

Impact of soaking, germination, fermentation, and roasting treatments on nutritional, anti-nutritional, and bioactive composition of black soybean (*Glycine max* L.)

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ABSTRACT

Legumes are plants of the family *Leguminosae* with seed pods that split into two halves. Black soybean seed coat contains numerous bioactive compounds having radical scavenging, anti-tumor, and anti-carcinogenic activities. This study was aimed to assess the effect of soaking, germination, natural fermentation, and roasting on nutritional and anti-nutritional components, minerals (Fe, Zn, Mn, and Cu), and bioactive components of the black soybean. The effect of soaking was studied at 12 and 24 h while that of germination at 24, 48, and 72 h. The results revealed that the phenolic contents augmented significantly ($P \le 0.05$) in germination, fermentation, and roasting by 11.49%, 8.96%, 2.95%. Further, there was an 11.84% and 22.13% increase in the protein contents during the germination and fermentation processes, respectively. The antioxidant activity of processed grains increased significantly ($P \le 0.05$) during germination, fermentation, and roasting by 72.51, 10.14, and 9.64%, respectively. The anti-nutritional compounds such as phytic acid and tannin contents decreased significantly ($P \le 0.05$) during processing treatments. Phytic acid decreased to the extent of 34.04, 51.06, and 13.47% and tannin contents as 47.22, 75, and 38.89%, after germination, fermentation, and roasting processes, respectively. A significant ($P \le 0.05$) increase in mineral contents was observed after the germination, fermentation, and roasting of the black soybean.

1. INTRODUCTION

There is great importance of pulses in human nutrition as these provide a sufficient amount of proteins, calories, vitamins, minerals, and other bioactive components [1]. The popularity of soybean is growing at faster rate as these are rich sources of micronutrients such as Fe, Zn, and Ca and have low glycemic index. 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 [2]. Besides nutraceutical components, seed comprises numerous essential isoflavones, namely, daidzein and genistein having medicinal properties [3]. The soybean seed coat is rich in several bioactive components having radical scavenging, anti-tumor, and anti-carcinogenic activity [4]. It is nutritionally rich and comprises higher contents of carbohydrates (30%), proteins (32.1–39.8%), fats (10.8–19.6%), dietary fibers (21.77–30.31%), and minerals (3.93–6.15%) including phosphorous,

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Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib - 173 101, Himachal Pradesh, India. E-mail: krishankumar02007@gmail.com iron, potassium, sodium, zinc, copper, and manganese. Besides, higher content of vitamin B comprising vitamin B1, B2, B3, B5, and B6 is present in different cultivars of soybean [5].

Black soybean (*Glycine max* L. Merrill) has recently received considerable attention due to its high nutritional value and availability as an ingredient in various foods and folk medicines in Asia. Out of the supposed medicinal components in black soybean, the common chemical components are anthocyanins [6], isoflavones such as phytoestrogens [7], oligosaccharides, and saponins [7]. Soybean contains anti-nutritional factors such as tannin and phytic acid. These anti-nutritional factors, especially phytates, are powerful chelating agents that reduce the bioavailability of divalent cations such as zinc, iron, and calcium by the formation of insoluble phytates [8].

Traditional processing treatments such as soaking, fermenting, germinating, and roasting have been utilized for improving the nutritional value of cereals and pulses [9]. The germination process is widely used in cereals and legumes for increasing the nutritive value mainly through the breakdown of anti-nutritional components [10]. Processing techniques responsible for decreasing the anti-nutritional factors as well as minimizing the losses of micronutrients are of great interest to scientists. The thermal, as well as biological processing

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treatments, have the potential to increase the bioavailability of nutrients in foods [9,11]. These techniques lead to a decline in antinutrients such as phytic acid and increase the solubility of minerals in foods resulting in an improved bioavailability of mineral contents in legumes and cereals. The study is aimed at investigating the changes in nutritional, anti-nutritional, and bioactive components of black soybean subjected to various processing treatments such as soaking, germination, fermentation, and roasting.

2. MATERIALS AND METHODS

2.1. Materials

The black soybean (VL-BHATT) used in the current research was procured from Vivekananda Parvatiya Krishi Anushandhan Sansthan, Almora. The chemicals utilized were bought from standard companies such as Merck India, BDH chemicals, Hi-Media, Qualigens, and Sigma.

