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Impact of diverse processing treatments on nutritional and anti-nutritional characteristics of soybean (*Glycine max* L.)

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ABSTRACT

Soybean (*Glycine max* L.) is considered as an important and widely consumed legume due to its higher nutritional and bioactive potential as well as better functional characteristics. It is a rich source of numerous nutritional components such as essential amino acids, protein, and various dietary components which are suitable for all age groups. This research is aimed to assess the effect of soaking, germination, fermentation (natural and with *Saccharomyces cerevisiae*), and roasting on nutritional characteristics, anti-nutritional components, minerals (Fe, Zn, Mn, and Cu), and bioactive components of soybean. The effect of soaking was studied at 12 and 24 h, while that of germination at 24, 48, and 72 h, fermentation for 12, 24, and 36 h interval, and roasting at a temperature of 180°C. The results revealed that the antioxidant activity increased significantly ($P \leq 0.05$) by 98.01% after 72 h of germination and 68% after the 36 h of fermentation with *S. cerevisiae*. Further, there was a 19.86 and 17.42% increase in the phenolic components during roasting and germination processes, respectively. The protein contents get increased significantly ($P \leq 0.05$) by 6.54, and 23% during germination and fermentation treatments, respectively. The anti-nutrients such as phytic acid and tannin contents declined significantly ($P \leq 0.05$) to the extent of 7.35, 27.94, and 58.82% and tannin contents as 8.70, 44.93, and 58.82%, after soaking, germination, and fermentation processes, respectively. There was a significant ($P \leq 0.05$) increase in mineral contents after processing treatments of soybean. Therefore, the processing treatments were quite effective in increasing the nutritional value as well as the bioactive components and decreasing the antinutritional components.

1. INTRODUCTION

Legumes are economical and nutritionally rich sources of nutrients containing low glycemic index carbohydrates, proteins, and micronutrients such as Fe, Zn, and Ca. Being rich in all the nutrients, the popularity of soybean is growing at faster rate. The production of soybean showed increasing trend from 454.50 kg/ha in 1961 to 927.80 kg/ha in 2020 in India. The overall production of soybean in world was 385.85 million metric tonnes during 2019–2020 [1]. Soybean (*Glycine max* L.) is the richest source of protein, essential amino acids, and other nutritional and bioactive components for making them a popular nutritional supplement to help people stay healthy, especially in rural areas of Asian countries [2]. Because of its high nutritional potential, it's not only preferred by vegetarians but also the non-vegetarian people of all age groups. Soybeans possess the most scientific interests, due to the presence of phytoestrogens such as isoflavones, which have been linked to the lower cholesterol levels, anti-cancer capabilities, and the risk of cardiovascular diseases [2,3]. Studies have revealed that the people in East Asia who consume soybean products frequently are less prone to develop certain chronic ailments, such as cardiovascular diseases and cancer [4]. Phenolic components, which are present in abundance in soybean, are a collection of bioactive components that possess health-promoting characteristics. These may act as antioxidants, helpful for lowering the risk of various lifestyle diseases such as atherosclerosis and coronary heart diseases, both of which are linked to the oxidation of low-density lipoproteins. These have also been reported to protect against certain types of cancer [5].

Anti-nutrients are inactivated by thermal procedures such as boiling, steaming, autoclaving, and roasting [6]. Different processing methods can be utilized to improve the bioavailability of numerous micronutrients in diets based on plant foods [7]. Soaking and germination are commonly utilized as traditional processing techniques and are considered as the simplest and economical techniques for improving the nutritional value and decreasing the anti-nutrients in food grains. Soaking is a common domestic technique for hydrating the seeds in water for a few hours [8] and decreasing the anti-nutritional compounds in cereals and pulses by leaching in water [9].

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The use of fermentation techniques to process the cereals and legumes for manufacturing complementary foods has been found to reduce the anti-nutritional compounds and improving the nutritional value of food products. This technique is being used in making a wide range of foods at domestic as well as commercial scales around the world. The fermentation process is beneficial in imparting the characteristic flavor and textural properties to the grains [10]. Roasting is a typical heating method, and roasted soybeans are frequently sold as snack foods. Thermal treatments can alter physical features such as color and break down cell walls and membranes which release insoluble ester bonds resulting in increased soluble phenolic compounds as well as enhancement in antioxidant properties. Roasting influences not only the physical qualities and flavor of food but also its chemical composition. The Millard reaction during the roasting treatment has been found to increase the antioxidant activity [11]. Keeping in view the above benefits of different processing treatments, the present investigation was planned to study the effect of processing treatments such as soaking, germination, fermentation (natural and with *Saccharomyces cerevisiae*), and roasting for improving the nutritional value, and bioactive potential of soybean as well as decreasing the anti-nutrients in soybean seeds.

2. MATERIALS AND METHODS

2.1. Materials

The soybean seeds (NRC-127 cultivar) were procured from ICAR-Indian Institute of Soybean Research, Indore, Madhya Pradesh, and seeds were reproduced at the experimental farms of the Eternal University, Baru Sahib. The chemicals and reagents of ultrapure grade were used in the present study. These were obtained from the standard companies of chemicals such as Qualigens, Hi-Media, Merck India, and Sigma-Aldrich.

2.2. Physicochemical Properties

The physicochemical evaluations of raw soybean (RS) grains and that of the processed (soaked, germinated, fermented, and roasted) ones were carried out at the laboratories of Eternal University, Sirmour, Himachal Pradesh, India.

2.2.1. Physical and functional characteristics

Physical parameters such as length, breadth, as well as thickness were determined with the help of Vernier caliper. The thousand-grain weight (TGW) was determined by measuring the weight of thousand grains of soybean and expressed in g [12]. The bulk density (BD) was evaluated as per the methodology expressed by Huang *et al*. [13]. The tap density was estimated as per the methodology described by Jones *et al*. [14], and the water absorption capacity (WAC) by the method specified by Sosulski [15] with minor modification. A similar methodology was also adopted to estimate the oil absorption capacity (OAC) [16] in which refined oil was used instead of water. The water solubility index (WSI) was estimated as per the method of Stojceska *et al*. [17] and the swelling capacity of grains was estimated as per the method of William *et al*. [18].

