

Physico-chemical characteristics and the effects of processing methods on nutritional and anti-nutritional quality of soybean (*Glycine max* L. Merrill)

ABSTRACT

The present investigation was carried out to determine the physicochemical characteristics and effects of processing methods on the nutritional and anti-nutritional quality of soybean. Soybean seeds were analyzed using physico-chemical to obtain the qualities. The effects of processing methods on the nutritional and anti-nutritional quality of soybean had an impact on the nutritional and anti-nutritional values. Soybeans were subjected to different processing methods which were sorted, washed dried and milled into flour (Sample A Control), soaked for 12h, mechanically dried at $60 \pm 2^\circ\text{C}$ for 10 h and milled into flour (Sample B), soaked for 12h, sprouted 72 h, mechanical dried at $60 \pm 2^\circ\text{C}$ for 10 h, roasted for 3 min and milled into flour (Sample C), boiled for 30 min in an open lid, mechanically dried at $60 \pm 2^\circ\text{C}$ for 10 h, roasted for 3 min and milled into flour (Sample D). The study revealed that raw seeds had 90.43 ± 3.93 g seed weight, 6.31 ± 0.41 mm length, 5.50 ± 0.29 mm breadth, 4.58 ± 0.35 mm height, 0.88 ± 0.04 mm sphericity, 753.01 ± 6.62 kg/m³ bulk density, 1181.03 ± 11.72 kg/m³ true density, 36.33 ± 0.79 % porosity, 0.13 ± 0.005 g hydration capacity, 1.25 ± 0.07 hydration index, 0.12 ± 0.01 mL swelling capacity, 1.62 ± 0.05 swelling index. The colour was recorded to be ($L^* 59.52$, $a^* 8.97$, $b^* 34.01$), Chroma (c^*) 35.17 , and hue angle (h°) 9.84 . Moisture increased (10.61-12.43%), crude protein (42.97 – 47.87%), crude fibre (9.44-11.57%), ascorbic acid (5.38-11.65 mg/100 g) and ash content (4.84-5.99%) while total carbohydrates and total energy decrease significantly i.e. (17.70-15.92%) and (439.69-397.82%), respectively. Similarly, the phytic acid content decreased from 8.12 to 5.19 mg/100g while the tannin decreased from 25.34 to 18.57 % and the protease inhibitor decreased from 7.12 to 5.01 %. The study showed that processing methods can significantly improve the nutritional qualities of soybeans while substantially reducing their anti-nutritional properties thereby boosting the nutrients' bioavailability.

Keywords: Soybean, nutritional value, anti-nutritional factors, soaking, sprouting, roasting

1. INTRODUCTION

Soybean is among the leguminous crop cultivated worldwide for food, feed and oil (Puozaaet al., 2023). Soybean output in the world is around 176.6 million tons per year, with an average

yield of 2.8 t/ha (FAO, 2017). It is recognized as the Queen, Golden, and Miracle of all leguminous crops because it has the maximum amount of protein, lipids, vitamins, and minerals. The protein quality of soybean can be compared to animal protein sources such as meat and milk (Fabiya and Hamidu, 2011). Soybean contains about 40-45% proteins and 18-22% oil (Goyal *et al.*, 2012) and is a rich source of vitamins and minerals. Soy milk is a rich source of calcium, which is useful for vegetarians and people who are lactose intolerant and unable to fully digest dairy products (Chuwae *et al.*, 2022). In addition, calcium from soy milk can be used to prevent osteoporosis. Recent research has shown that legume consumption has a variety of health benefits, including lowering the risk of cardiovascular disease, aiding in diabetes treatment, managing body weight, preventing cancer, and decreasing inflammation. (Duranti, 2006). Legumes are also known to contain a variety of bioactive substances such as lectins, saponins, enzyme inhibitors, phytates, polyphenols (tannins), and oxalate (Pasqualone *et al.*, 2020). These bioactive substances are secondary metabolites that plants produce largely to protect themselves from harmful environmental circumstances. (Abdel-Aal, 2016), whereas others are reserve compounds, such as defence proteins (Bowman-Birk inhibitors and Kunitz inhibitors), which are stored in seeds as energy pools in preparation for germination (Muzquiz *et al.*, 2012). These chemicals' effects might be either unfavourable or good (Muzquiz *et al.*, 2012). As a result, depending on the biochemical and physiological aspects, as well as their concentration, they might have both positive and detrimental effects. These bioactive compounds are secondary metabolites that plants manufacture primarily to defend themselves from hazardous environmental conditions (Gilani *et al.*, 2012).

