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Effect of germination on antioxidant capacity and nutritional quality of soybean seeds (*Glycinemax* (L.) Merr.)

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ABSTRACT

The present study investigated the effects of germination for 0, 12, 24, 36, 42, 48, 60 and 72 hours on nutritional and antioxidative characteristics of germinated soybean seeds, Glycine max (L.) Merr. After germinating at 25°C in dark condition, germinated seeds were freeze-dried and used for determination the nutritional as well as antinutritional components such as proteins, lipid, phytic acidand trypsin inhibitor. In addition, biochemical compounds in germinated soybean seeds such as phenolics, flavonoids, ascorbic acid (or vitamin C), α-tocopherol and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity in terms of IC50 were detected. Results showed that germination caused significant ($p \leq 0.05$) increases in protein content of soybean seeds. The SDS-PAGE patterns showed that proteins in germinated soybean seeds were unchanged for 60 hours of germination. However, lipid content, antinutritional factors (phytic acid and trypsin inhibitor) significantly ($p \leq 0.05$) decreased. It was found that total phenolic content, total flavonoid content, vitamin C and α–tocopherol contents increased during soybean seeds germination and tended to reach the maximum values after 60 hours of germination. Germinated soybean seeds had lower IC50 or higher antioxidantcapacity. Thus, the present study revealed that germination significantly affects the nutritional and antioxidant properties of soybean seeds.

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1 INTRODUCTION

Nowadays, there is a wide interest in the effects of processing on the nutritional value, especially on antioxidant compounds of legumes. Indeed, soybean seeds, *Glycine max* (L.) Merr. contain high protein and lipid as well as many bioactive compounds with antioxidant activity (Sefatie *et al.*, 2013) that can contribute to health promotion in the prevention of cancers including breast and prostate cancers, cardiovascular diseases, bone health, and diabetes (Clarkson, 2002; Jayagopal *et al.*, 2002; Yan and Spitznagel, 2009). For these reasons, soybeans are widely used in food industry and occupy an important place in human nutrition worldwide (Singh *et al.*, 2014). On the other hand, soybean seeds have been reported to contain adequate amounts of antinutrients such as phytic acid (PA) and trypsin inhibitor (TI), oligosaccharides (Alonso *et al.*, 2000). The antinutrients may be known as those substances generated in natural food stuffs by the normal metabolism of species as well as by different mechanisms and they limited the biological utilization of existing nutrients of these grains (Liener, 1994).

Germination processes have been developed in some countries to overcome some of the disadvantages of soybeans, for example, undesirable flavour and the presence of anti-nutritional factors such as PA and TI (McKinney *et al.*, 1958; Suberbie *et al.*, 1981; Vanderstoep, 1981). Germination is considered as one of the best methods to be applied in the improvement of nutritional profile of the seeds (Fordham *et al.*, 1975; Warle *et al.*, 2015), For instance, germination can increase the amount of vitamin and available mineral. Germination improves protein digestibility (Sattar *et al.*, 1989; Ghanem and Hussein, 1999; Bau *et al.*, 2000; Preet and Punia, 2000). Especially, it can also lead to modification of bioactive constituents (Paucar-Menacho *et al.*, 2010).

The aim of the present study was to examine the effects of germination on the nutritive value, the content of bioactive phytochemicals as well as the antioxidant capacity of soybean seeds.

2 MATERIALS AND METHODS

2.1 Soybeans and germination process

Soybean variety MTD 760 was supplied from Department of Genetics and Plant Breeding, College of Agriculture and Applied Biology, Can Tho University.

Soybeans were cleaned and rinsed with cleaned water before being soaked for 12 hours to reach the equilibrium moisture content at ambient temperature. Soaking process was carried out in drinkable water containing 1 mg/L gibberellic acid and the ratio of soybean seeds and water as 1: 5. The soaked beans were drained, rinsed and placed in a germination chamber in dark condition. Watering the seeds was set up for two minutes every 4 hours with cleaned water automatically. The germination process was carried out at 25^{o}C for 0, 12, 24, 36, 48, 60 and 72 hours. Raw soybean seeds (as control treatment) and germinated soybean seeds were freeze-dried for analyzing the contents of protein, lipid, protein profile patterns, PA, TI, total phenolic content (TPC), total flavonoid content (TFC), vitamin C, α-tocopherol and IC50 value. The extraction procedure for analysis of the antioxidant compounds followed the study results of (Lien *et al.*, 2015), α-tocopherol content was detected in soybean oil.