2.2.2. Chemical parameters

Moisture content (%) of grains was estimated by following the hot air oven-drying method [19]. The equipment Fibroplus FBS 08P (Pelican Inc.) was used to determine the crude fiber, Soxoplus SPS 06 AS (Pelican Inc.) for crude fat, and Kjelodist CAS VA (Pelican Inc.) was used to estimate the crude proteins and ash contents as per the methods defined by Ranganna [20]. The total carbohydrates were assessed by deducting the measured moisture, crude protein, ash, crude fat, and crude fiber from 100. The calorific value (kcal/100 g) was determined using the factors of 4.0, 9.10, and 4.2 kcal/g for crude protein (Nx6.25), fats, and carbohydrates, respectively. The mineral components such as iron, zinc, manganese, and copper were assessed using Atomic Absorption Spectrometer (AA240FS, Agilent Technology, CA, USA) [19].

The antioxidant activity (%) (DPPH radical scavenging activity) was estimated as per the methodology stated by Bouaziz *et al*. [21] with minor modifications. Tannins (%) were determined using the technique evaluated by Saxena *et al*. [22]. The extraction, as well as the quantification of the phytic acid in all the samples, was estimated by Gao *et al*. [23], with slight changes. Total phenolic contents (TPCs) were determined using the Folin–Ciocalteu reagent by following a slightly modified method of Ainsworth and Gillespie [24] and were expressed as mg GAE/100 g.

2.3. Processing Treatments

The processing treatments such as soaking as well as germination of grains were conducted as per the technique described by Egli *et al*. [25] with minor modification. The soybean seeds were cleaned by removing the foreign impurities and soaking of grains was done in distilled water in the ratio of 1:5. The seeds were soaked for 12 and 24 h at room temperature conditions and then dried in the hot air oven at 40°C for 24 h. The grains were stored at 4°C after packaging in airtight pouches for further analysis. For germination treatment, the seeds after soaking in distilled water were subjected to the germination process after dividing into many groups (20 g for each group) according to the treatments given for different periods. The seeds after the steeping process were drained off and then covered with the wet muslin cloth. The process of germination was conducted in the incubator for 0 (control), 24, 48, and 72 h at a temperature of 25°C. During germination treatment, the water was sprinkled intermittently over the muslin cloth to keep it moist. After each germination time treatment, the seeds were dried at 40°C for 24 h in hot air oven. The roasting treatment of seeds was conducted as per the methodology defined by Sindhu *et al*. [26] on a heated plate at 180°C for 10 s. The roasted seeds were then cooled and milled. The flour was screened through a 60 mesh sieve size and stored in airtight sealable packets at 4°C.

The natural fermentation process was carried out as per the method of Park *et al*. [27] with minor modification. Samples of soybean flour (20 g) were gently mixed with 60 ml distilled water. The fermentation was carried out in an incubator at 37°C for 12, 24, and 36 h. The microorganisms naturally present in the seeds caused fermentation. The samples collected at each time point were oven-dried at a temperature of 50°C, pulverized in a blender, and then sieved through a 60 mesh sized sieve, before being sealed in polyethylene bags at 4°C.

Fermentation with baker's yeast (*S. cerevisiae*) was conducted as per the method described by Day and Morawicki [28] with minor modification. Soybean flour (20 g) was mixed properly with 120 ml of distilled water in a 500 ml conical flask and was autoclaved. The pH of the solution was checked to ensure that it is >4.5, and 0.4 g of *S. cerevisiae* was added to it. After proper mixing, the conical flask was put in the water bath at 37°C for 12, 24, and 36 h. The fermented flours after each fermentation time were oven-dried at 50°C, packed in airtight pouches, and stored at 4°C until further analysis.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characteristics of Raw Grains

3.1.1. Physical and functional properties

The data on physical as well as functional properties of raw grains are presented in Table 1. The length, width, and thickness of soybean grains were 6.6 mm, 5 mm, and 5.65 mm, respectively. Sakare *et al*. [29] reported the length, width, and thickness of soybean seeds as 7.71, 6.71, and 5.34 mm, respectively. The tap and BD of soybean were determined as 0.74 and 0.71 g/cm^3, respectively. The values for WAC, WSI, OAC, and SC of soybean grains were observed as 1.93 ml/g, 5.47 g/g, 3.37 ml/g, and 249.67%, respectively. Sakare *et al*. [29] reported the BD, WAC, WSI, and OAC of soybeans as 0.455 g/ml, 1.690 g/g, 25.66%, and 1.956 g/g. The TGW of soybean grains was 152.15 g. Gandhi *et al*. [30] described that the TGW of different cultivars varied from 93.5 to 184.0 g and was analogous with the values detected in the present study.

3.1.2. Nutritional, anti-nutritional, and bioactive characteristics

The nutritional and anti-nutritional components, as well as bioactive characteristics of RS are depicted in Table 2. The values for crude fat, crude fiber, crude protein, ash, and carbohydrate contents in raw grains of soybean were observed as 19.86, 7.0, 38.35, 5.43, and 21.18%, respectively. Similar findings were also reported by Joshi and Varma [31] during the nutritional evaluation of soybean. They observed a crude fat content of 19.2%, crude fiber 6.5%, crude protein 40.2%, ash content 4.8%, and carbohydrate content 21.18%. The antioxidant activity in RS was observed as 38.22% DPPH radical scavenging activity. Malenčić *et al*. [32] reported 22.87–48.17% antioxidant activity in different cultivars of soybean. Tannin and phytic contents of soybean were found as 0.207 and 1.36%, respectively. The TPC content observed in soybean was 14.35 mg GAE/100 g. Kumari *et al*. [33] reported tannin, phytic, and TPC of 2.18 mg/g, 22.50 mg/g, and 6.24 mg/g in soybean grains, whereas Sharma *et al*. [34] found TPC ranging from 14 to 36.2 mg/g in soybean seeds. The mineral contents such as Cu, Mn, Fe, and Zn have been reported as 12.10, 21.28, 61.43, and 41.65 mg/kg, respectively. Özcan and Al Juhaimi [35] reported the Cu content of 14.1 mg/kg, Mn 22.8 mg/kg, Fe 64.9 mg/kg, and Zn contents as 45.5 mg/kg in soybean.