Raw soybeans contain several nutritional factors such as trypsin inhibitors, lectins, saponins, enzyme inhibitors, phytates, polyphenols (tannins), and oxalate which reduce the nutritional value of legumes and cause health problems for both humans and animals when they are consumed in large quantities (Sharma *et al.*, 2011). Trypsin inhibitors can block either trypsin or chymotrypsin, lowering dietary protein breakdown, amino acid absorption, and hence digestibility (Roy *et al.*, 2010). These anti-nutrients should be eliminated to increase the nutritional content and sensory appeal of legumes for successful usage as prospective human food. Processing procedures can also increase the nutritional value of soybeans by enhancing the bioavailability of amino acids, vitamins, and protein digestibility (Prodanov *et al.*, 2004). Okagbare and Akpodiete (2006) also reported that seed treatment methods to remove nutrient factors were a major challenge for most farmers. Against this background, this study attempts to evaluate the impact of soybean treatment methods on the nutritional and anti-nutritional value of soybeans. To germinate soybeans, the seeds were

cleaned, washed and soaked in water (1:3) for 12 hours. The seeds were removed and germinated by spreading the seeds on a germination box. The box was sprinkled with water twice a day until the seeds began to germinate. The germinated seeds were dried in an oven, roasted and ground into flour.

2. MATERIALS AND METHODS

The current study was carried out at the Department of Food Science and Technology, College of Horticulture Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni-173230, Solan-HP, India, to investigate nutritional quality and alternative techniques of processing soybean seeds. 20 kg of yellow soybean seeds were bought at the Solan local market. The chemicals and reagents utilized in this study were of analytical quality and were obtained from Loba Chemie, International Scientific and Surgicals in Solan (HP). PET containers were also provided by the same provider. Three replicates were used for each treatment and analysis, and the results were computed on a dry weight basis.

2.1 Physical Characteristics

Seed weight

In three replications, thousands of soybean seeds were counted and weighed. The average weight was computed and expressed in grams per thousand seeds.

Seed dimensions

Using a digital Vernier calliper, the length, breadth, and thickness of 10 randomly chosen soybean seeds were measured. The mean values were computed and reported in millimetres.

Sphericity

Mohesenin's method (1970) was used to examine the sphericity of 10 randomly chosen soybean seeds.

$$\text{Sphericity } (\Phi) = \frac{(\text{LWT})^{1/3}}{L}$$

Bulk density

The bulk density of soybean seeds was determined using the technique recommended by Varnamkhastiet *al.* (2008). The preset volume of the empty beaker was filled with seeds. To close the largest air gap possible, the beaker was tapped. The formula below was used to calculate the density of the weight of seeds needed to fill the beaker:

$$\text{Bulk density (kg/m}^3\text{)} = \frac{\text{Weight of seeds}}{\text{Total volume}}$$

True density

The real density of the seeds was assessed using the toluene (C₇H₈) displacement technique, as advised by Pradhan *et al.* (2008). The volume of 50 soybean seeds was calculated by

pouring them into a graduated 50 mL cylinder that held 25 mL of toluene. It was noted that the toluene level increased. Following that, true density was determined using the provided formula and represented as kg/m³.

$$\text{True density (kg/m}^3\text{)} = \frac{\text{Weight of seeds}}{\text{Volume of seeds}}$$

Porosity

By using the formula proposed by Mohsenin (1970) and basing the estimate on bulk and real density, the porosity of seeds was determined. The per cent porosity was calculated using the formula below.

$$\text{Porosity (\%)} = 1 - \frac{\text{Bulk density}}{\text{True density}} \times 100$$