2.2 Determination of the nutritional components

Total protein contents were determined by Kjeldahl method and total lipid contents were determined by Soxhlet method. All chemical components were displayed as percent on dry basic. The soybean protein subunits were fractionated by SDS-PAGE analysis.

2.3 Determination of the antinutritional components

The PA in the extract was determined according to a colorimetric assay described by Gao *et al*. (2007). The pink colour of the Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid) is due to the reaction between ferric ion and sulfosalicylic acid with a maximum absorbance at 500 nm. In the presence of phytate, the iron becomes bound to the phosphate ester and is unavailable to react with sulfosalicylic acid, resulting in a decrease in pink colour intensity. The PA content was calculated from the calibration curve of PA standard and expressed as milligrams per gram of dry matter of sample (PA, mg/g).

The determination of trypsin inhibitor activity (TIA) is based on the reaction of trypsin with a synthetic substrate N-α-benzoyl-DL-arginine-p-nitroanilide (BAPA). As a result of that reaction, yellow p-nitroaniline is formed, and its maximum absorbance at 410 nm is proportional to its concentration (Hamerstrand *et al.*, 1981). The TI content is expressed as mg per gram of dry matter of sample (TI, mg/g).

2.4 Determination of the TPC, TFC, vitamin C, α-tocopherol and antioxidant capacity (IC50)

The TPC was estimated by Folin-Ciocalteu method (Jiang *et al.*, 2013). Reduction of phosphomolybdic-phosphotungstic acid (Folin reagent) to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds. Absorbance of sample was read at 760 nm against the blank using a spectrophotometer. The total phenolic content of samples was expressed as milligrams garlic acid equivalents per gram of dry matter (mg GAE/g).

The TFC was determined by the Dowd method (Meda *et al*., 2005) with slight modification (adding $NaNO_2$ 5% solution in test sample and absorption reading after 30 minutes of reaction). The standard quercetin (Sigma–Aldrich Chemie, Germany) was used to build up a standard curve. Thus, the results were expressed as milligrams of quercetin equivalents (QE) per gram of dry matter sample (mg QE/g).

Ascorbic acid (vitamin C) content was determined by redox titration with iodine (Mussa and Sharaa, 2014).

Vitamin E (α-tocopherol) content was determined by Emmerie-Emmerie Engel reaction. The reduc-

tion by tocopherol of ferric ions to ferrous ions which then forms a red complex with α,α'-dipyridyl that can be read at optimum wave length of 520 nm (Rutkowski and Grzegorczyk, 2007).

Antioxidant activity of the phytochemicals extracted from soybean was assessed by measuring their radical scavenging activity that was measured by the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay used stable DPPH radical as a reagent. The DPPH radical scavenging activity was evaluated from the difference in peak area decrease of the DPPH radical detected at 517 nm between a blank and a sample (Liu *et al.*, 2011). Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract (µg/ml) to obtain IC50 value in mg (dry matter)/ml. IC50 is defined as the amount of antioxidant material required to scavenge 50% of free radicals in the assay system. The IC50 values are inversely proportional to the antioxidant activity.

2.5 Statistical analysis

The data were submitted to analysis of variance (ANOVA) by Portable Statgraphics Centurion 15.2.11.0 and expressed as mean values and standard deviation.

3 RESULTS AND DISCUSSION

3.1 Effect of germination on protein and lipid of soybean

Total protein and lipid contents of raw and germinatedsoybeans are presented in Table 1. The change in protein content during soaking was not significant difference. However, germination process caused significant increases in protein content. Similar results were reported in mung bean (Sattar *et al.*, 1989), mungbean, chickpea and cowpea (Uppal and Bains, 2012), sorghum seed (Narsih *et al.*, 2012) and lentil seeds (Fouad and Rehab, 2015). The increase in protein content was attributed to loss in dry weight, particularly carbohydrates through respiration during germination (Uppal and Bains, 2012). According to Bau *et al.* (1997), the increase in protein content was due to the synthesis of enzyme such as proteases during seed germinating or a compositional change following the degradation of other constituents.