2.2. Physicochemical Characteristics

The physicochemical assessment of raw soaked, germinated, roasted, and fermented black soybean was done at the laboratories of the Eternal University, Baru Sahib, Sirmour, India. The moisture content was estimated by following the air-oven drying method of AOAC [12]. The crude fiber was determined using Fibroplus (Pelican Inc.) and crude protein contents using Kjeloplus (Pelican Inc.). The crude fat contents were assessed by the equipment Soxoplus (Pelican Inc.), and ash contents were measured, as per the technique defined by Ranganna [13]. The total carbohydrates were calculated by deducting the sum of moisture, protein, fat, fiber, and ash content out of 100. The calorific value (kcal/g) was calculated by multiplying the contents of crude protein, fats, and carbohydrates with the Atwater factors of 4.0, 9.10, and 4.2, respectively [14]. The minerals were estimated as per AOAC [11] using Atomic Absorption Spectrometer (Agilent Technology). The antioxidant activity (AA) (%) was assessed according to the methodology discussed by Bouaziz et al. [15] and expressed as DPPH radical scavenging activity (% inhibition). The tannins (%) were determined as per the procedure defined by Saxena et al. [16]. The anti-nutrient phytic acid was evaluated as per the techniques given by Gao et al. [17]. Total phenolic contents (TPC) were determined using Folin–Ciocalteu reagent as discussed by Ainsworth and Gillespie [18] and expressed as mg GAE/100g.

2.3. Processing Treatments of Black Soybean

The soaking as well as the germination of black soybean seeds was conducted as per the methodology given by Egli *et al.* [19]. For soaking treatment, the grains of black soybean were cleaned manually and dipped in distilled water (1:5) for 12 and 24 h at ambient conditions and were subjected to oven-drying for 24 h at a temperature of 40°C. For germination, the 30 g seeds were taken for each treatment and immersed in 120 ml of water in a beaker for 16 h. The seeds were drained off, spread on a tray, and covered with a wet muslin cloth. The germination was conducted at 24, 48, and 72 h at 25°C in an incubator. The muslin cloth was kept wet by sprinkling with water frequently during germination. Seeds after germination were dried at a temperature of 40°C in a hot air oven for a period of 24 h.

For roasting treatment, seeds were heated on a hot plate at 180°C for 10 s. The roasted seeds were cooled and converted to fine flour by a kitchen grinder (Sujata, Powermatic Plus) at high speed to obtain flour of fine particle size. The flour was sieved through a 60-mesh screen and stored in an airtight container at 4°C until further analysis. The

process fermentation was conducted by mixing 100 g of black soybean flour with 300 ml of distilled water and kept in an incubator at 37°C for 72 h. The microorganisms naturally present in soybean flour caused fermentation. The samples were mixed thoroughly every 12 h and the fermentation was conducted for different time intervals, that is, 12, 24, and 36 h. The samples collected at each period were oven-dried at 50°C, ground in a blender, and sieved by passing through a 60-mesh sieve [20] [Figure 3].

2.4. Statistical Analysis

The IBM SPSS Statistics 26 software was used for the analysis of data using one-way analysis of variance. The values in tables were presented as mean \pm Standard Deviation and changes in values were considered significant at the level of $P \le 0.05$.

3. RESULTS AND DISCUSSION

3.1. Nutritional, Functional, and Anti-nutritional Components of Raw Black Soybean

The data on nutritional as well as anti-nutritional components are presented in Table 1. Black soybean contains 10.57% of moisture, 16.36% of crude fat, 8.23% of crude fiber, 3.08% ash content, 24.67% crude protein, and 75.33% of carbohydrates. Sumangala and Kulkarni [21] reported that moisture, fat, protein, fiber, ash, carbohydrate, and calorific value of black soybean were 8.58%, 17.86%, 42.35%, 6.30%, 5.82%, 19.60%, and 408.00 kcal/100g, respectively. Black soybean was found a rich source of polyphenolic, antioxidant, and anthocyanin components. It contained 56.56 mg GAE/100g of polyphenolic components, 27.69% AA, and 614.41 mg CGE/100g (Cyanidin 3-glucoside equivalent/100 g) of anthocyanin content. Choi *et al.* [22] reported that the AA, TPC, and anthocyanin content of black soybean ranged between 32.3 and 39.6%, 19.87–46.25 mg GAE/g, and 45.47–1085.89 mg/100 g, respectively.