Table 1: Physical and functional characteristics of raw grains of soybean

Parameters	Values
Length (mm)	6.60±0.36
width (mm)	5.00±0.20
Thickness (mm)	5.65±0.31
1000 kernel wt. (g)	152.15±0.88
Tap density (g/cm^3)	0.74±0.01
Bulk density (g/cm3)	0.71±0.01
WAC (ml/g)	1.93±0.08
WSI (g/g)	5.47±0.03
OAC (ml/g)	3.37±0.15
Swelling capacity (%)	249.67±0.58

Values in the table are presented as mean±SD

Table 2: Nutritional, anti-nutritional, antioxidant activity, phenolic, and mineral content of raw grains of soybean

Parameters	Values
Moisture (%)	8.17±0.20
Fat (%)	19.86±0.34
Fiber (%)	7.00±0.22
Ash (%)	5.43±0.08
Protein (%)	38.35±0.18
Carbohydrate (%)	21.18±0.20
Calorific value (Kcal/100 g)	423.11±2.58
Antioxidant activity (%)	38.22±0.20
Tannin (%)	0.207±0.002
Phytic acid (%)	1.36±0.04
Total phenolic content (mg GAE/100 g)	14.35±0.09
Cu (mg/kg)	12.10±0.18
Fe (mg/kg)	61.43±0.35
Zn (mg/kg)	41.65±0.66
Mn (mg/kg)	21.28±0.58

Values in the table are presented as mean±SD

3.2. Effect of Processing Treatments on the Nutritional, Anti-nutritional, and Bioactive Compounds

The soybean seeds after the soaking, roasting, germination, and fermentation treatments were subjected to physicochemical analysis. The alterations reported in physicochemical features of RS were recorded after the soaking treatment of 12 (S12) and 24 h (S24), roasting (RosG), germination treatment for 24 (G24), 48 (G48), and 72 h (G72), natural fermentation for 12 (NF12), 24 (NF24), and 36 h (NF36), and yeast (*S. cerevisiae*) fermentation for 12 (SCF12), 24 (SCF24), and 36 h (SCF36). The results of analytical studies conducted on processed soybean are discussed under the following headings.

3.2.1. Effect of soaking as well as roasting treatments on the nutritional components, antioxidant activity, and total phenolic components of soybean

The physicochemical characteristics of soybean grains after roasting and soaking treatments are presented in Table 3. Moisture content ranged between 8.12 (RosG) and 8.20% (S12). There was a non-significant ($P \leq 0.05$) increase in moisture content during the soaking treatment. Joshi and Varma [31] observed a similar change in processed grains after soaking and roasting treatments. The fat content ranged from 19.34 to 19.86% during the processing of soybean. It was observed highest in RS (19.86%) but it decreased to 19.34% resulting in a non-significant ($P \leq 0.05$) decrease of 2.62% in fat content. The fiber content increased from 7.00 (RS) to 7.26% (S24) and ash content from 5.43 (RS) to 5.74% (RosG) during soaking and roasting treatments, respectively. Soaking as well as roasting both significantly ($P \leq 0.05$) increased the crude fiber content by 3.71 and 3.43%, respectively. Ash content ranged from 5.43% (RS) to 5.74 (S24). There was a non-significant ($P \leq 0.05$) increase in ash content during soaking (5.71%) and roasting (4.97%) treatments. The protein content ranged from 38.35% (RS) to 38.69% (S24). The values increased non-significantly ($P \leq 0.05$) by 2.13% during the roasting of soybean. There was a non-significant ($P \leq 0.05$) decline in carbohydrate and calorific values of soaked and roasted grains and values declined from 21.18 to 20.65% and 423.11 to 418.63 Kcal/100 g, respectively.

The antioxidant activity ranged from 38.22% (RS) to 66.02% DPPH radical scavenging activity (S24). There was a significant ($P \leq 0.05$) increase in antioxidant activity after the S24 (72.74%) treatment. Roasting is a type of heat treatment that uses dry heat treatment to produce the Maillard reaction and it has been shown to increase the antioxidant rich Maillard reaction products [36,37]. The improved oxidative stability of some roasted seed oils has been connected to the Maillard process [38]. There was a 19.86% increase in polyphenol content in soybean grains during the roasting process. The roasted grains were observed to have the highest polyphenolic content (17.20%) and it was recorded lowest in RG (14.35%). Results were comparable with Joshi and Varma [31] who reported an increase in TPC content by 15.55% during the roasting of soybean.

3.2.2. Effect of germination treatments on nutritional components, antioxidant activity, and total phenolic components of soybean

The changes in nutritional components, antioxidant activity, and the TPC of soybean are described in Table 4. The moisture content of soybean ranged from 8.09 to 8.17%. It was observed highest in RS (8.09%) and lowest in G72 (8.13%). The fat content of treated grains ranged between 18.68 and 19.86% after soaking and germination treatments, respectively. It was observed highest (19.86%) in RS but it decreased to 18.68% after G72. Warle *et al*. [39] observed a similar decrease in fat content of soybean after 96 h of germination. Similarly, Joshi and Varma [31] reported 1.56% declines in fat content after 48 h of germination. The utilization of fat content as an energy source during germination is thought to be the cause of the decrease in fat content. According to Mostafa *et al*. [40], fat content gets decreased due to high metabolic activity in the grains during the germination processing of grains.

During germination processing of soybean grains, a significant ($P \leq 0.05$) increase (14.14%) in the fiber content was observed, and values increased from 7.00% (RS) to 7.99% (G72). Joshi and Varma [31] reported a 22.62% increase in soybean after G48. The crude fiber contents increased significantly during germination processing, which mainly consists of cellulose, lignin, and hemicelluloses [41,42] as the plant cells manufacture diverse cellular constituents. A diet rich in dietary fiber is recommended because the dietary fiber delays the release of glucose from food [43], which may be beneficial to the person suffering from diabetes. There was a non-significant ($P \leq 0.05$) change in ash content during the germination process and values ranged between 5.43% (RS) and 5.95% (G72). The protein content increased significantly ($P \leq 0.05$) from 38.35% (RG) to 40.86% (G72) causing a 6.54% increase in protein contents during the germination processing of soybean grains. Results are equivalent with the finding of Joshi and Varma [31] who reported a significant ($P \leq 0.05$) increase in protein content during soaking, germination, and roasting processes. The increase in protein content during germination treatment is due to the mobilization of stored nitrogen to make it available for the formation of high-quality proteins required for the young plant's development. The seed germination comprises the mobilization of protein reserves in the cotyledon, as well as the production of new proteins which is required for the growth of sprouts [44]. Many enzymes are stimulated during germination, and some proteins are generated by a sequence of biochemical reactions. Alternatively, protease can hydrolyze several other proteins also. The resultant protein content is influenced by the interactions of proteolysis and protein synthesis processes [45].

The carbohydrate and calorific values of germinated soybean ranged from 18.40 to 21.18% and 410.68 to 423.11 kcal/100 g, respectively.