Lipid content of soaked seeds did not alter significantlyafter soaking. However, germination process caused significant reductions in oil content (Table 1). Uppal and Bains (2012) reported that soaking and germination did not change in lipid content of mungbean, chickpea and cowpea; however, Fouad and Rehab (2015) as well as Dhaliwal and Aggarwal (1999) indicated that the lipid content decreased with increases in germination time for lentil and soybean, respectively. Narsih *et al.* (2012) noted that the lipid content in sorghum seeds decreased as both soaking and germination time increases.

Table 1: Total protein and lipid contents of raw and germinated soybean seeds

Germination time (hour)	Total protein content (%)	Total lipid content (%)
Untreated soybean seeds (control)	40.35^{ab}±0.08	18.18^{f}±0.10
0 (Soaked)	40.18^{a}±0.21	18.15^{ef}±0.08
12	40.65^{b}±0.18	18.12^{ef}±0.04
24	41.28^{c}±0.40	18.01^{de}±0.14
36	41.76^{d}±0.18	17.91^{d}±0.05
48	43.36^{e}±0.34	17.55^{c}±0.16
60	44.08^{f}±0.29	17.23^{b}±0.10
72	44.07^{f}±0.11	16.70^{a}±0.12

Data are expressed as mean ± standard deviation (SD). Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p< 0.05$) by LSD test

In the present study, lipid content decreased significantly in samples germinated from 36 to 72 hours. Uvere and Orji (2002) as well as Inyang and Zakari (2008) assumed that germination process resulted in the increased activity of lipolytic enzymes, which hydrolyzed the lipid into fatty acid and glycerol leading to decrease in the amount of lipid. Germination also enhances the hydrolysis of complex organic compounds which are insoluble in the seeds, and forms more simple organic compounds that are water soluble. Another reason, the decrease in lipid content of seed could be due to total solid loss during soaking prior to germination (Wang *et al.*, 1997). In addition, energy used for respiration during germination comes in part from lipid degradation (El-Adawy, 2002).

The SDS-PAGE analysis of total proteins from different soybean seed extracts obtained at different germination times is presented in Figure 1. In soybean seeds, the major storage source proteins are β-conglycinin (7S) and glycinin (11S), that were identified during germination. Bands for the subunits of β-conglycinin and acidic and basic polypeptides of glycinin of soaked sample as well as germinated samples (from 12 to 60 hours) are wider and more intensely stained than those of soybean seeds. During 60 hours of germination, these major protein bands look unchanged. Germination for 72 hours, the α and α' components of β-conglycinin

showed the little degradation, while no decline in the β subunit is noted. The catabolism of glycinin can be discerned in the disappearance of its acidic and basic chains. For 72 hours of germination, the acidic chains slightly decreased, but there is no observable decrease in the basic chains of glycinin.