Anti-nutritional factors such as tannin and phytic content were reported as 0.24 mg/g and 1.51 mg/g, respectively. Rusydi and Azrina [23] reported phytic and tannin content in black soybean flour as 0.63 mg/g

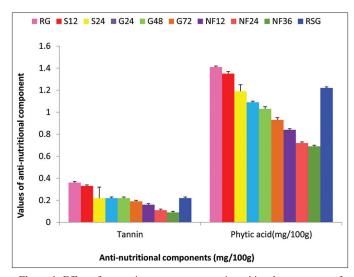


Figure 1: Effect of processing treatments on anti-nutritional components of black soybean (RG-Raw grains, S12-Soaking for 12 h, S24-Soaking for 24 h, RSG-Roasted grains, G24-Germination for 24 h, G48- Germination for 48 h, G72-Germination for 72 h, NF12-Natural fermentation for 12 h, NF24-Natural fermentation for 24 h, NF36- Natural fermentation for 36 h).

and 0.056 mg/g, respectively. The mineral contents such as Cu, Mn, Fe, and Zn have been reported as 0.76, 0.46, 5.97, and 1.21 ppm, respectively.

3.2. Effect of Processing Treatments on Nutritional and Antinutritional Components of Black Soybean

3.2.1. Nutritional characteristics

The various changes in nutritional and anti-nutritional characteristics of black soybean were determined after soaking treatment of 12 (S12) and 24 h (S24), germination treatment of 24 (G24), 48 (G48), and 72 h (G72), natural fermentation treatment of 12 (NF12), 24 (NF24), and 36 h (NF36), and roasting treatments (RS). The moisture contents decreased significantly from 10.57 in raw grains (RG) to 9.38 (G72) and fat content from 16.36 (RG) to 15.88% (G72), respectively, during the soaking and germination processing [Table 2]. A decrease of 11.25% in moisture content was recorded in samples subjected to

Table 1: Nutritional, anti-nutritional, and bioactive composition of black souhean

soybean.	
Parameters	Raw grains
Moisture (%)	10.57 ± 0.45
Fat (%)	16.36±0.01
Fiber (%)	8.23±0.03
Ash (%)	3.08 ± 0.01
Protein (%)	24.67 ± 0.02
Carbohydrates (%)	37.48 ± 0.44
Calorific value (kcal/100g)	415.05±1.85
Tannin contents (mg/g)	0.36±0.01
Phytic acid (mg/g)	1.41 ± 0.01
Antioxidant activity (% inhibition)	27.69±2.65
Total phenolic components (mg GAE/100 g)	56.56±0.79
Anthocyanin contents (mg CGE/kg)	614.41±3.22
Copper (ppm)	$0.76{\pm}0.02$
Manganese (ppm)	$0.46{\pm}0.02$
Iron (ppm)	5.97±0.03
Zinc (ppm)	1.21±0.03

Values in the table are presented as mean±SD

germination for 72 h. Warle *et al.* [24] reported a 10% decrease in the fat content of soybean after germination.

The moisture content of fermented grains declined significantly ($P \le 0.05$) from 10.57 (RG) to 8.98% (NF36) [Table 3]. Babalola and Giwa [25] reported a decrease in moisture content after 5 days of fermentation treatment of soybean. There was an 8.61% decrease in fat content and values decreased from 16.36 (RS) to 14.95% (NF72). Inyang and Zakari [26] reported a 27.32% decline in fat content in pearl millet after fermentation processing. Xu et al. [27] observed a decline in fat content in grain after the fermentation process and associated it with higher lipolytic action of enzymes during the fermentation that caused the hydrolysis of fat components.

In the roasting process, the moisture content declined significantly $(P \le 0.05)$ from 10.57% to 6.17%, whereas fat content increased from 16.36% to 17.59% resulting in 7.51% increase in fat content [Table 4]. Oboh et al. [28] reported that fat content increased by 18.19% in yellow maize during roasting. They associated it with the breakdown of the bonds in the endosperm of maize due to high temperature resulting in the effective mobilization of the oil content in maize after the roasting process. The fiber content in black soybean increased significantly from 8.23 to 9.94% resulting in a 20.77% rise during soaking and germination processing. Warle et al. [24] reported a 29.16% increase in crude fiber after G36 treatment and it has also been found to be associated with a significant augmentation in the cellular constituents such as cellulose, hemicelluloses, and lignin during the germination process. In case of fermentation, the crude fiber content decreased significantly ($P \le 0.05$) from 8.23% to 7.46% resulting in a 9.23% decrease after NF36 treatment. Babalola and Giwa [25] reported that the crude fiber decreased from 20.16 to 17.20% after 5 days of fermentation treatment. This can be due to the degradation of the fiber components by fermenting microbes. In the process of roasting, there was a significant ($P \le 0.05$) decrease in crude fiber content and it declined from 6.23% to 6.12%. A similar decrease in crude fiber content has been reported by Oboh et al. [28] during the roasting of soybean seeds and attributed it to the structural changes in the cell wall.