Table 3: Effect of soaking and roasting treatments on nutritional components, antioxidant activity, and total phenolic components of soybean

Parameters	Raw soybean	Soaking		Roasting
		12 h	24 h	
Moisture (%)	8.17±0.20^{a}	8.20±0.05^{a}	8.19±0.05^{a}	8.12±0.07^{a}
Fat (%)	19.86±0.34^{a}	19.61±0.19^{ab}	19.47±0.16^{ab}	19.34±0.04^{b}
Fiber (%)	7.00±0.22^{a}	7.15±0.12^{a}	7.26±0.12^{a}	7.24±0.50^{a}
Ash (%)	5.43±0.08^{a}	5.71±0.16^{a}	5.74±0.15^{a}	5.70±0.28^{a}
Protein (%)	38.35±0.18^{c}	38.65±0.25^{a}	38.69±0.68^{a}	38.44±0.35^{a}
Carbohydrate (%)	21.18±0.20^{a}	20.68±0.19^{a}	20.65±0.62^{a}	21.16±0.64^{a}
Calorific value (Kcal/100 g)	423.11±2.58^{a}	419.90±1.10^{a}	418.65±1.15^{a}	418.63±3.02^{a}
Antioxidant activity (%)	38.22±0.20^{d}	60.93±0.16^{c}	66.02±0.12^{a}	62.81±0.20^{b}
Total phenolic content (mg GAE/100 g)	14.35±0.09^{d}	14.64±0.04^{c}	14.98±0.06^{b}	17.20±0.09^{a}

Values in the table are presented as mean±SD; values within rows sharing the same letters are not significantly different according to Duncan's LSD *post hoc* analysis at $P \leq 0.05$

Table 4: Effect of germination treatments on nutritional components, antioxidant activity, and total phenolic components of soybean

Parameters	Raw (0 h)	Germination		
		24 h	48 h	72 h
Moisture (%)	8.17±0.20^{a}	8.13±0.02^{a}	8.09±0.08^{a}	8.13±0.04^{a}
Fat (%)	19.86±0.34^{a}	18.95±0.47^{ab}	18.85±0.49^{ab}	18.68±0.09^{ab}
Fiber (%)	7.00±0.22^{c}	7.42±0.09^{b}	7.55±0.22^{b}	7.99±0.12^{a}
Ash (%)	5.43±0.08^{b}	5.75±0.24^{a}	5.89±0.11^{a}	5.95±0.19^{a}
Protein (%)	38.35±0.18^{c}	39.93±0.18^{b}	39.94±0.03^{b}	40.86±0.44^{a}
Carbohydrate (%)	21.18±0.20^{a}	19.82±0.46^{b}	19.25±0.44^{b}	18.40±0.53^{c}
Calorific value (Kcal/100 g)	423.11±2.58^{a}	415.41±1.73^{b}	413.97±3.42^{bc}	410.68±0.38^{c}
Antioxidant activity (%DPPH)	38.22±0.20^{d}	68.89±0.31^{c}	72.68±0.33^{b}	75.68±0.48^{a}
Total phenolic content (mg GAE/100 g)	14.35±0.09^{d}	15.32±0.13^{c}	16.26±0.25^{b}	16.85±0.09^{a}

Values in the table are presented as mean±SD; values within rows sharing the same letters are not significantly different according to Duncan's LSD *post hoc* analysis at $P \leq 0.05$

The carbohydrate contents were observed highest in RS (21.18%) which get decreased to 18.40% (G72) after the germination process. Similarly, the calorific value was observed highest in RS (423.11 kcal/100 g) and lowest in G72 (410.68 kcal/100 g). Activated enzymes resulted in the hydrolysis of energy providing components such as lipids and carbohydrates during germination resulting in a significant decline in carbohydrates as well as the calorific value [46].

The antioxidant activity of germinated grains increased from 38.22 to 75.68% (DPPH radical scavenging activity) resulting in a significant ($P \leq 0.05$) increase of 98.01% after G72 treatment and the results are comparable with the findings as revealed by Kumari *et al*. [47] after S12 (71.02%) and G72 (311.46%) treatments. During the germination process, the synthesis of water-soluble vitamins (Vitamin C) or other compounds with antioxidative properties, such as polyphenols, may improve total antioxidant activity.

The TPC ranged between 14.35 (RG) and 16.85 mg GAE/100 g (G72). The TPC content enhanced significantly ($P \leq 0.05$) by 17.42% during the germination process. A similar increase of 9.62% (G72) in TPC was observed during the germination treatment of soybean by Kumari *et al*. [47].

3.2.3. Effect of fermentation treatments on nutritional components, antioxidant activity, and total phenolic components of soybean

The changes in nutritional components, antioxidant activity, and TPC of soybean during fermentation are described in Table 5. The moisture and fat content of grains subjected to natural (NF) and *S. cerevisiae* (SCF) fermentation treatments varied slightly but non-significantly ($P \leq 0.05$). Moisture content ranged between 8.08% (NF12) and 8.17% (RS). Whereas, the values for fat content ranged between 19.18 and 19.86%. It was reported highest in RS (19.86%) but decreased to 19.18% after NF36. The fat content decreased by 3.43% after NF36 and 2.82% after SCF36. The reduction in fat content observed could be ascribed to the action of lipolytic enzymes during the process of fermentation [48,49].

During the fermentation treatment, the values for fiber content ranged between 5.10 and 7.00%. The crude fiber contents declined significantly ($P \leq 0.05$) during fermentation by 27.14% in soybean seeds which were comparable with the findings of Felix and Francis [48]. They reported that the crude fiber content decreased by 37.14% after 72 h of fermentation in African locust beans. According to Igbabul *et al*. [50], a decrease in fiber content after fermentation indicates softening of fibrous tissues and enhanced digestibility due to the conversion of complex carbohydrates and lignocelluloses into simple sugars. A non-significant ($P \leq 0.05$) change in ash content was observed during germination and values ranged between 5.27 (NF36) and 5.43% (RS). The protein contents increased significantly ($P \leq 0.05$) from 38.35% (RG) to 47.17% (SCF36) causing a 23% increase in soybean grains during the fermentation process. Similar observations were reported during the fermentation of lupin seeds as reported by Romero-Espinoza *et al*. [51]. During the fermentation process, an upsurge in protein content can be attributed to the release of nitrogen when microbes used carbohydrates as a source of energy [52]. A similar observation has been reported by Pranoto *et al*. [53] during the fermentation of sorghum seeds.

Carbohydrate and calorific values of fermented soybean ranged from 14.96 to 21.31% and 421.90 to 427.10 Kcal/100 g, respectively. The carbohydrate contents were observed highest in RS (21.31%) which get decreased to 14.90% (SCF36) after the fermentation process. A significant ($P \leq 0.05$) decrease in carbohydrate components was seen due to their use as an energy source during the fermentation process.