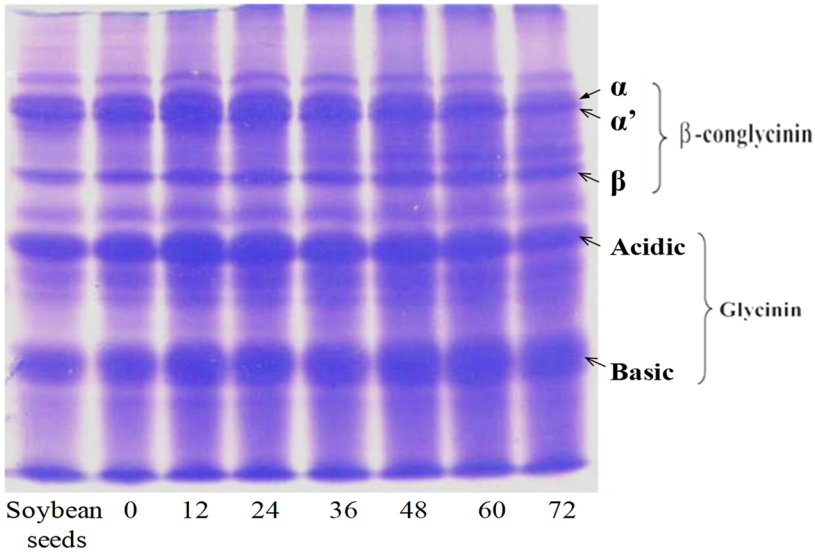

Fig. 1: SDS-PAGE profile of soybean extract at different germination times

A similar pattern of degradation was observed by Wilson *et al.* (1986) for the storage proteins of the soybeans during germination. They found that the acid chains of glycinin and both the α and α' subunits of β-conglycinin decreased after 3 days. However, there was no observable decrease in the basic chains of glycinin and β subunit of β-conglycinin until after 6 days of germination. The present results allow concluding that germination of soybean up to 60 hours did not change in the protein subunit pattern.

3.2 Effect of germination on antinutrients of soybean seeds

Legume seeds such as soybeans contain the considerable amount of antinutrients that are harmful to human consumption in raw state. Common antinutrients in soybean seeds are PA and TI. The changes in these factors in soybean seeds during soaking and germination are displayed in Table 2.

Soaking and germination caused significant decreases in PA and TI content. These decreases were gradually and significantly increased with increasing germination time.

PA content in raw soybeans is 28.57 mg/g. After soaking and germinating for 72 hours, PA content reduced to 26.82 and 16.12 mg/g, respectively (Table 2). These reductions are approximately 6.13 and 43.6%. The decrease in PA level during soaking may be attributed to leaching the acid out into soaking water under the concentration gradient (Abdelrahaman *et al.*, 2007; Sokrab *et al.*, 2012; Olu *et al.*, 2014). In the study of Hooda and Jood (2003), the reducing of PA content in fenugreek (*Trigonella foenum graecum* L.) after soaking and germinating for 48 hours is from 588.2 (in seeds) to 535.1 and 340.3 mg/100g, respectively (about 9 and 42.2%).

Table 2: PA and TI contentsof raw and germinated soybean seeds

Germination time (hour)	PA (mg/g)	TI (mg/g)
Untreated soybean seeds (control)	28.57^{g}±0.25	83.83^{h}±0.47
0 (Soaked)	26.82^{f}±0.37	77.78^{g}±0.42
12	26.47^{f}±0.36	67.27^{f}±0.60
24	25.15^{e}±0.20	55.88^{e}±0.30
36	23.24^{d}±0.36	47.50^{d}±0.57
48	19.02^{c}±0.49	43.18^{c}±0.57
60	17.18^{b}±0.44	40.89^{b}±0.59
72	16.12^{a}±0.39	38.86^{a}±0.58

Data are expressed as mean ± standard deviation (SD). Values given represent means of three determinations. Values followed by the same letter are not significantly different (P< 0.05) by LSD test

The reduction apparently in PA content during germination due to increased phytase activity in the germinated grains was also reported in some studies (Larsson and Sandberg, 1992; El-Adawy, 2002; Khattak *et al.*, 2007; Shimelis and Rakshit, 2007). Hydrolysis of phytates catalysed by phytase during germination led to the liberation of inorganic phosphates for plant growth from organic phosphorus containing compound including phytate (Shimelis

and Rakshit, 2007). There were 20-70% or more of the PA hydrolyzed during germination depending on the type of seeds and the increase in phytase activity (Reddy *et al.*, 1982). Sokrab *et al*. (2012) found that the reduction of PA in corn ranged from 81.88 to 84% for 6 days after germination, depending on the variety. Germination of lentil seeds at 25ºC for 6 days reduced the PA by 73.76% (Fouad and Rehab, 2015). PA has been considered to be one of the factors responsible for reducing minerals bioavailability, therefore, its reduction during germination may have a part in enhancement of nutritional quality of soybean seeds (Shimelis and Rakshit, 2007).