Hydration capacity

For the analysis of the hydration capacity of seeds, the method suggested by Sood *et al.* (2002) was used. After being weighed, fifty seeds were incubated in a 150 mL conical flask with 100 mL of water. The excess water was removed from the flask after it had been left at room temperature for 24 hours, and the seeds were dried by wiping them with absorbent paper. In order to determine the hydration capacity, the enlarged seeds were reweighed. The equation is given below:

$$\text{Hydration capacity per seed (}\frac{\text{g}}{\text{seed}}\text{)} = \frac{\text{W of seeds after soaking (g)} - \text{W of seeds before soaking (g)}}{\text{Number of seeds}}$$

Hydration index

According to Sood *et al.* (2002), the hydration index of the seed was calculated by dividing its weight by its capacity to retain water.

$$\text{Hydration index} = \frac{\text{Hydration capacity per seed}}{\text{Weight of one seed}}$$

Swelling capacity

50 mL of water was put into a 100 mL graduated cylinder before 50 seeds were inserted. It was observed the quantity of both raw and soaked seeds (Sood *et al.*, 2002). The following formula was used to evaluate the swelling capacity value:

$$\text{Swelling capacity per seed (mL/seed)} = \frac{\text{Volume of seeds after soaking} - \text{Volume of seeds before soaking}}{\text{Number of seeds}}$$

Swelling index

According to Sood *et al.* (2002), the swelling index of the seed was calculated by dividing the swelling capacity per seed by the volume per seed.

$$\text{Swelling index} = \frac{\text{Swelling capacity per seed}}{\text{Volume of one seed}}$$

Colour

The colour of soybean flour was measured in a Lovibond Colour Tintometer Model PFX-I series spectrocolourimeter in which RYBN colour units were obtained along with CIE readings i.e. L*, a* and b* values. Each sample was measured three times for colour (Ranganna, 2009). Changes in colour (ΔE), chroma (C*), and hue angle (h^0) were calculated by the formula suggested by (Goswami *et al.* 2015).

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Hue angle} = (h^0) = \tan^{-1}\left(\frac{b}{a}\right)$$

2.2 Sample Preparation

Different processing methods were employed to produce soybean flour. Soybean seeds were sorted, washed dried and milled into flour (Sample A =Control), soaked for 12h, mechanically dried at $60 \pm 2^\circ\text{C}$ for 10 h and milled into flour (Sample B), soaked for 12h, sprouted 72 h, mechanical dried at $60 \pm 2^\circ\text{C}$ for 10 h. roasted for 3 min and milled into flour (Sample C), boiled for 30 min in an open lid, mechanically dried at $60 \pm 2^\circ\text{C}$ for 10 h, roasted for 3 min and milled into flour (Sample D). Brief description of soybean sample processing is shown in Table 1.

Table 1. Soybean Processing Methods

Sample	Processing Description
Sample A	Control (un processed)
Sample B	Soaking for 12h, mechanically drying at $60 \pm 2^\circ\text{C}$ for 10 h and milling the soybean into flour
Sample C	Soaking the soybean for 12h, Sprouted for 72 h, mechanical dry at $60 \pm 2^\circ\text{C}$ for 10 h, roasting for 3 min and milling the soybean into flour
Sample D	Boiling the soybean for 30 min in an open lid, mechanically dry at $60 \pm 2^\circ\text{C}$ for 10 h, roast for 3 min and milled into flour

2.3 Chemical Characteristics

The technique recommended by AOAC (2012) was used to obtain the moisture content (%), ash content (%) and protein content (%). The (AOAC, 2010) technique was used to assess crude fibre, and the (AOAC, 2009) method was used to determine crude fat. The AOAC (2006) technique's differential approach was used to compute total energy (Kcal/100 g) and total carbs (%). β -carotene (mg/100 g) was determined using the Ranganna (2009) process. AOAC (2004) method was used to analyze the ascorbic acid.

2.4 Determination of Anti-Nutritional factors

Tannin was measured using the gravimetric method described by Makkar *et al.* (1996), whilst phytate was identified using the method described by Young and Greaves (1990). According to a technique developed by Ladd and Butler (1972), protease inhibitors were identified.

2.5 Statistical Analysis

For all parameters done for the evaluation of effect of soybean processing, analysis was done in triplicate and mean values was calculated. Data obtained were subjected to statistical analysis using SPSS version 20. Duncan's multiple range test (DMRT) was conducted for significant difference at a level of $p < .05$ for tested parameters.