Similarly, the antioxidant activity increased from 38.22 (RS) to 64.21 (SCF36) during the fermentation process resulting in a significant ($P \leq 0.05$) upsurge of 68% after the SCF36 treatment. The findings are similar to those of Moore *et al*. [54] who revealed that after the fermentation process, some yeast species increased DPPH radical scavenging activity in cereals. The TPC ranged from 14.35 to 19.65 (mg GAE/100 g) during the fermentation of soybean. It was reported highest in SCF36 (19.65 %) and lowest in the RS (14.35%). The values for TPC get increased by 28.43% during NF and 36.93% during the SCF process of fermentation.

3.2.4. Effect of the soaking, germination, roasting, and fermentation treatments on anti-nutritional components of soybean

The changes in anti-nutritional components during various processing treatments are represented in Figures 1 and 2. The tannin contents decreased from 0.207% (RS) to 0.114% (G72) during germination and 0.207 (RS) to 0.112% (SCF36) during fermentation processes. There was a significant ($P \leq 0.05$) decrease of 8.70%, 44.93%, and 45.89% in tannin content during soaking, germination, and fermentation treatments, respectively. A similar trend of decline in tannin contents has been reported by Kumari *et al*. [47] where tannin content decreased by 14.22% during soaking and 50.46% during the germination process.

Phytic content reduced from 1.36 (RG) to 1.26% (S24) during soaking, 1.36 (RG) to 0.98% (G72) during germination, and 1.36

Table 5: Effect of fermentation treatments on nutritional components, antioxidant activity, and total phenolic components of soybean

Parameters	Raw (0 h)	Natural fermentation			Fermentation with *Saccharomyces cerevisiae*		
		12 h	24 h	36 h	12 h	24 h	36 h
Moisture (%)	8.17±0.20ᵃ	8.08±0.02ᵃ	8.09±0.07ᵃ	8.16±0.01 ᵃ	8.12±0.04ᵃ	8.11±0.04ᵃ	8.14±0.02ᵃ
Fat (%)	19.86±0.34ᵃ	19.30±0.14ᵇ	19.28±0.03ᵇ	19.18±0.20 ᵇ	19.35±0.28ᵇ	19.33±0.22 ᵇ	19.30±0.07 ᵇ
Fiber (%)	7.00±0.22ᵃ	6.83±0.07ᵃᵇ	6.53±0.24ᵇ	6.15±0.05 ᶜ	6.04±0.22ᶜ	5.87±0.25 ᶜ	5.10±0.05 ᵈ
Ash (%)	5.43±0.08ᵃ	5.30±0.09ᵃᵇ	5.28±0.03ᵇ	5.27±0.04 ᵇ	5.37±0.12ᵃᵇ	5.35±0.09 ᵃᵇ	5.34±0.04 ᵃᵇ
Protein (%)	38.35±0.18ᶠ	39.23±0.18ᵉ	41.85±0.18ᵈ	44.48±0.80 ᵇ	39.81±0.36ᵉ	43.61±0.18 ᶜ	47.17±0.27 ᵃ
Carbohydrate (%)	21.18±0.20ᵃ	21.27±0.12ᵃ	18.95±0.23ᵇ	16.77±0.97 ᵈ	21.31±0.38ᵃ	17.74±0.30ᶜ	14.96±0.28ᵉ
Calorific value (Kcal/100 g)	423.11±2.58ᵇ	421.90±0.87ᵇ	422.48±0.54ᵇ	422.89±0.96ᵇ	424.84±2.68ᵃᵇ	424.85±2.51ᵃᵇ	427.10±0.44ᵃ
Antioxidant activity (%DPPH)	38.22±0.20ᵍ	46.82±0.48ᶠ	51.88±0.12ᵉ	52.80±0.16 ᵈ	61.71±0.47ᶜ	62.74±0.21ᵇ	64.21±0.58ᵃ
Total phenolic content (mg GAE/100 g)	14.35±0.09ᶠ	17.20±0.09ᵉ	17.83±0.12ᵈ	18.43±0.16ᶜ	17.93±0.06ᵈ	18.91±0.09ᵇ	19.65±0.07ᵃ

Values in the table are presented as mean±SD; values with in rows sharing the same letters are not significantly different according to Duncan's LSD *post hoc* analysis at $P \leq 0.05$

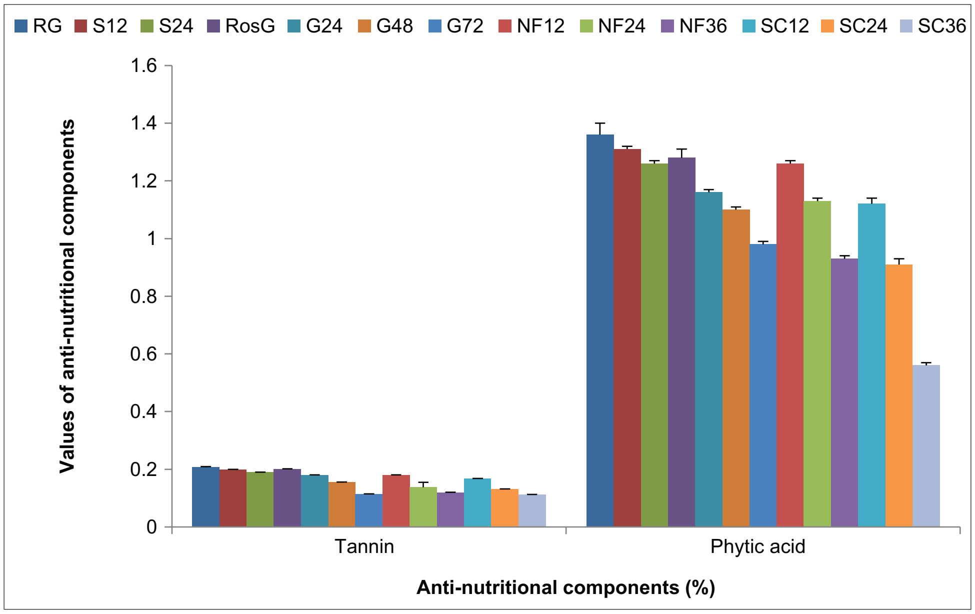

Figure 1: Effect of processing treatments on the anti-nutritional components of soybean (RG – Raw grains, S12 – Soaking for 12 h, S24 – Soaking for 24 h, RosG – Roasted grains, G24 – Germination for 24 h, G48 – Germination for 48 h, G72 – Germination for 72 h, NF12 – Natural fermentation for 12 h, NF24 – Natural fermentation for 24 h, NF36 – Natural fermentation for 36 h, SC12 – Fermentation with *Saccharomyces cerevisiae* for 12 h, SC24 – Fermentation with *S. cerevisiae* for 24 h, and SC36 – Fermentation with *S. cerevisiae* for 36 h)

