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Changes in Antioxidants and Nutritional Properties During Germination of Soybean

Abstract

Soybeans are a valuable source of nutrients and bioactive compounds. However, their nutritional quality can be limited by the presence of antinutrients, such as phytic acid and trypsin inhibitors. Germination is a natural process that can enhance the nutritional value of legumes by reducing antinutrients and increasing the bioavailability of nutrients. The objective of this study was to evaluate the effect of soaking and germination on the antioxidant and nutritional attributes of soybean. Results showed that the germination process led to a significant increase ($p \leq 0.05$) in the content of phenolic, flavonoids, and antioxidation activity (%) compounds following the 72-hour germination compared to samples from ungerminated soybeans. Strong correlations were concluded between antioxidant activity and total phenolic (R^2 0.92), flavonoids (R^2 0.82), and ascorbic acid (R^2 0.99). The germination process also enhanced proteases activities, lipase activity, and protein digestibility. Concurrently, there was a significant reduction in phytic acid from 99.20 ± 2.56 to 73.39±0.85, mg/100g as well as trypsin inhibitors from 5.73±0.116 to 2.91±0.126 mg/g. Germination led to these alterations contributed to an enhancement in the antioxidant and nutritional characteristics of soybean.

Keywords: Soybean, Germination, Phytic acid, Protein digestibility, Phenols, Antioxidants

1. Introduction

Despite the widely recognized nutritional and physiological benefits of soybean products, their consumption is still limited in many countries due to disagreeable beany tastes. Asian countries, however, have embraced soybean products more readily. To create products that appeal to global consumers, researchers are exploring advanced methods to enhance the properties of traditional soy foods (1).

Germination is a conventional strategy for improving the nutritional and sensory quality of soybeans (2). During germination, soaked soybeans sprout, activating enzymes that break down complex nutrients into simpler, more bioavailable forms. This process increases levels of beneficial compounds, including vitamins (especially vitamin C), minerals, antioxidants, and phytochemicals (3-5). Germinated soybeans also contain higher levels of free amino acids, making them a valuable plant-based protein source, especially for vegetarians. Additionally, germination reduces the levels of antinutritional factors like trypsin inhibitors and phytic acid, improving nutrient absorption (5,6). This enhanced digestibility ensures better utilization of the nutrients in germinated soybeans.

Germinated soybeans exhibit higher antioxidant activity than ungerminated soybeans (3,6). Moreover, studies have shown that consuming germinated soybeans can lower cholesterol levels, improve blood lipid profiles (7,8).

Germinated soybeans can be used in various food preparations, adding texture, flavor, and nutritional value. Their fresh, crunchy texture and mild, nutty taste make them popular in salads, stir-fries, soups, and sandwiches.

Germination is a cost-effective and efficient method for improving soybean quality. This study aimed to investigate the effects of the germination process on the chemical (proximate) and functional components of soybeans at ambient temperature. By analyzing changes in total phenolic content, flavonoids, antioxidant capacities, L-ascorbic acid, antinutritional factors, and digestibility, this research will provide insights into the variations in functional components and guide manufacturers in producing germinated soybeans with high nutritional value.

2. Materials and methods

2.1. Soaking and Germination of soybeans

To sterilize the seeds, they were steeped in a 1% sodium hypochlorite solution for around 15 minutes and then rinsed in distilled water. The sterilized seeds were soaked for 16 hours in water (1:10 (w/v), drained, spread on try, and dried in a hot air oven at 45°C for 24 hours. For germination, the soaked seeds were spread on try and covered with a wet muslin cloth at 25±2°C in the dark for 72h. After 72h, the germinated soybeans were harvested and then dried at a temperature of 45°C in a hot air oven for a period of 24h.

