate solution treatments was recorded in SL 790. The nutritional value of native proteins is improved by heat treatments as they are converted to more digestive denatured forms as well as result into inactivation of heat labile enzymes such as lipoxygenase, trypsin inhibitor and urease (Savage *et al.*, 1995; Mubarak, 2005). Soaking and boiling of the legumes resulted in the reduction in protein content that can be attributed to the progressive solubilization and leaching out of the nitrogenous substances (Osman, 2004). Among all the genotypes, the reduction in oil content was highest in SL 791 when seeds were soaked in acid/alkaline solution or cooked (unsoaked). The decrease in fat content after cooking of presoaked seeds can be attributed to high lipolytic enzyme activities which break down the triacylglycerol to simple fatty acid, sterols, esters and polar lipids (Osman, 2007). The total soluble sugars and sucrose content increased nonsignificantly in soybean seeds soaked in water or acid/alkaline solution or followed by boiling for 30 min (Table 3). This increase can be due to the breakdown of the complex carbohydrates which were bound in the raw samples.

There was a significant reduction in the antinutritional compounds, such as total phenols, tannins, phytate, saponins and TIA after soaking (Table 4). Soaking of soybean seeds in different solutions had a marked reducing effect on their total phenolic compounds and tannin content and the maximum reduction was observed in 1% citric acid treatment. Most of the genotypes showed more than 85% reduction in their phenolic content after citric acid soaking followed by cooking and maximum reduction of phenolic content was seen in SL 992 (85%) followed by SL 790 (83.5%). Reduction in tannins was maximum in soaking and /or cooking in distilled water and raw cooked (unsoaked) SL 791 seeds. Acid/alkali treatments resulted in maximum decrease in tannins content in SL 989 followed by SL 791. The decrease in free phenolics during soaking may simply be due to leaching out into soaking solution (Ramakrishna et al., 2006) and differences on distribution and contents of phenolic compounds in the seed coat and cotyledons between the tested seeds (Xu and Chang, 2008). The decrease in condensed tannins in soybean after soaking in the present studies is also in agreement to earlier reports by Alonso *et al.* (2000). A loss of 25% tannin content of blackgram and greengram as a result of overnight soaking in water (Rao and Deosthale, 1982) was reported. Whereas soaking in sodium bicarbonate solution reduced phenolics and tannins by 72% and 78% respectively (Vijayakumari *et al*., 2007). Raw seeds cooked for 30 min in boiling water resulted in significant decrease in total phenolic (72%) and tannins (30.5%) contents. Seeds soaked in water, bicarbonate and citric acid solution and then cooked resulted in significant decrease in total phenols and tannins as compared to raw seeds and the percent loss of total phenols and tannins were 43, 66, 77% and 43, 50, 60% respectively. This could be due to decomposition of phenols or formation of their complexes with protein during heating or due to leaching out in the soaking medium (Khandelwal *et al*., 2010) when soaking solution was being discarded.

Phytic acid and saponins decreased significantly in soybean genotypes when the soaking was done in distilled water, citric acid and bicarbonate solutions. Cooking of raw seeds also showed significant decrease in phytate and saponin content by 57 and 66% respectively. When soybean seeds were soaked in distilled water, citric acid and bicarbonate solutions and then cooked, phytate and saponins decreased (45, 73, 61% and 56, 77, 71%) significantly. Maximum decrease in phytate and saponin content was observed in seeds soaked in citric acid and then cooked for 30 min. Among different genotypes, R-5 showed maximum per cent reduction in phytate content in all the treatments followed by SL 992 and DS 2614. Raw seeds of genotype SL 592 contained highest content of saponins and the reduction was about 80% after cooking of seeds soaked in citric acid followed by PK 1042. Genotypes SL 791 and SL 793 which initially contained low amount of saponins showed maximum reduction of about 90% after soaking in acid/alkali and/ cooking treatments. Phytic acid is water soluble and maximum reduction of phytic acid content in soybean has been reported at pH 5.5 (Ford *et al*., 1978). Soaking also enhances the acitivity of phytase enzyme present in the seed (Kumar *et al*., 2010). The higher reduction of phytic acid content in citric acid in present studies might be due to its higher solubility in acidic medium. Vidal-Valverde *et al.* (1994) also reported that soaking lentils in citric acid solution for 9 h caused more phytic acid removal than soaking in water or sodium bicarbonate solution whereas maximum reduction in phytic acid in seeds soaked in distilled water as compared to raw as well as soaking in sodium bicarbonate solution was reported (Vijayakumari *et al*., 2007). Soaking caused 42.8-48.9% reduction in phytic acid content (Khattab and Arntifield, 2009) that could be due to the presence of phytic acid as a water soluble salt (probably potassium phytate) in dried legumes (Crean and Haisman 1963). Soaking of pulses reduced 5-20% saponins (Jood *et al*., 1988, Kataria *et al*., 1989) that could be due to their leaching out into soaking medium.

When soaking of soybean seeds was carried out in water, citric acid and bicarbonate solutions, TIA decreased by 8, 41 and 27% respectively (Table 4). There was significant decrease in TIA in citric acid and sodium bicarbonate soaking as compared to control value. TIA values further decreased (26, 65 and 75% respectively) when soybean seeds were soaked in water, sodium bicarbonate and citric acid solution and cooked for 30 min. Genotype Sel-P with maximum TIA showed up to 80% reduction after various soaking/cooking treatments. Genotypes SL 903, NR-05-154, SL 989, SL 773 and DS 98-2 with initial TIA activity ranging from 30-40.5 mg/g showed about 85-90% reduction when seeds were soaked in citric acid and cooked. In twelve genotypes maximum decrease in TIA was observed in cooked seeds (unsoaked). Soaking of soybean seeds in water at 22^{o}C for 24 h did not affect TIA (Liu and Markakis, 1987). Similar observations were made in our studies that overnight distilled water soaking at 25^{o}C decreased the TIA activity non-significantly but the decrease was significant when soaking was carried out in acid/alkaline solution. Our results are in agreement to earlier reports by different authors who observed reduced TIA in kidney bean (Ramakrishna *et al*., 2006; Shimelis and Rakshit, 2007) and different legumes (Khattab and Arntfield, 2009; Rasha *et al*., 2011). The cooking of pre soaked seeds appears to be most effective method for reducing TI activity. The further loss during boiling is due to the heat sensitive nature of TIA (Shimelis and Rakshit, 2007; Rasha *et al*., 2011). Soaking in distilled water reduced TIA by 10.22 -19.85% and heat treatment (boiling, roasting, microwave cooking) brought about total removal of Trypsin inhibitor (Khattab and Arntfield, 2009).

Conclusions

The results of present studies have shown that soaking and cooking treatments reduce the antinutrients and consequently increased some of the nutrients in soybean. These are the most simple and inexpensive methods for bringing reductions in phenolic content, tannins, TIA and other water soluble antinutrients. Genotypic variability in soybean w.r.t processing treatments was also recorded.

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