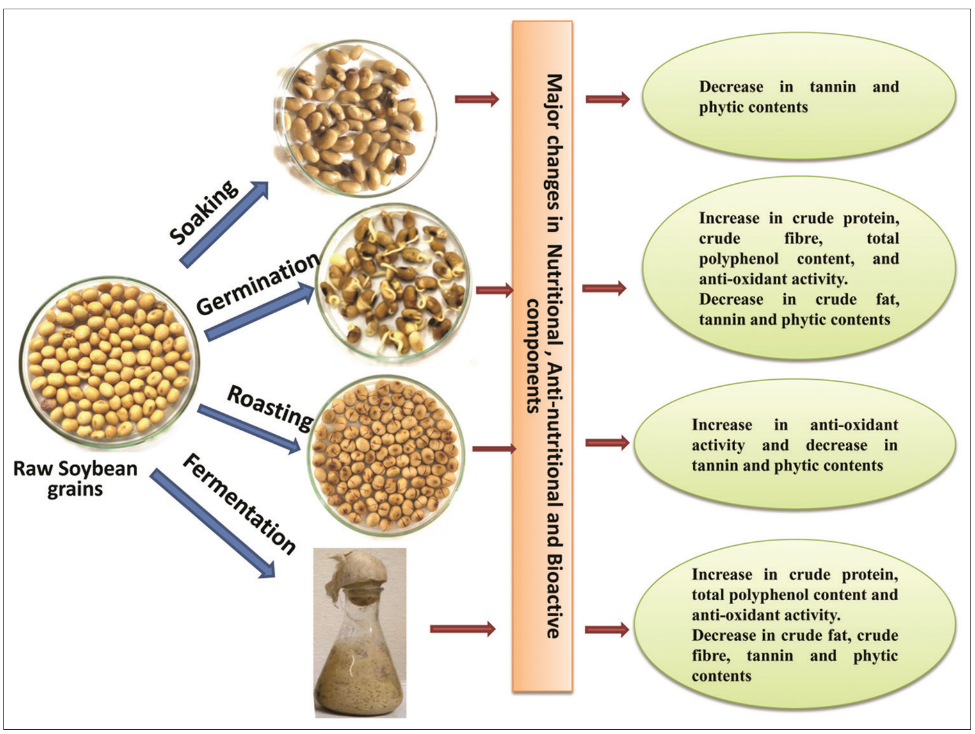

Figure 2: Schematic representation of processing treatments and major effects on nutritional, anti-nutritional, and bioactive components

(RG) to 0.56% (SCF36) during fermentation process. The values of phytic contents (%) diminished significantly ($P \leq 0.05$) by 7.35% during soaking 27.94% during germination and 58.82% during the fermentation treatments. A similar decline of 9.78% has been found by Kumari *et al*. [47] during the soaking process. Egli *et al*. [25] and Chitra *et al*. [55] reported a decline of 26.43% and 38.85% in phytic contents during the germination treatment. Luo *et al*. [56] stated that the significant ($P \leq 0.05$) reduction in phytic acid during germination treatment was due to the enhancement in activity of enzyme phytase resulting in increased availability of minerals. There was a 58.82% reduction in phytic acid and similar findings have been reported by Tope [57-59] where the phytic acid in lima bean seeds get decreased to 85.71% during the fermentation with *S. cerevisiae*. Decreases in tannin content with the increase in fermentation time agreed closely with the

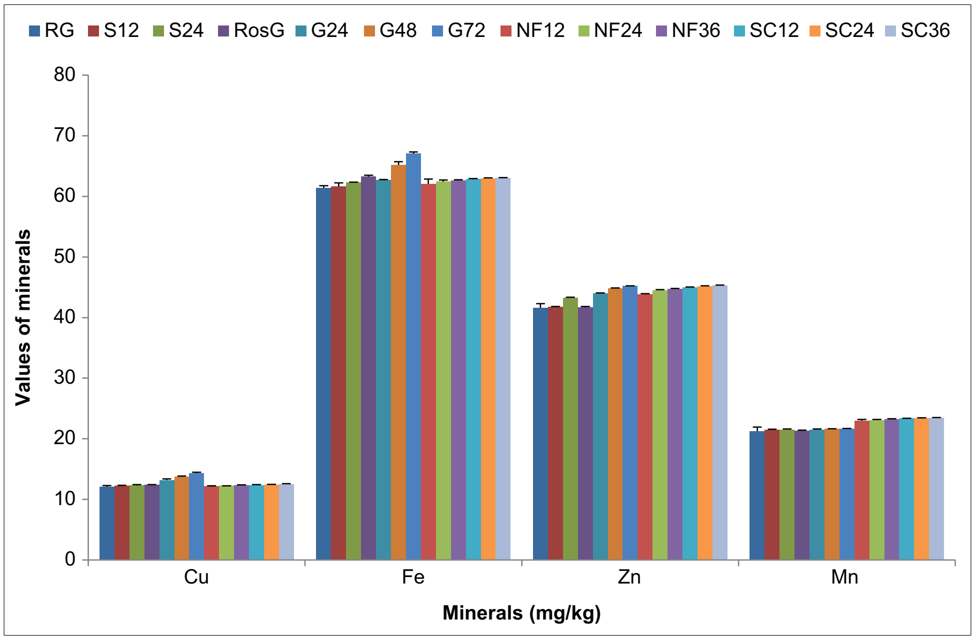

Figure 3: Effect of processing treatments on the mineral composition of soybean (RG – Raw grains, S12 – Soaking for 12 h, S24 – Soaking for 24 h, RosG – Roasted grains, G24 – Germination for 24 h, G48 – Germination for 48 h, G72 – Germination for 72 h, NF12 – Natural fermentation for 12 h, NF24 – Natural fermentation for 24 h, NF36 – Natural fermentation for 36 h, SC12 – Fermentation with *Saccharomyces cerevisiae* for 12 h, SC24 – Fermentation with *S. cerevisiae* for 24 h, and SC36 – Fermentation with *S. cerevisiae* for 36 h)

findings of Effiong and Umoren [57] during processing treatments in horse-eye beans (*Mucuna urens*). According to Molin [58], during the fermentation treatment by *Lactobacillus*, the tannase activity breaks down the tannin-protein complex, resulting in decreased tannin content in fermented grains. The anti-nutrient contents were reduced in fermented samples due to leaching and further microbial activities [60].