RESULTS AND DISCUSSIONS

3.1 Physical Characteristics

When designing machinery and equipment for sorting, separating, transporting, processing, and storing soybeans, it is essential to consider their physical characteristics. Designing such equipment and machines without taking this into consideration may yield poor results. As a result, determining and taking into account these features play a crucial role. The physical characteristics of soybean seeds flour are shown in Table 2. The seed weight of thousand seeds was recorded to be 90.43g which is lower than the value investigated by Puzozaa *et al.* (2023) and in conformity with the range by Sharma *et al.* (2016). The difference may be attributed to soybean cultivars. The average length noted was 6.31 mm, which is lower than the range analyzed by Kuzniar *et al.* (2016). Breadth was 5.50 mm which is lower than the range by Kuzniar *et al.* (2016). Height was 4.58 mm and sphericity was 0.88 mm. Soybean seed had 752.01 kg/m^3 bulk density, 1181.03 kg/m^3 true density, 36.33% porosity, 0.13g/seed hydration capacity, 1.25 hydration index, 0.12mL/seed swelling capacity and 1.62 swelling index. The seed dimensions in the present study are almost near to

the range given by Nwakonobi and Idike (2002), Wandkaret *et al.* (2012), Kuzniar *et al.* (2016) and Sumangala and Kulkarni (2019).

Based on the soybean yellow variety as shown in Table 1, the measurement results indicated that the lightness value was recorded to be 59.52 which is lower than the range (76.94-85.81) analyzed by Abadi *et al.*, (2022). The difference may be due to particle size difference. The smaller the particle size, the greater the L value. a* indicate the colour range from green to red with (-a= greenness; + a= redness). The value of a* was analyzed as 8.97. which showed the level of redness is higher as compared to the range investigated (1.10-2.04) by Abadi *et al.*, (2022). The value of b* presents the colour range from yellow to blue with (-a= blueness; + a= yellowness). The value of b* or yellowness level (34.01) is in line with the range (32.44-37.07) of Abadi *et al.*, (2022). The chroma investigated to be 35.17 which is within the range (32.74-37.29) reported by Abadi *et al.*, (2022). The value of 9.84 Hue was smaller than the range (87.01-88.67) reported in the study by Abadi *et al.*, (2022). The difference may be due to particle size difference.

Table 2: Physical characteristics of soybean seeds (yellow variety)

Physical parameters	Determined values
Seeds weight (g/1000 seeds)	90.43 ± 3.39
Length (mm)	6.31 ± 0.41
Breadth (mm)	5.50 ± 0.29
Height (mm)	4.58 ± 0.35
Sphericity (mm)	0.88 ± 0.04
Bulk density (kg/m ³)	752.01 ± 6.62
True density (kg/m ³)	1181.03 ± 11.72
Porosity (%)	36.33 ± 0.79
Hydration capacity (g/seed)	0.13 ± 0.05
Hydration index	1.25 ± 0.02
Swelling capacity (mL/seed)	0.12 ± 0.01
Swelling index	1.62 ± 0.05
Colour	
L*	59.52 ± 0.96
a*	8.97 ± 1.08
b*	34.01 ± 0.57
Chroma (c*)	35.17 ± 5.32
Hue angle (h°)	9.84 ± 7.86

