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SHORT COMMUNICATION

Impact of soaking and germination durations on antioxidants and anti-nutrients of black and yellow soybean (*Glycine max*. L) varieties

Sweta Kumari · Veda Krishnan · Archana Sachdev

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Abstract From nutritional point of view, the nutrient-anti-nutrient homeostasis determines the food quality and germination, a simple household process is potent enough to alter this balance. This study was conducted in black (Kalitur) and yellow (Pusa 9712) soybean varieties comparing the effect of soaking and germination on various antioxidants (total phenols, flavonoids, Vitamin C and total antioxidant capacity) and anti-nutrients (tannins, phytate, RFOs and trypsin inhibitors) on them. A significant ($P<0.05$) decrease in the levels of total phenols (11 %, 6 %), total flavonoids (9 %, 5 %), condensed tannins (16 %, 14 %), phytates (13 %, 10 %), RFOs (11 %, 9 %), trypsin inhibitors (9 %, 8 %) and an increase in the levels of Vitamin C (62 %, 78 %) and the total antioxidant capacity (51 %, 71 %)in both Kalitur as well as Pusa 9712 respectively were observed after soaking. Further on comparingthe controls with different germination durations,maximum up to 72 h resulted insignificant ($P<0.05$) reductions in the levels of tannins (68 %, 50 %), phytates (65 %, 49 %), RFOs (60 %, 45 %) and trypsin inhibitors (70 %, 57 %),were observed in both black and yellow soybean varieties respectively. An increase in the total polyphenols (17 %, 10 %), flavonoids (21 %, 11 %), Vitamin C (403 %, 308 %) and antioxidant capacity (410 %, 311 %) was however observed in the black and yellow soybean respectively compared to controls. The results implicated the role of the germination and an increase in its duration in reducing the anti-nutrients, enriching the antioxidants and thus improving the nutritive value of soy sprouts. Sprouts being a part of the present diet conscious society, 72 h old black soybean sprouts can be considered as better substitutes than their yellow counterparts.

S. Kumari · V. Krishnan · A. Sachdev (✉)
Division of Biochemistry, Indian Agricultural Research Institute (IARI), New Delhi, India
e-mail: archanabiochem307@gmail.com

Keywords Soybean · Germination · Soaking · Antioxidants · Antinutrients

Abbreviations

ANFs	Anti nutritional factors
TPC	Total polyphenolic content
GAE	Gallic acid equivalent
TFC	Total flavonoid content
CUPRAC	Cupric ion reducing antioxidant capacity
RFOs	Raffinose family of oligosaccharides
TIA	Trypsin inhibitor activity
BAPA	Benzoyl-DL-arginine-P-nitroanilide TAC, Total antioxidant capacity
TAC	Total antioxidant capacity
TIU	Trypsin inhibitor unit

Soybean (*Glycine max*. L.), due to its rich nutritional profile (protein 40 %, oil 20 %) is considered as a high quality complete food for the development and maintenance of health. Even though credited as 'Super foods',the nutritional quality is however dictated by high amounts of anti-nutritional factors (ANFs) which lead to malnutrition situations, afflicting human populations mainly in the developing countries.

Various traditional methods like soaking, dehulling, germination etc. have been reported to be beneficial for enhancing the nutritive value and the organoleptic acceptability of legumes, as these bring changes in nutrient/anti-nutrient homeostasis. Germination is one of the most metabolically active stage, is known to alleviate the effects of ANFs (phenolics, tannins, saponins, protease inhibitors and phytic acid) and also to increase ascorbic acid, riboflavin content and bioavailability of trace elements (Fasuyi 2005).

Previous studies have mainly focussed on the chemical composition of soybean as affected by the traditional processes, but a comparative trend of the nutrient-antinutrient content in soybean is lacking. Thus the present study was conducted with an aim to investigate the impact of soaking and germination durations on the contents of antioxidants and anti-nutrients in Kalitur and Pusa 9712.

Soybean varieties were obtained from Division of Genetics (IARI), were cleaned, washed and soaked in distilled water (1:10, w/v) for 12 h at 22–25 °C and later germinated for a period of 24 h, 48 h, and 72 h. Germinated seeds were frozen for 12 h to stop the germination process and later dried at 50± 5 °C for 16-18 h and ground using the stainless steel grinder. Raw seeds were used as control.

