

Original Research Article

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

ABSTRACT

The present investigation was carried out to determine the physico-chemical characteristics and effects of processing methods on the nutritional and anti-nutritional quality of soybeans. Soybean seeds were analysed using physico-chemical approaches, in order to obtain a detailed profile on these qualities. 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, mechanically-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 soybean 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/seed 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. After processing, Moisture content 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 decreased significantly (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 overall results of the current study revealed that the effects of processing methods on the nutritional and anti-nutritional quality of soybeans had an impact on the nutritional and anti-nutritional values. Further, 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.

Comment [H1]: It is better to mention where you soaked
Example:soaked in water

Comment [H2]: This is not the Degree sign.
Insert the correct symbol.

Comment [H3]: It is better to mention where you soaked
Example:soaked in water.

Comment [H4]: Results are expressed only for the raw soybean seeds,
Results after applying the processing methods??

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Comment [H6]: Better if you can avoid these types of abbreviated terms in the abstract

Comment [H7]: If the word limit permits, better if you could add a sort of a conclusion statement, at the end of the abstract.

Keywords: Soybean, physico-chemical characteristics, processing methods, nutritional values, anti-nutritional factors, quality, soaking, sprouting, roasting soybean

1. INTRODUCTION

~~Soybean is among the leguminous crop cultivated worldwide for food, feed and oil (Puozaa *et 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 (Chuwa *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~~ defense 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~~ The effects of these chemicals might be either good or unfavourable ~~unfavorable~~ or good (Muzquiz *et al.*, 2012). As a result, depending on the biochemical and physiological aspects, as well as their concentrations, 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).~~

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Soybean is among the leguminous crops cultivated worldwide for food, feed, and oil (Puozaa *et 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 soybeans 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, unable to fully digest dairy products (Chuwa *et al.*, 2022). In addition, calcium from soy milk can be used to prevent osteoporosis.

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 anti-nutrient factors, were a major challenge for most farmers. Against this background, this study attempts to evaluate the impact of soybean treatment-processing 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-Himachal Pradesh (HP), India, to investigate nutritional quality and alternative techniques of processing soybean seeds. ~~20 kg of Y~~yellow soybean seeds (20.00 kg) were bought from the Solan local market. The chemicals and reagents utilized in this study were of analytical grade quality and were obtained from Loba Chemie, International Scientific and Surgicals in Solan (HP), India. Polyethylene terephthalate (PET) containers were also provided by the same supplier provider. Three replicates were used for each treatment and analysis, and the results were computed on a dry weight dry-weight basis.

2.1 Physical Characteristics

Comment [H9]: This part should come under Methodology.

Here you can add 2 to 3 sentences to elaborate on the research gap and please emphasize your objectives.

Seed weight

In three replications, ~~thousands of~~ 1000 soybean seeds were counted and weighed. The average weight was computed and expressed in grams per thousand seeds.

Comment [H10]: To compute the weight results on dry weight basis, you have to oven-dry the seeds. Better if you could mention here the time-temperature combination used in the oven drying process.

Seed dimensions

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

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Sphericity

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

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

Comment [H11]: This citation is not included in your references list (at the end of the manuscript).

Also, you can briefly (in 1-2 sentences) mention what you did here/how did you perform Mohesenin's method. As an author you should provide sufficient technical details to allow others to replicate and build on published results.

Bulk density

The bulk density of soybean seeds was determined using the technique recommended by Varnamkhasti *et 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:

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

Comment [H12]: LWT?

Is this an abbreviation? If so, you have to define the terms of this equation.

True density

The real density of the seeds was assessed using the toluene (C₇H₈) displacement technique, as ~~advised by described in~~ 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 based on~~ the estimates on bulk and real density ~~ies~~, the porosity of seeds was determined. The ~~per cent~~ percent porosity was calculated using the ~~following~~ formula ~~below~~:

Comment [H13]: This citation is not included in your references list (at the end of the manuscript).

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

Hydration capacity

For the analysis of the hydration capacity of seeds, the method suggested by Sood *et al.* (2002) was used. After being weighed, ~~50~~ fifty seeds were incubated in a ~~conical flask (150.0 mL)~~ conical flask filled with ~~water (100.0 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

Comment [H14]: To compute the weight results on dry weight basis, you have to oven-dry the seeds. Better if you could mention here the time-temperature combination used in the oven drying process.

wiping them with absorbent paper. In order to determine the hydration capacity, the enlarged seeds were re-weighed. The equation is given below:

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

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 by its weight.

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

Swelling capacity

~~50 mL of w~~Distilled Water (50.0 mL) was put into a ~~100 mL~~ graduated cylinder (100.0 mL) ~~and before~~ 50 soybean 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) spectrophotometer in which RYBN colour units were obtained along with Commission Internationale de l'Éclairage (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°) were calculated by the formula suggested by (Goswami *et al.* 2015).

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

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

2.2 Sample Preparation

Comment [H15]: Quantity??
Is that the volume of raw and soaked seeds?
How did you measure the volume of seeds here?

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Different processing methods were employed to produce soybean flour. 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 (brand ??). The box was sprinkled with water twice a day until the seeds began to germinate. The germinated seeds were dried in oven (mention the brand of the oven and time -temperature combination for oven drying), roasted and ground into 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, mechanically 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 seed samples processing is shown in Table 1.

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Comment [H18]: Mention how you monitored the seed germination. Did you measure the length of the germination tube or just take the visual observations on seedling growth?