L*=lightness; a*=redness and greenness; b*=yellowness and blueness

3.2 Chemical Characteristics

The results of traditional processing of soybean seeds had effects on nutritional characteristics as shown in Table 3. Moisture content (%) was recorded to be 10.61, 11.76, 12.43 and 10.95 in Samples A, B, C and D, respectively. The moisture increased significantly with the processing method in which sample C showed the highest value. The results are in line with the results of Pele *et al.* (2016). The increase in moisture content in sample C may be due to the absorption of soaking water into the seeds' coat during sprouting (Chuwaet *al.*, 2022). However, sample C is more susceptible to microbial spoilage than samples A, B and D. Food samples with lower moisture content have a longer shelf life and better product quality because chemical and physical degradation are less likely at lower moisture levels. (Intipunya and Bhandari, 2010). The crude protein content (%) of the control (sample A), B, C and D were 42.97, 47.55, 47.87 and 42.74, respectively. The results showed that there is a significant increase in the protein content of sample C as compared with the other samples, with sample A having the lowest protein content. The increase in the protein content of sample C could be due to sprouting which increased the bioavailability of the crude protein in the soybeans. The high increase in the crude protein of sprouted soybean could be attributed to complex biochemical changes that occur during hydration and sprouting, which lead to the protein constituent being broken down by enzymes into simple compounds that are used to make new compounds. The disappearance of starch caused the hydrolytic activities of the sprouting enzymes to increase, which improved the component of total crude protein (Ramadan, 2012). The increase in crude protein content relative to sprouting is particularly significant from a nutritional standpoint as it would increase digestibility and absorption. The result however showed that there is no significant difference in the amount of protein in samples B and D, though soaking and sprouting significantly increase the amount of protein content when compared with sample A. The crude fat (%) of samples A, B, C and D were 21.89, 19.45, 16.14, and 22.78, respectively. The results showed that crude fat significantly decreased in sample C, while there is a significant increase in samples D and A. Decrease in fat content may be due to the depletion of the fat stored that contributed to the catabolic activities of the seeds during sprouting (Onimawo and Asugo, 2004). Another reason could be due to biochemical reaction and dissociation of lipid complexes as reported by Ragab *et al.* (2010). The implication of this however is that sample D will be more prone to rancidity than the other samples. The crude fibre (%) of samples A, B, C and were 9.44, 10.22, 11.57 and 9.17, respectively. A significant increase in fibre in sample C is due to the soaking and sprouting process which could be attributed to the disappearance of starch. The trend was also reported by Sood *et al.* (2002). The total carbohydrate (%) content of samples A, B, C

and D were 17.70, 16.37, 15.92, and 17.54, respectively. A high significant decrease in total carbohydrates was observed in the soaked and germinated seed flour (sample C). This may be due to sprouting, carbohydrate was used as a source of energy for embryonic growth which could explain the changes in carbohydrates content after sprouting. Uppal and Bains (2012) reported a 5.6% decrease and Jirapaet *et al.* (2001) reported a 2.34% decrease in carbohydrate content after 24 h of sprouting in cowpea. The results are in accordance with Pele *et al.* (2016) who reported a decrease in carbohydrates in sprouted soybean seed flour. The ascorbic acid content (mg/100g) of 5.38, 5.89, 11.65 and 5.28 were recorded in samples A, B, C and D, respectively. Soaking and germination increase ascorbic acid significantly in sample C as compared to other samples. The increase in ascorbic acid may be due to the enzymatic hydrolysis of starch by amylases and diastases that increase the availability of glucose for the biosynthesis of ascorbic (Desai *et al.*, 2010). The increase of ascorbic acid in different sprouted pulse seed flour has been reported by Shah *et al.* (2011). The β -carotene of the present study was significance difference with sample C having the lowest β -carotene and sample A with the highest β -carotene. The lowest β -carotene content in T3 may be due to oxidative degradation in thermal processing that results from the conversion of trans-cis isomerization of all transform. A similar trend has been observed by Khyade and Jagtap (2016) in germinated cowpea, black gram chickpea and yellow mustard. The ash content (%) of samples A, B, C and D were 3.83, 4.76, 5.27 and 6.75, respectively. Results showed that there is a significant difference in all samples. There is a significant increase in the ash content of sample D while sample C is significantly low. The effect of treatment on ash content shows higher significance on soaked and germinated seed flour as compared to the control. Similar results were reported by Ranhotra *et al.* (1977). An increase in ash content may be apparent due to the loss of starch (Lorenz, 1980). The total energy of 466.22 Kcal/100 g was found to be highest in sample D and lowest in sample C (397.82 Kcal/100 g). Significant low energy in soaked and germinated sample C is due to the low energy value of sprouted seed flour for fat and total carbohydrates (Uppal and Bains, 2012) in which energy depends on them during calculation (differential method). Seed sprouting involves energy use, which is provided by the breakdown of starch to sugars and lipids to free fatty acids resulting in a shift in nutrient profile.