The values for ash content diminished to a significant ($P \le 0.05$) level from 3.08% to 2.90% during the germination process. However, protein content augmented significantly from 24.67% to 27.59%. An increase of 11.83% in protein content was recorded after G72 treatment. Warle *et al.* [24] reported a 7.27% decrease in ash content and a

Table 2: Effect of soaking and germin	ation treatments at different	time intervals on the n	utritive value of black soybean.
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Parameters	Time interval (h)						
		Soaking			Germination		
	0	12	24	24	48	72	
Moisture (%)	10.57±0.45ª	$9.98{\pm}0.45^{\mathrm{b}}$	9.76±0.45 ^b	9.83±0.03 ^b	9.62±0.03 ^b	$9.38{\pm}0.06^{\mathrm{b}}$	
Fat (%)	16.36±0.01ª	16.34±0.03ª	16.29±0.03 ^b	16.15±0.02°	$16.04{\pm}0.02^{d}$	15.88±0.03°	
Fiber (%)	8.23±0.03°	$9.14{\pm}0.03^{\rm d}$	$9.16{\pm}0.02^{d}$	9.47±0.02°	$9.66{\pm}0.02^{b}$	9.94±0.03ª	
Ash (%)	$3.08{\pm}0.01^{a}$	$2.94{\pm}0.04^{\rm b}$	$2.92{\pm}0.01^{b}$	$2.92{\pm}0.01^{b}$	$2.90{\pm}0.02^{\text{b}}$	$2.89{\pm}0.03^{\rm b}$	
Protein (%)	$24.67{\pm}0.02^{\rm f}$	26.15±0.06 ^e	$26.85{\pm}0.03^{\rm d}$	26.98±0.01°	27.18 ± 0.02^{b}	$27.59{\pm}0.06^{\rm a}$	
Carbohydrates (%)	39.09±0.44ª	$37.45{\pm}0.38^{\mathrm{b}}$	$37.02{\pm}0.40^{\rm bc}$	$36.65{\pm}0.06^{\rm cd}$	$36.61{\pm}0.05^{\rm cd}$	$36.31{\pm}0.11^{d}$	
Calorific value (kcal/100g)	415.05±1.85 ^b	411.26±1.65°	460.49±3.69ª	$406.86{\pm}0.07^{d}$	406.51±0.23 ^d	405.66±0.26 ^d	
Antioxidant activity (% inhibition)	27.69±2.65°	$32.09{\pm}0.06^{\rm d}$	33.28±0.16°	33.19±0.24°	46.43±0.35 ^b	$47.77{\pm}0.19^{a}$	
Total phenolic components (mg GAE/100g)	56.56±0.79°	$58.59{\pm}0.29^{\rm d}$	$60.14{\pm}0.02^{\circ}$	$60.97 \pm 0.09^{\circ}$	$61.06{\pm}0.01^{\rm b}$	63.06±0.01ª	
Anthocyanin contents (mg CGE/kg)	614.41±3.22ª	$605.93{\pm}1.85^{a}$	605.55±3.76ª	$601.31{\pm}0.38^{\rm b}$	598.06±1.06°	593.34±2.50°	

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≤0.05

Table 3	: Effect of	fermentation	treatment a	at different	time i	intervals on	the nutritive	value	of black soybean.	
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Parameters	Time interval (h)			
	0	12	24	36
Moisture (%)	$10.57{\pm}0.45^{a}$	9.31±0.03 ^b	9.27±0.21 ^b	$8.98{\pm}0.01^{b}$
Fat (%)	16.36±0.01ª	15.36 ± 0.20^{b}	15.12±0.05°	$14.95 \pm 0.02^{\circ}$
Fiber (%)	8.23±0.03ª	$7.97{\pm}0.04^{\rm b}$	7.62±0.04°	$7.46{\pm}0.04^{\rm d}$
Ash (%)	3.08±0.01ª	$2.89{\pm}0.02^{\text{b}}$	$2.88{\pm}0.02^{\rm bc}$	2.84±0.04°
Protein (%)	$24.67{\pm}0.02^{d}$	28.04±0.02°	28.93±0.02b	30.13±0.01ª
Carbohydrates (%)	39.09±0.44ª	36.43 ± 0.20^{b}	36.18±0.20 ^b	35.64±0.09°
Calorific value (kcal/100g)	415.05±1.85ª	403.25±1.15 ^b	$403.88{\pm}0.83^{\rm b}$	405.12±0.25 ^b
Antioxidant activity (% inhibition)	27.69±2.65°	28.99 ± 0.46^{b}	29.96±2.01b	30.50±0.42ª
Total phenolic components (mg GAE/100g)	56.56±0.79 ^b	59.40±0.31 ^b	61.48±0.38ª	61.63±0.46 ^a
Anthocyanin contents (mg CGE/kg)	614.41±3.22ª	596.39±1.51 ^b	586.19 ± 3.72^{b}	588.90±2.55 ^b