2.2. Determination of antioxidant properties:

1. Sample Extraction

The soybean powder of soymilk samples were extracted according to (9) and (10) with some modification using an acidified methanol solution (methanol: Hydrochloric acid, 99:1.0 v/v). A 2 g sample of soybean powder was agitated in 10 mL of acidified methanol on a platform shaker at room temperature for 16 hours in complete darkness. The mixture was centrifuged at 10,000 g for 10 minutes at 4°C (Hettich centrifuge EBA 8, Zentrifugen D-78532 Tuttlingen, Germany). The supernatant was filtered through Whatman No. 42 filter paper and kept in the dark at 5 °C to determine antioxidant activity, TP content, and TF concentration in soybean samples.

2. Total phenol Content (TPC)

The total phenolic content (TPC) was quantified using a Folin-Ciocalteu method as per reference (11). The TPC were computed and represented as milligrams of gallic acid equivalents per gram of soybean (mg of GAE/100g).

3. Total Flavonoid Content (TFC)

The TFC was calculated using a colorimetric technique described in (12). The TFC was determined and represented as milligrams of quercetin equivalents per gram of soybean (mg QE/100g).

4. Ascorbic acid Content

The determination of ascorbic acid content was conducted using the 2,6-dichlorophenol indophenol (DCPIP) dye method (13). This colorimetric approach involves reducing DCPIP with ascorbic acid, which results in a color shift that may be measured spectrophotometrically. The ascorbic acid content was then reported as milligrams per 100 grams of sample (mg/100g).

5. Antioxidant activity

The overall potential antioxidant activity of the soybean seed sample extract was evaluated by its ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, using a modified version of the DPPH assay (14). To conduct the test, 250 µl of the sample extract was mixed with 4 ml of the DPPH working solution. After allowing the mixture to stand for 30 minutes, its absorbance was measured at 517 nm against a blank that contained absolute methanol instead of the sample. The activity is expressed as a percentage relative to the control.

$$\%\text{DPPH scavenging activity} = 1 - \left(\frac{A\ \text{sample}}{A\ \text{control}}\right) * 100$$

2.3. Nutritional methods:

1. Protease activity -

The protease activity of soybean samples was assessed using the O-phthaldialdehyde (OPA) method, with modifications based on (15). The protease activity was expressed as a degree of hydrolysis according to the following equation:

$$DH = \frac{Att - Bt\ 0}{C\,24} * 100$$

Att is the optical density (OD) of the hydrolyzed sample at time t, Bt0 is OD of the unhydrolyzed sample at time zero (control sample), and C24 is OD of the hydrolyzed sample after 24 hours.

2. Lipase activity

The lipase activity was measured using the titrimetric technique described in (16). Olive oil with Arabic gum in a pH 8.0 phosphate buffer served as the substrate. Lipase activity is the percentage of free fatty acids released after a 90-minute incubation, and ultimately expressed as U/g/min.

$$\text{Unit/g/min} = (\text{volume of NaOH}(\text{ml}) * \text{molarity of NaOH} * 1000 * 0.67)/(\text{sample weight}\ (g$$

*0.67 because time conversion from 90 min to 60 min (1h)

3. Digestibility

The study evaluated soybean protein digestibility in vitro using gastric and intestinal enzymes (pepsin and trypsin respectively) accordance with the procedure outlined by (17). Samples were dissolved in phosphate buffer solution and mixed with trypsin and pepsin solutions. The mixture was incubated at 37 degrees Celsius for 90 minutes. After removing portions (after 0, 30, 60 and 90-min incubation), digestion was stopped with 24% trichloroacetic acid. Samples were filtered and total nitrogen was determined using the Kjeldahl method (18).[3] %digestibility was calculated using the equation.

digestibility%= (N in sample – N in undigested sample)/N in sample × 100.

4. Total phytic acid

The total phytic acid content in soybeans was determined using the Wade reagent method. The reagent was $FeCl_3 \cdot 6H_2O$ and sulfosalicylic acid in distilled water at a ratio of 1:10. Soybean samples (0.5[23] g) were mixed with 10 mL of 2.4% (0.64N) HCl in Falcon tubes and shaken for 16 hours at 300 rpm and room temperature. After centrifugation, the supernatant was mixed with NaCl, and a portion was further diluted

and mixed with Wade reagent. After centrifugation, absorbance was measured at 500 nm against water. Phytic acid content was calculated as g/100g soybean (19).