Total phenolic content (TPC) per gram of seed flour was determined according to Emmons et al. (1999). Calibration plot was expressed as gallic acid (2-10 μg/ml) equivalents/g DW. Total flavonoid content (TFC) was also estimated using Ordonez et al. (2006). The calibration plot was generated by using rutin solutions at concentrations 12.5–100 μg/ml in methanol solution. TFC was expressed as mg rutin equivalents/g DW. Vitamin C was estimated by visual titration method of reduction of 2, 6-dicholorophenol-indophenol dye (Gupta et al. 2005) and was estimated using the relationship: Vitamin C (mg/100 g)=Titre value×dilution factor×mg of dye×10. Total antioxidant capacity (TAC) was measured using cupric ion reducing antioxidant capacity (CUPRAC) assay method by Apak et al. (2004) and is expressed as trolox equivalents (μmol TE/g). Tannins were estimated by vanillin-HCl method of Price and Butler (1977). Catechin solution (0-100 ppm) was used for standard curve and the results were expressed as mg of catechin equivalent/g DW. The phytic acid and RFOs were estimated using assay kits (Megazyme International, Ireland) and were expressed as mg/g DW. Trypsin inhibitor activity (TIA) was measured by Hamerstrand et al. (1981) using benzoyl-DL-arginine-P-nitroanilide (BAPA) as substrate. TIA was expressed as TIU/mg DW. Experiments performed in triplicates, data were analysed using the one-way ANOVA using SPSS 15.0 and difference between means was compared by the Duncan's Multiple Range Test.

Effect of soaking and germination on total polyphenols, flavonoids, Vitamin C and TAC was studied (Table 1). Kalitur was found to contain higher amounts of antioxidants than the Pusa 9712. The TPC, TFC, Vitamin C and TAC were 8.12 mg/g, 1.81 mg/g, 6.2 mg/100 g and 8.01μgTE/g, respectively in Kalitur and 6.24 mg/g, 1.13 mg/g, 3.50 mg/100 g and 3.14 μgTE/g, respectively in Pusa 9712. A significant loss ($P<0.05$) of TPC and TFC was observed in the present study after soaking 9-11 % in black and 5-6 % in yellow compared to the raw dry controls. Observed losses may be the result of either leaching of these compounds into the soaking medium or due to poor extractability, as lower molecular weight phenolic compounds are known to polymerize, thus making them insoluble in water (Veronique 2005). Alternatively during the period of soaking, the enzyme polyphenol oxidase is presumably activated resulting in the degradation and consequent loss in the polyphenol content (Rusydi and Azrina 2012).

In contrast to soaking, significant ($P<0.05$) increase in TPC and TFC were however observed after 24 h, 48 h and 72 h of germination in black as well as yellow soybean compared to the raw controls (Table 1). Increase in TPC and TFC after 72 h of germination was 17 % and 21 %, respectively in black and 10 % and 11 %, respectively in the yellow soybean. Increase in flavonoid content during germination might also be due to the activation of enzymes involved in the biosynthesis of flavonoids from large molecular weight polyphenols like tannins (Drewnowski and Gomez-Carneros 2000).

A significant ($P<0.05$) increase in Vitamin C and TAC after soaking was observed in both black and yellow soybean. Vitamin C and TAC were 10.10 mg/100 g and 14.30 μgTE/g, respectively in 12 h soaked black soybean and 5.28 mg/100 g and 5.37 μgTE/g, respectively in yellow soybean soaked for the same period. Increase in Vitamin C and TAC was higher in black soybean (62 % and 78 %, respectively) than yellow soybean (51 % and 71 %, respectively). After 24 h, 48 h and 72 h of germination a further increase in Vitamin C and TAC was however observed. After 72 h of germination, the increase in Vitamin C and TAC was 403 % and 410 %, respectively in Kalitur compared to 308 % and 311 %, respectively in Pusa 9712. The increased TAC observed after soaking and germination might be a response, which initiates after breaking dormancy, to protect the hypocotyl growth against oxidative reactions triggered by environmental factors. Flavonoids can directly scavenge molecules of active oxygen including hydrogen peroxide, singlet oxygen, and superoxide, hydroxyl, peroxyl radicals and are effective for peroxynitrite, a highly reactive oxidant formed when superoxide reacts with nitric oxide (Gould et al. 2002). The increase of TAC could be due to the synthesis of hydro soluble Vitamins (Vitamin C) or other compounds such as polyphenols, with antioxidative characteristics during the germination process. In general, the accumulated total polyphenols, flavonoids, Vitamin C and TAC observed in the present study showed a consistent trend (72 h sprouts>48 h sprouts>24 sprouts) compared to the raw controls.