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≤0.05

Table 4: Effect of roasting treatment on	the nutritive value of black soybean.
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Parameters	Raw grains	Roasted grains
Moisture (%)	10.57 ± 0.45	6.17±0.02
Fat (%)	$16.36{\pm}0.01$	17.59 ± 0.05
Fiber (%)	6.23 ± 0.03	6.12 ± 0.02
Ash (%)	$3.08{\pm}0.01$	2.99 ± 0.02
Protein (%)	24.67 ± 0.02	22.17±0.02
Carbohydrates (%)	39.09±0.44	44.96 ± 0.07
Calorific value (Kcal/100g)	415.05 ± 1.85	352.45±0.28
Tannin content (mg/ g)	$0.36{\pm}0.01$	$0.22{\pm}0.01$
Phytic acid (mg/ g)	1.41 ± 0.01	$1.22{\pm}0.01$
Antioxidant activity (% inhibition)	27.69±2.65	30.36±0.25
Total Phenolic Components (mg GAE/ 100 g)	56.56±0.79	58.23±0.23
Anthocyanin contents (mg CGE/ kg) 614.41±3.22	584.41±3.22

Values in the table are presented as mean±SD

19.99% increase in protein content during germination. In the case of fermentation, a significant decrease (3.08-2.84%) in ash content was observed during the fermentation process. Constant and Kablan [29] reported a 54.14% decrease in ash content after fermentation of lima bean. The protein contents increased significantly from 24.67% to 30.13% in black soybean resulting in a 22.13% increase in proteins after NF36 treatment. Barampama and Simard [30] reported an increase in protein content after fermentation of common bean. The enrichment in proteins after fermentation can be due to an increase in nitrogen content released when microorganisms used carbohydrates for energy [31]. In roasting, a non-significant change in ash content was observed and values decreased from 3.08% to 2.99%. Oboh et al. [28] reported that roasting did not cause a significant change in the ash content (1.93-2.00%) during the roasting of white maize flour. The carbohydrate content of black soybean declined from 39.09% to 36.31% during germination.

Warle *et al.* [24] found that the germination of soybean reduced the carbohydrate content from 22.1% to 17.9 (%). Similarly, a significant decrease ($P \le 0.05$) in the calorific value from 415.05 to 405.66 kcal/100g was observed after germination of the black soybean. During fermentation, the levels of carbohydrates and calorific value of fermented flour decreased significantly. The values for carbohydrates decreased from 39.09 to 35.64% and calorific values from 415.05 to

405.12 kcal/100g. According to Đorđević *et al.* [32], fermentation caused a significant decline in the carbohydrate contents of the maize.

Whereas during roasting, the values of carbohydrates increased significantly from 39.09% to 44.96%, and calorific values decreased from 357.84 to 352.45 kcal/100g. As the carbohydrates in plant food are generally calculated by difference, the decreased amounts of crude protein, fiber, and moisture contents after the roasting treatment resulted in increasing the values of carbohydrates [28].

3.2.2. TPC, AA, and Anthocyanin

The TPC augmented significantly from 56.56 to 63.06 mg GAE/100g during the soaking and germination processing of grains. There was an 11.49% rise in TPC after G72 treatment. Lin and Lai [33] reported that the TPC of soybeans gets increased after germination and it increased further with an increase in germination time.

After fermentation, there was an 8.96% rise in the TPC after 36 h of fermentation. Similar results have been obtained by Lin and Tanaka [34] who demonstrated that fermentation treatment enhanced the phenolic content of soybean. In roasting, the TPC decreased significantly from 56.56 to 58.23 mg GAE/100g resulting in an augmentation of 2.95% in TPC after the roasting of the black soybean.