5. Trypsin Inhibitor Activity

The modified method outlined by (20) was employed to assess trypsin inhibitor activity. Casein solution (1% in PBS, pH 7.0, 0.02M $CaCl_2$) served as the substrate. Finely ground soybean samples (100 mesh) were dissolved in 0.01 mol/L NaOH to achieve a pH of 8.4-10. Duplicate sets of test tubes were prepared, one for the standard and the other for the sample. Sample extract and trypsin solution were added to sample tubes, while phosphate buffer was added to standard tubes. After a 15-minute incubation at 37°C, substrate solution was added and incubated for an additional 10 minutes. The reaction was stopped with 30% acetic acid, and the mixture was filtered for absorbance measurement at 410 nm using a spectrophotometer. For blank preparation, trypsin solution was added after acetic acid. The trypsin inhibitor activity, measured in Trypsin Inhibitor Units per milliliter (TIU/mL), can be calculated using the following formula:

Trypsin inhibitor activity/(TIU/mL) = $(\mathrm{Ar} - \mathrm{Abr}) - ¿¿$

Ar represents the absorbance reading of the standard solution. Abr is the absorbance of the standard blank solution.[7] As denotes the absorbance of the sample solution.[7] Abs indicates the absorbance of the sample blank solution. F is the dilution factor applied. A 0.04 corresponds to the sample weight utilized in the analysis. This equation allows for the determination of trypsin inhibitor activity by comparing the absorbance values of standard and sample solutions, while accounting for their respective blanks and any dilution performed.

[12] Statistical analysis .2.5

The differences in the antioxidants and nutritional attributes of raw, soaked and germinated soybeans were analyzed using the one-way analysis of variance (ANOVA) procedure of MINITAB vision 19 statistical software for windows (2010). Dunken test was applied to compare the mean values of the different compounds. Probability less than 0.05 was considered significant ($p \leq 0.05$). Mean values with standard deviation media (SD) are reported. All data were analyzed using vision 19MINITAB statistical software for windows (2010).

3. Results

: Antioxidant properties .3.1

1. Total phenol

Data in Table 1, reveal that phenol contents in soybean seeds increased from 108.80 ± 20.50 mg GAE/100g (milligrams equivalents of gallic acid) in raw soybean to 111.78 ± 4.45 and 161.41 ±11.67 mg/100g in soaked and germinated seeds, respectively. No significant differences were observed between raw and soaked beans, but germination significantly increased phenol content.

2. Total Flavonoids

The impact of soaking and germination on flavonoid contents were shown in Table 1. The total flavonoids in soybean seed extracts significantly increased from 14.64±1.02 mg QE/100g (quercetin equivalents), in raw seeds to 67.95±0.45 mg QE/100g in soaked seeds, and further to 87.18±7.10 mg QE/100g in germinated seeds. The total isoflavone content in whole soybean usually ranges from 50 to 450 mg/100 g raw weight (21).

3. Ascorbic acid

Ascorbic acid is an antioxidant that protects body cells against the effects of free radicals. The results in Table 1 showed the concentration of ascorbic acid in raw, soaked, and germinated soybean. The results clearly demonstrated that the soaking and germination process significantly increased the levels of ascorbic acid from 3.47±0.50 mg/100g to 5.62±0.76 and 11.08±0.29 mg/100g respectively. These results in a good agreement with those obtained by Kumari et al., (3) where, they reported values, 3.5; 5.28 and 14.3 mg/100 g in yellow soybean in the same order. Villares et al. (22) reported ascorbic acid values in soybean varies from 0.03 to 35 mg/100 g raw weight. However, Xue et al. and Saini et al., (23,24) did not detect ascorbic acid in raw soybean.