3.2.5. Effect of the soaking, germination, roasting, and fermentation treatments on the mineral content of soybean

The data regarding the changes in mineral components during various processing treatments are represented in Figure 3. Cu content increased from 12.10 (RS) to 12.38 mg/kg (RosG) during roasting, 12.10 (RS) to 14.33 mg/kg (G72) during germination, and 21.53 mg/kg (SCF36) during the fermentation treatments. Similarly, the Fe content increased significantly ($P \leq 0.05$) from 61.43 (RS) to 63.25 (RosG) during roasting, 67.08 (G72) during germination, and 63.03 mg/kg (SCF36) during fermentation treatments. The Zn content increased from 41.65 (RS) to 43.30 mg/kg (S24), 45.22 mg/kg (G72), and 45.30 mg/kg (SCF36) during soaking, germination, and fermentation treatments, respectively. In the case of Mn content, the values increased from 21.28 (RG) to 21.50 mg/kg (S24), 21.65 mg/kg (G72), and 23.42 mg/kg (SCF36) during the soaking, germination, and fermentation processing treatments, respectively. During the germination process, the values for Cu, Fe, Zn, and Mn contents increased by 18.43, 9.20, 8.57, and 1.74%, respectively. Similarly, during the fermentation process, there was 3.55, 2.60, 8.76, and 10.06% increase in the values for Cu, Fe, Zn, and Mn contents, respectively. Özcan and Al Juhaimi [35] reported similar increasing trends during the roasting as well as the sprouting of soybean cultivars.

4. CONCLUSION

The objectives of this study claim the effect of processing techniques on nutritional value, anti-nutritional compounds, as well as bioactive components of soybean. It was observed that there was a significant ($P \leq 0.05$) increase in protein contents, the total phenolic components, and antioxidant activity during the germination and fermentation treatments. Mineral contents were found to get increased significantly ($P \leq 0.05$) after processing treatments. Anti-nutritional components bind the micronutrients resulting in their decreased bioavailability. The leaching of tannins in water during the soaking process and their additional degradation during the processing treatments and increased activity of enzyme phytase caused the decline of anti-nutrients such as tannins and phytic acid and increasing the bioavailability of micronutrients in the processed grains. Therefore, it can be concluded that the use of processing techniques proved to improve the nutritional quality as well as the functionality of soybean seeds. The processed soybean can be used for value addition of bakery and extruded functional food products with decreased anti-nutrients and increased bioavailability of nutrients.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors state that no conflicts of interest exist in this study.

9. ETHICAL APPROVALS

There is no involvement of experiments on animals or human beings.

10. DATA AVAILABILITY

Not applicable.

11. PUBLISHER'S NOTE

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REFERENCES

1. Food and Agriculture Organization. Joint Report on Crop and Livestock Products. Food and Agriculture Orgaganiztaion of United Nation Series No. 100. Rome, Italy: Food and Agriculture Orgaganiztaion; 2021.
2. He FJ, Chen JQ. Consumption of soybean, soy foods, soy isoflavones and breast cancer incidence: Differences between Chinese women and women in Western countries and possible mechanisms. Food Sci Hum Wellness 2013;2:146-61.
3. Krishnan HB, Kim WS, Oehrle NW, Alaswad AA, Baxter I, Wiebold WJ, *et al*. Introgression of leginsulin, a cysteine-rich protein, and high-protein trait from an Asian soybean plant introduction genotype into a North American experimental soybean line. J Agric Food Chem 2015;63:2862-9.
4. McCue P, Shetty K. Health benefits of soy isoflavonoids and strategies for enhancement: A review. Crit Rev Food Sci Nutr 2004;44:361-7.
5. Emmons CL, Peterson DM. Antioxidant activity and phenolic content of oat as affected by cultivar and location. Crop Sci 2001;41:1676-81.
6. Liu K. Expanding soybean food utilization. Food Technol (Chicago) 2000;54:46-58.
7. Hotz C, Gibson RS. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. J Nutr 2007;137:1097-100.
8. Embaby HE. Effect of heat treatments on certain antinutrients and *in vitro* protein digestibility of peanut and sesame seeds. Food Sci Technol Res 2010;17:31-8.
9. Singh B, Singh JP, Shevkani K, Singh N, Kaur A. Bioactive constituents in pulses and their health benefits. J Food Sci Technol 2017;54:858-70.
10. Oyedoyin O, Olatunji O. Fate of pathogenic bacteria during fermentation of cereal porridge ("ogi")-A weaning food formula. Niger Food J 2009; 27: 19-24.
11. Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agric Food Chem 2002;50:4959-64.
12. American Association for Clinical Chemistry. Approved Methods of the American Association of Cereal Chemists. Washington, DC: American Association for Clinical Chemistry; 2000.
13. Huang S, Shiau C, Liu T, Chu C, Hwang DF. Effects of rice bran on sensory and physico-chemical properties of emulsified pork meatballs. Meat Sci 2005;70:613-9.
14. Jones D, Chinnaswamy R, Tan Y, Hanna M. Physiochemical properties of ready-to-eat breakfast cereals. Cereal Foods World 2000;45:164-8.
15. Sosulski F. The centrifuge method for determining flour absorption in hard red spring wheats. Cereal Chem 1962;39:344-50.
16. Kaur M, Sandhu KS, Arora A, Sharma A. Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: Physicochemical and sensory properties. LWT Food Sci Technol 2015;62:628-32.
17. Stojceska V, Ainsworth P, Plunkett A, İbanoğlu E, İbanoğlu Ş. Cauliflower by-products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks. J Food Eng 2008;87:554-63.
18. William P, Nakoul H, Singh K. Relationship between cooking time and some physical characteristics in chick pea (*Cicer arietinum* L.). J Sci Food Agric 1983;34:492-6.
19. AOAC. Official Methods of Analysis. Washington, DC: AOAC; 1990. p. 684.
20. Ranganna S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. New York: Tata McGraw-Hill Education; 1986.
21. Bouaziz M, Fki I, Jemai H, Ayadi M, Sayadi S. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chem 2008;108:253-62.
22. Saxena V, Mishra G, Saxena A, Vishwakarma K. A comparative study on quantitative estimation of tannins in *Terminalia chebula*, *Terminalia belerica*, *Terminalia arjuna* and *Saraca indica* using spectrophotometer. Asian J Pharm Clin Res 2013;6:148-9.
23. Gao Y, Shang C, Maroof M, Biyashev R, Grabau E, Kwanyuen P, *et al*. A modified colorimetric method for phytic acid analysis in soybean. Crop Sci 2007;47:1797-803.
24. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2007;2:875-7.
25. Egli I, Davidsson L, Juillerat M, Barclay D, Hurrell R. The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feedin. J Food Sci 2002;67:3484-8.
26. Sindhu R, Beniwal SK, Devi A. Effect of grain processing on nutritional and physico-chemical, functional and pasting properties of amaranth and quinoa flours. Indian J Tradit Knowl 2019;18:500-7.
27. Park J, Sung JM, Choi YS, Park JD. Effect of natural fermentation on milled rice grains: Physicochemical and functional properties of rice flour. Food Hydrocoll 2020;108:106005.
28. Day CN, Morawicki RO. Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. J Food Qual 2016;2018:3964392.
29. Sakare P, Jadhav M, John H. Study on physical properties of soaked soybean and functional properties of germinated soy flour. J Inst Eng (India) A 2020;101:787-94.
30. Gandhi A, Nenwani M, Ali N. Some physico-chemical characteristics of soybean, *Glycine max* Merr. Food Chem 1985;17:71-4.
31. Joshi P, Varma K. Effect of germination and dehulling on the nutritive value of soybean. Nutr Food Sci 2016;46:595-603.
32. Malenčić D, Popović M, Miladinović J. Phenolic content and antioxidant properties of soybean (*Glycine max* (L.) Merr.) seeds. Molecules 2007;12:576-81.
33. Kumari S, Krishnan V, Jolly M, Sachdev A. *In vivo* bioavailability of essential minerals and phytase activity during soaking and germination in soybean (*Glycine max* L.). Aust J Crop Sci 2014;8:1168-74.
34. Sharma S, Goyal R, Barwal S. Domestic processing effects on physicochemical, nutritional and anti-nutritional attributes in soybean (*Glycine max* L. Merill). Int Food Res J 2013;20:3203-9.