Anthocyanin contents decreased significantly ($P \le 0.05$) from 614.41 to 593.34 mg CGE/kg resulting in a 3.42% decline in anthocyanin contents after 72 h of germination. During fermentation, the anthocyanin contents decreased significantly from 614.41 to 588.90 mg CGE/kg. In roasting, the anthocyanin content decreased from 614.41 mg CGE/kg to 584.41 mg CGE/kg. Lemos *et al.* [35] found that roasting at 150°C for 45 min did not significantly influence the anthocyanin content in Baru nuts. There was a 72.51% rise in the AA in black soybean grains during the germination and grains after G72 treatment possessed the highest AA (47.77%) and it was observed lowermost in raw grains (27.69%). In the case of fermentation, the AA enhanced from 27.69–30.50% resulting in a 10.14% increase in values. During roasting, the AA increased significantly ($P \le 0.05$) from 27.69% to 30.36%. There was a 9.64% increase in AA during roasting. Lemos *et al.* [35] reported higher AA after roasting Baru nuts.

3.2.3. Anti-nutritional Components

The changes in anti-nutritional components during various processing treatments are represented in Figure 1. The tannin contents declined significantly from 0.36 mg/g to 0.19 mg/g in black soybean. There was

a 47.22% decline in tannin content after G72 treatment. Shimelis and Rakshit [36] found a significant reduction in tannins contents because of the leaching out of tannins in water during soaking and germination. Similarly, the phytic acid decreased significantly ($P \le 0.05$) by 34.04% after the G72 treatment. Egli *et al.* [19] reported a 56.09% decline in phytic content after the germination process. Luo *et al.* [37] postulated that phytic acids may get reduced due to higher activity of phytase enzyme during germination treatment.

In fermentation, the tannin contents decreased from 0.36 to 0.09 mg/g. There was a significant reduction (75%) in tannin contents after NF36 treatment. Similarly, the values for phytic content declined from 1.41 mg/g to 0.69 mg/g resulting in a 72% reduction in phytic

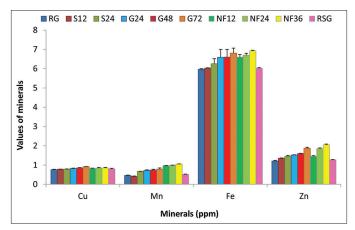


Figure 2: Effect of processing treatments on mineral contents of black soybean. (RG-Raw grains, S12-Soaking for 12h, S24-Soaking for 24 h, RSG-Roasted grains, G24-Germination for 24 h, G48-Germination for 48 h, G72-Germination for 72 h, NF12-Natural fermentation for 12 h, NF24-Natural fermentation for 24 h, NF36- Natural fermentation for 36 h).

content after the fermentation process. Towo *et al.* [38] reported that fermentation caused a higher decline in phytic acid than other treatments and found it to be due to the development of low pH during fermentation. Reale *et al.* [39] stated that microbial phytase in microorganisms hydrolyzes phytic acid to inorganic phosphate and inositol at low pH and is responsible for decreasing the phytic acid in the fermented products [40]. During roasting, the tannin contents diminished from 0.36% to 0.22% causing a 38.88% decline in tannin contents. Similarly, the phytic acid content reduced significantly from 1.41% to 1.22% resulting in a 13.47% reduction in phytic content after roasting of grain for 2 min.

3.2.4. Mineral Components

The data regarding the changes in mineral components during various processing treatments are represented in Figure 2. A significant rise in mineral contents of black soybean was observed after processing treatments. The values for the Cu content augmented from 0.76 to 0.91 ppm during the soaking and germination processing. The Mn content increased from 0.46 ppm to 0.79 ppm, Fe content increased from 5.97 to 6.81 ppm, and values for Zn content increased from 1.27 to 1.87 ppm during the soaking and germination treatment. Results are similar to the findings of Guardianelli *et al.* [41] who found an increase in zinc, copper, and manganese by 15, 6.93, and 5%, respectively, after 24 h of germination in amaranth seeds.