4. Antioxidants Activity

The results presented in Table 1 illustrate the effect of soaking and germination on the antioxidant capacity of soybeans, as measured by their ability to scavenge DPPH free radicals. While no significant difference was observed between raw and soaked beans (54.76 ± 1.29% vs. 58.48 ± 2.57%), a notable increase in antioxidant activity was seen during germination, reaching 65.60 ± 1.24%. [16] Previous studies (25), reported varying levels of free radical scavenging activity in raw soybeans, with values ranging from approximately 57% to 79% depending on the extraction method used. The current study showed higher antioxidant activity compared to those reported by Xue et al. (23).

[6] Correlations .5

The relations between antioxidant activity and total phenolic compounds, total flavonoids and ascorbic acid were statistically assessed as shown in Fig.1. Positive and high correlations were observed ($p \leq 0.05$) between antioxidant and ascorbic acid (R2 =0.996), flavonoids (R2 =0.816) and phenolic compounds (R2 = 0.916) respectively. The most significant correlation was observed with ascorbic acid, which showed a substantial increase (319%, rising from 3.47±0.50 to 5.62±0.76 and 11.08±0.[2]29 mg/100g) following the soaking and germination processes.

The present study revealed increase of total phenolic compound, flavonoids and ascorbic acid during germination process. The same trend was obtained by Guzmán-Ortiz et al. (9).

3.2. Nutritional composition

1. Protease activity

During a three-day germination process, the degree of protein hydrolysis (DH) in soybeans increased significantly. Germinated soybean has the highest protease activity. The DH in raw soybeans was 29.71%, rising to 36.65% after soaking and further to 59.82% after germination (Table 2).

2. Lipase activity

The specific activity is a ratio of lipase activity (Unit/g) and protein content (mg/g), being expressed as units per mg of total proteins. Table 2 indicates the effect of germination treatment on lipase activity measured by titration method and expressed as U/g/min. A significant change in the lipase activity, due to the soaking and germination processes was observed. In soaked and germinated soybean, activities of lipase were increased by about 25% (8.45 ±0.05 U/g/min) and 87.46% (12.27±0.98 U/g/min) higher than control (6.75±0.05 U/g/min).

3. Digestibility

According to the data in Fig. 2 a significant increase in protein digestibility is observed soon after the first 30 h of germination reaching its maximum at 90 h. The protein digestibility expressed as NPN (Fig.2) showed a rise of 48%, 59%, and 62% for raw, soaked, and germinated samples respectively when assessed with trypsin after a 90-minute incubation. Similarly, these percentages were 50%, 53%, and 56% in the same order when tested with pepsin.

4. Phytic acid

Table 3 displays the phytic acid content in raw, soaked, and germinated soybeans. The data indicate a significant (P 0.5) reduction in phytic acid in soaked (1.50 ± 0.04 mg/100g) and germinated (1.24 ± 0.01 mg/100g) soybeans compared to raw beans (1.68 ± 0.04 mg/100g). Phytic acid decreased by 10.51% and 26.02% in soaked and germinated beans, respectively.

5. Trypsin inhibitor

Cámara et al. (26) reported lower trypsin inhibitor values of 1.91 mg/g for Argentina, 2.83 mg/g for Brazil, and 3.0 mg/g for USA soybeans, which are significantly lower than the average concentration (5.73 ± 0.12 mg/g) observed in the present study. As evident from Table 3, here, significant (P 0.05) differences were observed in trypsin inhibitor levels among raw, soaked, and germinated soybeans. The concentration of trypsin inhibitor in raw soybeans was 5.73 ± 0.12 mg/g. Pesic et al. (27) determined the Kunitz trypsin inhibitor (KTI) type, the most represented part of soybean trypsin inhibitor in 12 genotypes and reported values ranging from 4.28 to 6.85 mg/g, with an average of 4.94 mg/g. However, Khan et al. (28) found KTI values ranging from 0.07 to 15.9 mg/g in full-fat soy flour, with an average value of 6.81 mg/g in 102 soybean genotypes.