Table 3: Chemical characteristics of soybean seeds (yellow variety) of different processing methods

Parameters	Sample A	Sample B	Sample C	Sample D
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	(control)			
Moisture content (%)	10.61 ± 0.55 ^c	11.76 ± 0.32 ^b	12.43 ± 0.21 ^a	10.95 ± 3.98 ^c
Crude protein (%)	42.97 ± 0.72 ^b	47.55 ± 3.54 ^c	47.87 ± 0.23 ^a	42.76 ± 0.12 ^c
Crude fat (%)	21.89 ± 0.56 ^b	19.45 ± 0.76 ^c	16.14 ± 0.27 ^d	22.78 ± 1.12 ^a
Crude fibre (%)	9.44 ± 0.08 ^c	10.22 ± 0.52 ^b	11.57 ± 0.27 ^a	9.17 ± 0.09 ^c
Total carbohydrates (%)	17.70 ± 0.80 ^a	16.37 ± 1.34 ^b	15.92 ± 0.87 ^d	17.54 ± 0.35 ^c
Ascorbic acid (mg/100g)	5.38 ± 0.17 ^d	5.89 ± 0.75 ^b	11.65 ± 0.19 ^a	5.28 ± 0.05 ^c
β-carotene (mg/100 g)	2.05 ± 0.02 ^a	1.97 ± 0.04 ^b	1.59 ± 0.06 ^c	1.37 ± 0.07 ^c
Ash (%)	4.84 ± 0.27 ^c	5.03 ± 0.05 ^b	5.99 ± 1.34 ^a	4.76 ± 3.34 ^c
Total energy (Kcal /100 g)	439.69 ± 3.58 ^b	430.73 ± 4.32 ^c	397.82 ± 6.18 ^d	466.22 ± 2.87 ^a

Means sharing the same superscript letter in rows are not significantly different from each other (DMRT, $p \leq 0.05$)

Effects of processing methods on the anti-nutritional properties of soybeans flour.

The results of the effects of traditional processing methods on the anti-nutritional factors of soybeans are presented in Table 4. The phytic acid (%) of samples A, B, C and D were 8.12, 7.76, 5.19 and 6.45, respectively. Results showed that there is a significant decrease in phytic acid of entire samples. Soaking and sprouting soybeans reduced phytic acid levels in sample C. Osman (2007) also reported a reduction in phytic acid content in different processing methods (soaking, cooking and germination). The tannin content (mg/100g) of samples A, B, C and D were 25.34, 23.55, 18.23 and 23.26, respectively. Results showed that there was a significant difference in the tannin content of the samples. Tannin content was significantly decreased in samples C, D and B. This could be attributed to a significant effect of soaking, sprouting and roasting which significantly decreased the tannin. The protease inhibitor of samples A, B, C and D were 7.12, 6.95, 5.01 and 6.55% respectively. The results showed a significant difference in protease inhibitor of the entire sample. The protease inhibitor of samples B, C and D is significantly decreased; due to soaking, sprouting and roasting which are significant in its reduction.

Table 4: Antinutritional factors of soybean seeds (yellow variety) of different processing methods

Parameters	Sample A (control)	Sample B	Sample C	Sample D
Phytic acid (%)	8.12 ± 0.91 ^a	7.76 ± 0.15 ^b	5.19 ± 0.17 ^d	6.85 ± 0.11 ^c
Tannin (mg/100 g)	25.34 ± 0.34 ^a	23.55 ± 3.76 ^b	18.57 ± 0.13 ^d	23.26 ± 0.67 ^b
Protease inhibitor (%)	7.12 ± 0.88 ^a	6.95 ± 0.09 ^b	5.01 ± 0.54 ^d	6.55 ± 0.79 ^c

Means sharing the same superscript letter in rows are not significantly different from each other (DMRT, $p \leq 0.05$)

3. CONCLUSION

The study highlighted the impact of conventional processing methods on the nutritional and anti-nutritional qualities of soybeans. Soaking and germination decrease significantly crude fat, carbohydrates, β -carotene and total energy in soybean seed flour while increasing significantly moisture, crude protein, crude fibre, ascorbic acid and ash. Furthermore, the results showed that processing methods considerably decreased the anti-nutritional qualities of soybeans. Soaking and sprouting lowered significantly the total carbohydrates and fat which may be an advantage for overweight and obese people who need to consume less carbohydrates and low-fat foods. The positive values in colour signify that the soybean seeds used in this study were of high quality.

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