Similar change was observed during fermentation treatment of black soybean seeds. The Cu content augmented significantly ($P \le 0.05$) from 0.76 to 0.85, Mn from 0.64 to 0.99, Fe from 3.60 to 5.18, and Zinc contents from 0.64 to 0.86. Gabriel-Ajobiewe [42] reported an upsurge in the mineral content of jack beans after fermentation. The increase in the mineral contents indicated that minerals were released

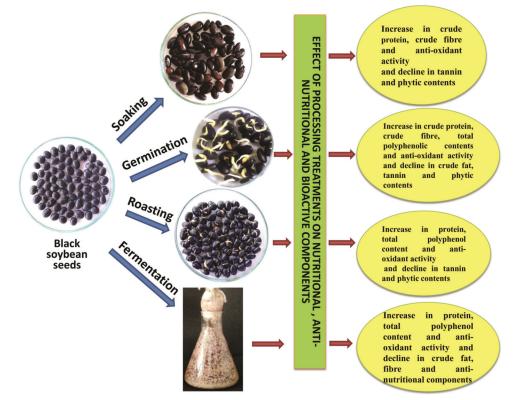


Figure 3: Schematic representation of processing treatments and major effects on nutritional, anti-nutritional, and bioactive components

from chelated complex compounds due to increased microbial activity during the fermentation process [43].

In the case of roasting, there was a slight increase in values for mineral contents in black soybean seeds. The Cu content increased from 0.76 to 0.80 ppm, Fe content from 5.97 to 6.02, Zn from 1.21 to 1.27, and Mn content from 0.46 to 0.51 ppm during the roasting treatment. Oboh *et al.* [28] reported an increase in mineral contents of different maize varieties during roasting.

4. CONCLUSIONS

This study aimed to evaluate the impact of processing techniques on the nutritional composition, anti-nutritional compounds, as well as bioactive potential of the black soybean. Germination, fermentation, and the roasting processes were found to decline the anti-nutrients such as tannin and phytic acid contents resulting in improving the nutritional quality of black soybean. The changes in macronutrients during germination have been attributed to the activation of endogenous enzymes and further increased energy requirements during the growth of the seedling. These processing techniques generally increased the soluble molecules and enhanced the digestibility of black soybean grains. These changes were quite positive to produce weaning foods, where high energy and low viscosity are desirable. Therefore, it can be concluded processing techniques improved the nutritional value as well as the functionality of black soybean seeds. The flour obtained from processed soybean grains can be incorporated with wheat flour for preparation 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.

7. CONFLICTS OF INTEREST

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

8. ETHICAL APPROVALS

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

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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REFERENCES

- 1. Deshpande S. Food legumes in human nutrition: A personal perspective. Crit Rev Food Sci Nutr 1992;32:333-63.
- 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.
- Nakamura A, Furuta H, Maeda H, Nagamatsu Y, Yoshimoto A. Analysis of structural components and molecular construction of soybean soluble polysaccharides by stepwise enzymatic degradation. Biosci Biotechnol Biochem 2001;65:2249-58.
- 4. Kennedy AR. The evidence for soybean products as cancer preventive agents. J Nutr 1995;125 Suppl 3:733S-43.
- Bhartiya A, Aditya J, Pal R, Chandra N, Kant L, Pattanayak A. Bhat (Black Soybean): A Traditional Legume with High Nutritional and Nutraceutical Properties from NW Himalayan Region of India; 2020.
- Koide T, Hashimoto Y, Kamei H, Kojima T, Hasegawa M, Terabe K. Antitumor effect of anthocyanin fractions extracted from red soybeans and red beans *in vitro* and *in vivo*. Cancer Biother Radiopharm 1997;12:277-80.
- Lee JH, Baek IY, Kang NS, Ko JM, Han WY, Kim HT, *et al.* Isolation and characterization of phytochemical constituents from soybean (*Glycine max* L. Merr.). Food Sci Biotechnol 2006;15:392-8.
- Reddy N, Salunkhe D. Interactions between phytate, protein, and minerals in whey fractions of black gram. J Food Sci 1981;46:564-7.
- Kayodé AP, Diversity, Users' Perception and Food Processing of Sorghum: Implications for Dietary Iron and Zinc Supply. Wageningen: Wageningen University and Research; 2006.
- Afify AE, El-Beltagi HS, Abd El-Salam SM, Omran AA. Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white *Sorghum* varieties. PLoS One 2011;6:e25512.
- Steiner T, Mosenthin R, Zimmermann B, Greiner R, Roth S. Distribution of total phosphorus, phytate phosphorus and phytase activity in legume seeds, cereals and cereal byproducts as influenced by harvest year and cultivar. Anim Feed Sci Tech 2007;133:320-34.
- 12. AOAC International. Official Methods of Analysis. Washington, DC: AOAC International; 1990. p. 684.
- Ranganna S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. New York: Tata McGraw-Hill Education; 1986.
- World Health Organization. Energy and Protein Requirements. WHO Technical Report Series No. 522 (and FAO Nutrition Meeting Report Series No. 52. Geneva: World Health Organization; 1973.
- 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.
- 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.
- Gao Y, Shang C, Maroof MS, Biyashev R, Grabau E, Kwanyuen P, et al. A modified colorimetric method for phytic acid analysis in soybean. Crop Sci 2007;47:1797-803.
- 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.
- 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.
- 20. Park YK, Jin YH, Lee JH, Byun BY, Lee J, Jeong KC, *et al.* The role of *Enterococcus faecium* as a key producer and fermentation condition as an influencing factor in tyramine accumulation in