Discussion

Current investigation revealed that the germinated soybean possessed higher benefit compounds, Phenol. Flavonoid, Ascorbic acid and Antioxidation activity. The total phenol contents in soybean cultivars ranged from 59.61 ± 1.70 (29) to 548.0 ± 21.0 mg GAE/100 g (9). Differences in reported values among studies may be due to differences in soybean genotypes, planting areas, and analysis methods. Ma et al., (30) attributed the increase in phenolic compounds of soybean during germination to the activity increase of the enzymes involved in the biosynthesis of phenolic acids such as of phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H) and 4-coumarate coenzyme A ligase (4CL). This increase in total Flavonoids in soaked and germinated soybean in this study was reported by Xua et al. and Yang et al. (23,31). Among the studied cultivars, yellow seed coats exhibited the highest flavonoid levels, whereas the Conquista cultivar, which also has a yellow seed coat but a black hilum, displayed the lowest flavonoid content (32). A study (9) found that the flavonoid content increased by 314.59% after germination for 6 days. The activation of enzymes involved in the manufacture of flavonoids from large molecular weight polyphenols, or glucosidase enzymes that create aglycones (33) resulted in the increase in flavonoid concentrations during germination (31).

Ascorbic acid as presented in Table 1 increased significantly during both soaking (61.96%) and the germination process (219%). These findings align with the previous outcomes (23,31). A similar increase in ascorbic acid levels was observed during soaking and germination. Previous studies demonstrated found that ascorbic acid was undetectable in the raw material but increased to 1.93 mg/g after germination and reached 93.33 mg/kg after 4 days of germination (23,31). The increase in ascorbic acid during germination could be attributed to the

activity increase of glutamic dehydrogenase which is the key enzyme in ascorbic acid biosynthesis (31).

A study by Guzmán-Ortiz et al. (9) found that the antioxidant activity of soybeans germinated for 2 days was not significantly, but it significantly increased during germination. Similarly, Xua et al. (23) observed a significant increase in antioxidant activity from approximately 10% in ungerminated soybeans to around 30% after 3 days of germination. Furthermore, several studies have observed substantial increases in antioxidant activity during germination, using various assays including ABTS and FRAP (9).

However, the relationship between isoflavones and antioxidant capacity was noted to depend on the structure of the isoflavones (34). In all cases, the germinated soybeans have the highest levels of these beneficial compounds. This suggests that germination process increases the levels of total phenol.

Germinated soybean increased the nutritional values of beans. Germination, as expected, led to an increase in DH. This aligns with previous research by Zahir et al. (35) who found higher protease activity in germinated soybeans. Similar findings were reported in other studies (6,23), where germinated soybeans had a higher DH compared to ungerminated controls.[5] These studies suggest that multiple protease types are involved in the germination process. Research by Bueno et al. (6) points towards a significant increase in trypsin-like proteases during germination. Additionally, Bueno et al. (6) identified specific soybean proteins like β-conglycinin and glycinin as targets for degradation by these proteases. Germination leads to the breakdown of these proteins into smaller polypeptides. For example, β-conglycinin degrades into at least six polypeptides, while the acidic component of glycinin is more readily hydrolyzed compared to the basic component. Overall, the results highlight the dynamic changes in protein composition and structure that occur during soybean germination. The activation of proteases plays a crucial role in mobilizing stored protein reserves within the soybean seed, providing essential nutrients for seedling growth during the early stages of germination. Similarly, germinated soybean has the highest lipase activity, these findings are in accordance with those obtained by Gadge et al. (36) where they reported values 10.37 U/ml/min for germinated soybean. However, these values are higher than those reported by Paul and Rakshit (37), they reported values 0.053 – 0.105 and 0.064 U /g respectively. Moreover, Paul and Rakshit (37) reported an increase in lipase activity of germinated soybean 1.75- and 2.2 times after 3 and 5 days compared with ungerminated beans. Chen et al. (29) reported an increase in lipase activity with concomitant increase of free fatty acid, glycerol, and phospholipids during soybean germination. [1] This may be due to the increase of lipase activities during germination process. The activation of the