35. Özcan MM, Al Juhaimi F. Effect of sprouting and roasting processes on some physico-chemical properties and mineral contents of soybean seed and oils. Food Chem 2014;154:337-42.
36. Oliviero T, Capuano E, Cämmerer B, Fogliano V. Influence of roasting on the antioxidant activity and HMF formation of a cocoa bean model systems. J Agric Food Chem 2009;57:147-52.
37. Anese M, Manzocco L, Nicoli MC, Lerici CR. Antioxidant properties of tomato juice as affected by heating. J Sci Food Agric 1999;79:750-4.
38. Cai L, Cao A, Aisikaer G, Ying T. Influence of kernel roasting on bioactive components and oxidative stability of pine nut oil. Eur J Lipid Sci Technol 2013;115:556-63.
39. Warle B, Riar C, Gaikwad S, Mane V. Effect of germination on nutritional quality of soybean (*Glycine Max*). J Environ Sci Toxicol Food Technol 2015;1:13-6.
40. Mostafa M, Rahma E, Rady A. Chemical and nutritional changes in soybean during germination. Food Chem 1987;23:257-75.
41. Laxmi G, Chaturvedi N, Richa S. The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. J Nutr Food Sci 2015;5:5.
42. Zheng Y, He R, Zheng L, Hua P, Hu Q, Huang X. Change of anti-nutrient and phytic acid contents in brown rice during sprouting period. J Chinese Cereals Oils Assoc 2006;21:1-4.
43. Riccardi G, Rivellese AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. Diabetes Care 1991;14:1115-25.
44. Rodríguez C, Frias J, Vidal-Valverde C, Hernández A. Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils. Food Chem 2008;108:245-52.
45. Zhang G, Xu Z, Gao Y, Huang X, Zou Y, Yang T. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. J Food Sci 2015;80:H1111-9.
46. Khandelwal S, Udipi SA, Ghugre P. Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. Food Res Int 2010;43:526-30.
47. Kumari S, Krishnan V, Sachdev A. Impact of soaking and germination durations on antioxidants and anti-nutrients of black and yellow soybean (*Glycine max*. L) varieties. Plant Biochem Biotechnol 2015;24:355-8.
48. Felix AE, Francis AK. Effect of traditional fermentation process on the nutrient and anti-nutrient content of maize and African Locust beans. J Food Nutr Res 2019;2:65-75.
49. Uvere PO, Onyekwere EU, Ngoddy PO. Production of maize-bambara groundnut complementary foods fortified prefermentation with processed foods rich in calcium, iron, zinc and provitamin A. J Sci Food Agric 2010;90:566-73.
50. Igbabul B, Amove J, Twadue I. Effect of fermentation on the proximate composition, antinutritional factors and functional properties of cocoyam (*Colocasia esculenta*) flour. Afr J Food Sci 2014;5:67-74.
51. Romero-Espinoza AM, Vintimilla-Alvarez MC, Briones-García M, Lazo-Vélez MA. Effects of fermentation with probiotics on anti-nutritional factors and proximate composition of lupin (*Lupinus mutabilis* sweet). LWT Food Sci Technol 2020;130:109658.
52. Onyango C, Noetzold H, Ziems A, Hofmann T, Bley T, Henle T. Digestibility and antinutrient properties of acidified and extruded maize-finger millet blend in the production of uji. LWT Food Sci Technol 2005;38:697-707.
53. Pranoto Y, Anggrahini S, Efendi Z. Effect of natural and *Lactobacillus plantarum* fermentation on *in-vitro* protein and starch digestibilities of sorghum flour. Food Biosci 2013;2:46-52.
54. Moore J, Cheng Z, Hao J, Guo G, Liu JG, Lin C, *et al*. Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. J Agric Food Chem 2007;55:10173-82.
55. Chitra U, Singh U, Rao PV. Phytic acid, *in vitro* protein digestibility, dietary fiber, and minerals of pulses as influenced by processing methods. Plant Foods Hum Nutr 1996;49:307-16.
56. Luo YW, Xie WH, Jin XX, Wang Q, He YJ. Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. CyTA J Food 2014;12:22-6.
57. Effiong O, Umoren U. Effect of multi-processing techniques on the chemical composition of horse eye bean (*Mucuna urens*). Asian J Anim Sci 2011;5:340-8.
58. Molin G. *Lactobacillus plantarum*: The Role in Foods and in Human Health. CRC press, New York; 2008: 353-393.
59. Tope AK. Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. Int J Adv Res 2014;2:1208-15.
60. Nwosu J. Effect of soaking, blanching and cooking on the anti-nutritional properties of asparagus bean (*Vigna sesquipedis*) flour. Nat Sci 2010;8:163-7.

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