Effect of soaking and germination durations on the anti-nutrients revealed higher amount of tannins, phytates, RFOs and trypsin inhibitors (TI) (3.12 mg/g, 28.90 mg/g, 10.20 mg/100 g and 75.32 mg/g, respectively) more in Kalitur compared to (2.18 mg/g, 22.50 mg/g, 8.60 mg/100 g and 65.40 mg/g respectively) in Pusa 9712 (Table 2). The overall significant ($P<0.05$) loss after soaking was in the range of 14-16 %, 10-13 %, 9-11 % and 8-9 % respectively when compared to raw soybean. Among the four anti-nutrients, the loss in the tannin content was relatively more due to the presence of water soluble tannins that might have leached into soaking medium. After 24 h, 48 h and 72 h of germination, a further significant

Table 1 Effect of soaking and germination method on antioxidants (total phenol, flavonoid, Vit. C and antioxidant activity) of black and yellow soybean

Treatments	Total Phenol (mg/g of flour)	Flavonoid (mg/g of flour)	Vit. C (mg/100 g of flour)	Total Antioxidant Capacity (μgTE/g of flour)
Black soybean				
Raw (Control)	8.12^{d}±0.02	1.81^{d}±0.01	6.20^{e}±0.04	8.01^{e}±0.03
12 h soaked	7.20^{e}±0.04 (-11)	1.65^{e}±0.02 (-9)	10.10^{d}±0.02 (+62)	14.30^{d}±0.02 (+78)
Germination				
24 h	8.57^{c}±0.03 (+5)	1.93^{c}±0.03 (+7)	16.20^{c}±0.03 (+161)	19.80^{c}±0.04 (+147)
48 h	8.98^{b}±0.02 (+10)	2.04^{b}±0.03 (+13)	23.20^{b}±0.04 (+274)	28.60^{b}±0.05 (+257)
72 h	9.53^{a}±0.03 (+17)	2.19^{a}±0.02 (+21)	31.21^{a}±0.04 (+403)	40.87^{a}±0.06 (+410)
Yellow Soybean				
Raw (Control)	6.24^{d}±0.02	1.13^{c}±0.02	3.50^{e}±0.02	3.14^{e}±0.03
12 h soaked	5.88^{e}±0.03 (-6)	1.07^{d}±0.02 (-5)	5.28^{d}±0.02 (+51)	5.37^{d}±0.03 (+71)
Germination				
24 h	6.49^{c}±0.04 (+4)	1.19^{b}±0.03 (+5)	8.60^{c}±0.04 (+146)	7.15^{c}±0.02 (+128)
48 h	6.65^{b}±0.03 (+7)	1.21^{b}±0.02 (+7)	11.30^{b}±0.03 (+222)	9.54^{b}±0.04 (+204)
72 h	6.84^{a}±0.08 (+10)	1.26^{a}±0.02 (+11)	14.30^{a}±0.04 (+308)	12.92^{a}±0.04 (+311)

[a] Values are means±SD of three determinations. Different superscripts in the same column among black and yellow soybeans with different letters are significantly (P<0.05) different. Figures in parentheses indicate percent change over control values

(P<0.05) loss of tannins, phytates, RFOs and TI were observed in the black as well as yellow soybean, compared to raw soybean (Table 2). After 72 h of germination, the decrease in the above anti-nutrients was 68 %, 65 %, 60 % and 70 %, respectively in Kalitur and 50 %, 49 %, 47 % and 57 % respectively in Pusa 9712. Reduction in the concentrations of anti-nutrients after 72 h of germination was higher in Kalitur (60-70 %) as compared to Pusa 9712 (47-57 %). In general, the reduction in antinutrients understudy,showed a consistent trend (72 h sprouts<48 h sprout<24 sprout< imbibed seeds<raw seeds). During germination, activated enzymes result in the hydrolysis of various components including carbohydrates, proteins, fibers, lipids as well as other phenolic compounds (Khandelwal et al. 2010) could justify the decreasing trend.