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Table 1. Soybean seeds Processing methods

Sample	Processing Description
Sample A	Control (unprocessed; <u>Soybean seeds sorting, washing, drying and milling into flour</u>)
Sample B	<u>Soybean seeds</u> <u>Soaking in water</u> for 12h, mechanically drying at $60 \pm 2^\circ\text{C}$ for 10 h and milling <u>the soybean</u> into flour
Sample C	<u>Soybean seeds</u> <u>Soaking in water</u> <u>the soybean</u> for 12h, <u>s</u> prouted for 72 h, mechanically <u>drying</u> at $60 \pm 2^\circ\text{C}$ for 10 h, roasting for 3 min and milling <u>the soybean</u> into flour
Sample D	Boiling the soybean <u>seeds</u> for 30 min in an open lid, mechanically <u>drying</u> at $60 \pm 2^\circ\text{C}$ for 10 h, roast for 3 min and milled <u>ing</u> 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 earbs carbohydrates (%). β -carotene content (mg/100 g) was determined using the

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Ranganna (2009) process. AOAC (2004) method was used to ~~analyze~~analyse the ascorbic acid content.

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 the software SPSS (IBM, Armonk, NY) version 20. Duncan's multiple range test (DMRT) was conducted for significant difference ~~differences~~ at a level of $p < .05$ ~~for tested parameters if the associated P-values < 0.05.~~

Comment [H20]: Can you briefly (in 1-2 sentences) mention what you did here/how did you perform each assay. As an author, you should provide sufficient technical details to allow others to replicate and build on published results.

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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, Therefore,~~ determination of ~~ing and taking into account~~ these features play a crucial role. The physical characteristics of soybean seeds flour are ~~shown listed~~ in Table 2. The seed weight of thousand seeds was recorded to be 90.43g which is lower than the value investigated by Poozaa *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~~analysed by Kuzniar *et al.* (2016) ~~while the~~ Breadth was 5.50 mm which is lower than the range expressed by Kuzniar *et al.* (2016). ~~Height was 4.58 mm and sphericity was 0.88 mm. Soybean seed had 752.01 kg/m³ bulk density, 1181.03 kg/m³ 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), Wandkar *et al.* (2012), Kuzniar *et al.* (2016) and Sumangala and Kulkarni (2019).

Comment [H21]: Do not repeat the results given in the table in the text as well.

Based on the soybean yellow variety as shown in Table 42, 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. Symbol a* indicates 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) as reported by Abadi *et al.*, (2022). The value of 9.84 Hue (9.84) 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.

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

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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 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 ^o)	9.84 ± 7.86

Comment [H24]: Recheck the symbol

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,

Comment [H25]: Since you have 4 tables in this manuscript, I suggest to indicate the data on nutritional characteristics as a bar chart or as a pie chart ----Sort of a diagrammatic indication would be more eye-catching than going through the tabulated data.

~~12.43 and 10.95 in Samples A, B, C and D, respectively.~~ The moisture content 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 (Chuwa *et 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 crude 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 proteins, in the soybeans. The significantly 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 content significantly decreased in sample C, while there is a significantly high counts-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 crude fibre content in sample C is due to the soaking and sprouting processes, 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 significantly high decrease in total

Comment [H26]: What is the basis of this statement?
Did you check it during your study?

Comment [H27]: However, fungi prefer low moisture content food items. Food spoilage by fungi and mycotoxin productions are yet possible

Comment [H28]: Do not repeat the results given in the table in the text as well.

Comment [H29]: Explain little bit how this happens

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Comment [H31]: Here the statistical analyses are incorrect. Recheck the superscripts.

Also how did you get a lower crude protein content in processed sample D, compared to the control sample A. You have to justify it.

Comment [H32]: Explain little bit how this happens and what is the connection between starch disappearance and crude fibre content increment?

Comment [H33]: Here the statistical analyses are incorrect. Recheck the superscripts.

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 Jirapa *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 increased the ascorbic acid content significantly in sample C, as when compared to other samples. The increase in ascorbic acid may be due to the enzymatic hydrolysis of starch by amylases and diastases, which in turns that increases the availability of glucose for the biosynthesis of ascorbic acid (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 content of the present study was significantly different with sample C having the lowest β -carotene and sample A with the highest β -carotene. The lowest β -carotene content in sample C 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 ash contents of the tested samples. There is a significant increase in the ash content of sample D while sample C is significantly low marked the lowest value. The effect of treatment on the ash content showed higher significance on soaked and germinated seed flour, as when 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 soybean seeds (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 those parameters, 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) after four different

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Comment [H36]: Not clear to the reader. Can you write this in a more explanatory manner.

Comment [H37]: Better to include scientific names

Comment [H38]: ?? Do you mean both ; sample B and sample C

Comment [H39]: Explain little bit how this happens and what is the connection between starch disappearance and ash content increment?

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processing methods

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Parameters	Sample A (control)	Sample B	Sample C	Sample D
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/fiber (%)	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

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

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Comment [H43]: DMRT - Is this an abbreviated term??

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Comment [H44]: Topic should be numbered as 3.3

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 content of the entire tested 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 B, C and D and B and T. This could be attributed to a significant effect of soaking, sprouting and roasting which significantly decreased deteriorated the tannins. 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 had significantly decreased; due to soaking, sprouting and roasting steps, which are significant in its reduction.

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

Comment [H45]: ??

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

Comment [H46]: In the rows highlighted, the statistical analyses are incorrect. Recheck the superscripts

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

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Comment [H47]: DMRT - Is this an abbreviated term??

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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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Comment [H48]: Recheck the referencing style with the journal guidelines.

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