The results from Table 2 clearly showed that there is a reduction in TI content of soybeans during soaking (7.22%) and germination (53.64%, after 72 hours). TIs are low molecular weight proteins, so it is quite possible to be extracted in the soaking medium (Kakati *et al.*, 2010). The decrease in TI activity during germination was observed by many researchers. For example, Khattab and Arntfield (2009) reported that soaking had reduced the TIA by 10.22-19.85% in cowpea, pea and kidney bean. Kakati *et al.* (2010) also reported that soaking of the green gram for 24 hours reduced TI content from 50.1 to 55.4% depending on the variety. Collins and Saunders (1976) reported that there was a reduction in TIA of soybean germinated for 3 days (13.2%). The TI content reduced from 47.7 to 49.4% in green gram germinated for 48 to 72 hours (Kakati *et al.*, 2010). The decrease in TIA during germination may be due to the mobilization and breakdown of chemical constituents including TI to produce an energy source used during the early stages of germination (Sangronis and Machado, 2007; Kakati *et al.*, 2010).

3.3 Effect of germination on antinoxidant capacity of soybean seeds

All of compounds that contributed to the antioxidant activity of soybean include phenolics, flavonoids, vitamin C and vitamin E. The changes of these compounds as well as antioxidant capacity (IC50) during germination of soybean seeds are expressed in Table 3. Most of these parameters increased significantly leading to an increase in antioxidant capacity after soaking. Normally, soaking process reduced TPC, TFC and vitamin C in seeds because of the water-soluble phenolics and vitamin C leaching into the soaking water (Xu and Chang, 2008; Segev *et al.*, 2011). However, the presence of gibberellic acid in soaking water resulted in the increase in TPC, TFC, vitamin C as well as α-tocopherol content in soybean seeds after soaking (Lien *et al.*, 2016).

Table 3: Antioxidant capacity of raw and germinated soybean seeds

Germination time (hours)	TPC (mgGAE/g)	TFC (mgQE/g)	Vitamin C (mg/g)	α-tocopherol (mg/g)	IC50 (mg/ml)
Soybean seeds	2.78^{a}±0.02	1.95^{a}±0.01	6.45^{a}±0.16	0.06^{a}±0.01	9.45^{g}±0.04
0 (Soaked)	2.99^{a}±0.02	2.13^{b}±0.03	8.17^{b}±0.13	0.19^{b}±0.01	9.19^{f}±0.03
12	5.81^{b}±0.34	4.51^{c}±0.10	11.10^{c}±0.47	0.21^{c}±0.01	8.35^{e}±0.21
24	7.02^{c}±0.17	5.85^{d}±0.09	11.74^{d}±0.39	0.25^{d}±0.02	7.26^{d}±0.13
36	7.92^{d}±0.18	6.35^{e}±0.05	12.47^{e}±0.43	0.27^{de}±0.02	6.26^{c}±0.11
48	8.27^{e}±0.04	7.05^{f}±0.18	13.23^{f}±0.36	0.27^{de}±0.01	5.51^{b}±0.06
60	8.70^{f}±0.08	7.43^{g}±0.17	13.58^{f}±0.45	0.28^{ef}±0.01	5.03^{a}±0.04
72	8.21^{e}±0.09	7.04^{f}±0.09	14.51^{g}±0.17	0.29^{f}±0.01	5.41^{b}±0.06

Data are expressed as mean ± standard deviation (SD). Values given represent means of three determinations. Values followed by the same letter are not significantly different (P< 0.05), by LSD test

The results in Table 3 show that the content of TPC, TFC, vitamin C, α-tocopherol, and antioxidant capacity of germinated soybeen seeds increased. According to Cevallos-Casals and Cisneros-Zevallos (2010), germination process generally increases the bioactive compounds including phenolics. In the present study, TPC and TFC tended to reach the maximum values for 60 hours of germination. They slow down in 72 hours of germination. This tenency in TPC and TFC changing has also been observed by several authors. For example, phenolic content increased from 1341.13 mg gallic acid/100g dry matter in raw lentil seeds to the maximum value of 1630.20 mg gallic acid/100g dm at the fifth day of germination and decreased to 1510.1 mg gallic acid /100g in samples germinated for 6 days (Fouad and Rehab, 2015). The TPC of soybean was significantly higher from the second day and reached a peak on the fourth day (6.67 mg GAE/g), which was almost 1.51 fold of the seeds, then reduced at the fifth and sixth days (Kou and Zhou, 2016). The increase in TPC and TFC during several beginning days of germination could be due to the biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses (Randhir *et al.*, 2004), and the decrease of TPC after that might be due to mobilization of stored

phenolics by the activation of enzymes such as polyphenol oxidase during germination (Vadivel and Biesalski, 2012).