After 72 h of germination, significant (P<0.05) decrease in phytic acid (49-65 %) was higher in Kalitur (65 %) than Pusa 9712 (49 %). Decrease in phytic acid content during germination could be due to an increase in phytase activity as reported in broad bean, chickpea, lentil (Egli et al. 2002).

Table 2 Effect of soaking and germination method on anti-nutritional factors of black and yellow soybean

Treatments	Tannin (mg/g DW)	Phytate (mg/g DW)	RFOs (mg/g DW)	Trypsin inhibitor activity (TIU/mg DW)
Black soybean				
Raw (Control)	3.12^{a}±0.04	28.90^{a}±0.06	10.20^{a}±0.02	75.32^{a}±0.8
12 h soaked	2.61^{b}±0.02 (-16)	25.20^{b}±0.05 (-13)	9.10^{b}±0.03 (-11)	68.30^{b}±0.6 (-9)
Germination				
24 h	2.17^{c}±0.03 (-36)	21.50^{c}±0.06 (-26)	7.90^{c}±0.04 (-23)	57.40^{c}±0.9 (-24)
48 h	1.84^{d}±0.01 (-49)	17.60^{d}±0.04 (-39)	6.80^{d}±0.02 (-33)	43.62^{d}±0.7 (-42)
72 h	1.01^{e}±0.02 (-68)	10.03^{e}±0.04 (-65)	4.06^{e}±0.03 (-60)	22.81^{e}±0.1 (-70)
Yellow Soybean				
Raw (Control)	2.18^{a}±0.03	22.50^{a}±0.04	8.60^{a}±0.04	65.40^{a}±0.7
12 h soaked	1.87^{b}±0.02 (-14)	20.30^{b}±0.05 (-10)	7.80^{b}±0.05 (-9)	60.23^{b}±0.9 (-8)
Germination				
24 h	1.62^{c}±0.02 (-26)	17.30^{c}±0.08 (-23)	6.90^{c}±0.02 (-20)	53.73^{c}±0.6 (-18)
48 h	1.35^{d}±0.03 (-38)	14.50^{d}±0.09 (-36)	5.81^{d}±0.06 (-33)	41.60^{d}±0.8 (-36)
72 h	1.08^{e}±0.04 (-50)	11.40^{e}±0.08 (-49)	4.72^{e}±0.04 (-45)	28.35^{e}±0.8 (-57)

[a] Values are means±SD of three determinations. Different superscripts in the same column among black and yellow soybeans with different letters are significantly (P<0.05) different. Figures in parentheses indicate percent change over control values

Similarly, a significant ($P<0.05$) reduction of RFOs was higher in Kalitur (60 %) than Pusa 9712 (45 %) was observed after 72 h of germination. In the present study a significant ($P<0.05$) reduction of TI from Pusa 9712 (57 %) and Kalitur (70 %) after 72 h of germination was comparable to reduction of TI observed in mung bean by (Mubarak 2005). They reported a statistically significant ($P<0.05$) decrease in TIA by soaking and dehulling processes and also a drastic reduction to 22 % during germination.

In conclusion, germination in general improved the Vitamin C and TAC, which showed a significant ($P<0.05$) reduction in the levels of tannins, phytates, RFOs and TI activities. Further enhancements in the mentioned parameters were observed up to 72 h of germination. The significant decreases in the levels of anti-nutrients after 72 h of germination were however higher in Kalitur (60-70 %) compared to Pusa 9712 >(47-newnbsp;%). Thus germination enriched the antioxidants and reduced the <?thyc=anti-nutrients and proved to be simple yet effective in enhancing the palatability of soybean. The decrease in anti-nutrients can help to tackle the existing constraints associated with soybean's nutritional index. This method, inexpensive in terms of time, energy and fuel can also be used in household processing of other legumes as well especially in the developing countries like India where legumes are an integral part of the daily meal.

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