lipase enzyme may be caused by the involvement of the $Ca^{+2}$and Mg^{2+}ions at lower concentration as catalysts in the reaction mixture (36). Tan et al. (38) attributed the activity of lipase enzyme during germination to the inhibition of lipase inhibitors such as tannin and phytic acid.[5] Based on the results shown in Fig. 2, it's evident that the protein digestibility using trypsin significantly increased in soaked and germinated samples compared to those using pepsin.[4] This improvement could be attributed to the reduction in trypsin inhibitors present in soaked and germinated samples (Table 3).[2]

This reduction in phytic acid during soaking may be due to the fact that phytic acid in raw legumes and cereals is mostly present as a water-soluble salt (likely potassium, magnesium, and manganese phytate), which is often lost in the steeping medium and rinsing during soaking (39).[2] Several authors have reported an inverse relationship between the decrease in phytic acid content and the increase in phytase activity during germination.[2] Murugkar (40) specifically noted a threefold to fivefold increase in phytase activity during germination, leading to an increase in inorganic phosphate and lower phosphoric esters. Wu et al. (5) documented a reduction of approximately 52.4% in phytic acid levels during the germination of soybeans. They also observed a phytic acid content of about 0.15 g/100 g in germinated soybeans, compared to approximately 0.31 g/100 g in ungerminated beans, representing a decrease of roughly 48.4%. Moreover, Pesic et al. (26) reported a decrease in phytic acid content during germination from 740 mg/100 g in ungerminated beans to 216 mg/100 g in germinated beans.

Soaking and germination can significantly reduce trypsin inhibitor levels in soybeans. These findings are in agreement with those obtained by (3), who found a 47.24% decrease in trypsin inhibitor levels in germinated (72 h) soybeans compared to ungerminated beans, and a decrease of 69.72% in black soybeans over the same period. Furthermore, Wu et al.[3] (5) found that the trypsin inhibitor content was reduced by 22.3% through germination.[1] The decrease in trypsin inhibitors during germination can be attributed to their hydrolysis into essential amino acids to support seedling growth (24) or to protein denaturation and their involvement in complex physiological metabolism at the early stage of germination.

4. Conclusion

Here, we found that soaking and germination significantly enhanced the antioxidant and nutritional properties of soybeans. Notably, these processes led to a substantial increase in essential components such as ascorbic acid (Vitamin C), total phenolic and flavonoid compounds, and DPPH radical scavenging capacity. Correlation analyses revealed a strong association between total phenolics, total flavonoids, and ascorbic acid levels, which collectively influenced antioxidant activity, with ascorbic acid demonstrating the most significant impact.[14] Moreover, soaking and germination improved

protease and lipase activities, protein digestibility, while simultaneously reducing phytic acid and trypsin inhibitor levels.[14] These findings underscore the effectiveness of these methods in enhancing the overall nutritional quality of soybeans.

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Authors' contributions

NMS and AAM designed this study and wrote the introduction and discussion sections. NMS, JT and ASZ drafted the methods and results, as well as performed the statistical analysis. JT prepared the references. HE and EMA prepared the manuscript for submission.[26] All authors read and approved the .final manuscript

Corresponding author

Ezzat M. Awad, Institute of Specific Prophylaxis and Tropical Medicine [ISPTM], Center for Pathophysiology, Infectiology and Immunology [CePII], Medical University of Vienna, Vienna, Austria, E-mail: ezzat.awad@meduniwien.ac.at[7] and Jameel Al-Tamimi, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451[7], Kingdom of Saud Arabia. jtamimi@ksu.edu.sa

Consent for publication

The authors generated all of the data from our laboratory tests. We have all agreed to the publishing .of the data given in this paper

Conflicts of interests

.The authors have not disclosed a conflict of interest

Competing interests

.The authors declare that they do not have any conflicting interests

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