Ascorbic acid increased significantly during germination ($p<0.05$) and reached the maximum level after 72 hours of germination (Table 3). Several authors reported that germination caused an increase of vitamin C content in legumes (Ahmad and Pathak, 2000; Doblado *et al.*, 2007; Masood *et al.*, 2014; Kou and Zhou, 2016). Vitamin C content of soybean increased by 91.3% after sprouting for 3 days (Ahmad and Pathak, 2000). Vitamin C had not been found in raw mung bean and chickpea, but it appeared 37.0±1.5 and 20.0±0.5 mg/100g in mung bean and chickpea, respectively after 120 hours of germination (Masood *et al.*, 2014). According to Davey *et al.* (2000), the difference in level of ascorbic acid biosynthesis during germination might be affected by legume type, maturity, climatic conditions, light conditions, harvesting and grain storage methods. The accumulation of ascorbic acid during seed germination could be due to reactivation of enzyme (L-Galactono-γ-lactone dehydrogenase) involved in the oxidation of L-galactono-1, 4-lactone to ascorbic acid. The activity of this enzyme increased with seed germination (Xu *et al.*, 2005).

The α-tocopherol contents were found to increase with germination time and reach the maximum value after 72 hours (Table 3). An increase in the α-tocopherol content after germination also reported for soybean (Vasantharuba *et al.*, 2007), mung bean, soybean and black bean (Kou and Zhou, 2016) and sorghum (Suryanti *et al.*, 2016). Suryanti *et al.* (2016) showed the highest α-tocopherol contents were obtained at the fourth day of germination. According to Vasantharuba *et al*. (2007), the increase in vitamin E content during germination may be due to increased lipoxygenase enzyme activity of seeds.

Germination was also suggested as a powerful strategy to increase antioxidant activity in seeds (Fernandez-Orozco *et al.*, 2006). The antioxidant activity of germinated soybean seeds evaluated by IC50 values is shown in Table 3. Germinated soybean seeds expressed a good antioxidant potential, and the IC50 value of germinated soybean seed tended to reduce to minimum value after 60 hours for germination. The reduction of IC50 value (the increase in antioxidant activity) resulted from of the biosynthesis of phenolic compounds and vitamin C during germination.

A high correlation between free radical scavenging and the phenolic contents in seeds and seedlings was reported by many authors (e.g., Arabshahi-Delouee and Urooj, 2007; Giannakoula *et al.*, 2012; Gao *et al.*, 2014). In this study, the TPC negatively correlated to their IC50 ($r = –0.96$). Giannakoula *et al.* (2012) found that TPC in lentil seeds significantly correlated to their total antioxidant capacity ($R^2= 0.99$). The high TPC and antioxidant activity in germinated soybean make them interesting and useful for daily human diet.

4 CONCLUSIONS

Soybean processing methods are very important to utiliseeffectively the nutritional source and bioactive compounds in seeds. The high content of antinutrients caused the difficulty in digestion of soybean products. The results of this study showed that germination significantly reduced certain unwanted antinutrient components such as PA and TI. Because of the leaching of water-soluble solid during soaking and germination, soybean protein content increased, but the subunit patterns unchanged during 72 hours of germination. In addition, germination process increased remarkably TPC, TFC, vitamin C and α-tocopherol contents as well asantioxidant capacity of soybean seeds. Germination, so, is a good way to enhance the nutritional and antioxidant properties of soybean seeds. The germinated soybeans will not only help with the prevention and treatment of various human diseases but in improving the market of various traditional soybean foods with the development of bioactive components.

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