Cheonggukjang. Foods 2020;9:915.

- Sumangala S, Kulkarni UN. Acceptable qualities of black soybean genotypes (*Glycine max*). Pharm Innov 2019;8:1125-8.
- Choi YM, Yoon H, Lee S, Ko HC, Shin MJ, Lee MC, et al. Isoflavones, anthocyanins, phenolic content, and antioxidant activities of black soybeans (*Glycine max* (L.) Merrill) as affected by seed weight. Sci Rep 2020;10:1-13.
- Rusydi MM, Azrina DA. Effect of germination on total phenolic, tannin and phytic acid contents in soy bean and peanut. Int Food Res J 2012;19:673-7.
- Warle B, Riar C, Gaikwad S, Mane V. Effect of germination on nutritional quality of soybean (*Glycine Max*). ISOR J Environ SCi Toxicol Food Technol 2015;9:13-6.
- Babalola RO, Giwa OE. Effect of fermentation on nutritional and antinutritional properties of fermenting Soy beans and the antagonistic effect of the fermenting organism on selected pathogens. Int Res J Microbiol 2012;3:333-8.
- Inyang C, Zakari U. Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "Fura"-a Nigerian cereal food. Pak J Nutr 2008;7:9-12.
- Xu Y, Xia W, Yang F, Kim JM, Nie X. Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with Pediococcus pentosaceus. Food Chem 2010;118:512-8.
- Oboh G, Ademiluyi AO, Akindahunsi AA. The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. Int J Food Sci Technol 2010;45:1236-42.
- Constant AK, Kablan T. Effect of spontaneous fermentation time on physicochemical, nutrient, anti-nutrient and microbiological composition of Lima Bean (*Phaseolus lunatus*) flour. J Appl Biosci 2021;162:16707-25.
- Barampama Z, Simard RE. Effects of soaking, cooking and fermentation on composition, *in-vitro* starch digestibility and nutritive value of common beans. Plant Foods Hum Nutr 1995;48:349-65.
- Oghbaei M, Prakash J. Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. Cogent Food Agric 2016;2:1136015.
- Đorđević TM, Šiler-Marinković SS, Dimitrijević-Branković SI. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chem 2010;119:957-63.

- Lin PY, Lai HM. Bioactive compounds in legumes and their germinated products. J Agric Food Chem 2006;54:3807-14.
- Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. Appl Microbiol Biotechnol 2006;69:627-42.
- Lemos MR, de Almeida Siqueira EM, Arruda SF, Zambiazi RC. The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts (*Dipteryx alata* Vog.). Food Res Int 2012;48:592-7.
- Shimelis EA, Rakshit SK. Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. Food Chem 2007;103:161-72.
- 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.
- Towo E, Matuschek E, Svanberg U. Fermentation and enzyme treatment of tannin sorghum gruels: Effects on phenolic compounds, phytate and *in vitro* accessible iron. Food Chem 2006;94:369-76.
- Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J Agric Food Chem 2007;55:2993-7.
- Khetarpaul N, Chauhan B. Effect of fermentation on protein, fat, minerals and thiamine content of pearl millet. Plant Foods Hum Nutr 1989;39:169-77.
- Guardianelli LM, Salinas MV, Puppo MC. Chemical and thermal properties of flours from germinated amaranth seeds. J Food Meas Charact 2019;13:1078-88.
- Gabriel-Ajobiewe RA. Nutritional composition of *Canavalia* ensiformis (L.)(Jack Beans) as affected by the use of mould starter cultures for fermentation. Trends Appl Sci Res 2011;6:463-71.
- 43. Gabriel R. Effect of Fermentation on the Nutritional and Antinutritional Content of Jack Beans, *Canavalia ensiformis* L. MSc Thesis. Akure, Nigeria: The Federal University Technology; 2002.

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