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The Changes in Antioxidant Capacity of Soybean (*Glycine max* (L.) *Merrill*) and Mung Bean (*Vigna radiate* (L.)*Wilczek*) during Germination Process

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Abstract

This study aim to determine effects of the germination time on the changes in antioxidant capacity of soybean (*Glycine max* (L.) *Merrill*) and mung bean (*Vigna radiate* (L.)). Antioxidant compounds, such as total polyphenol content (TPC), total flavonoids content (TFC), free radical scavenging capacities (DPPH), vitamin E content and vitamin C contents were studied. TPC were measured by Folin-Ciocalteu method. TFC were determine by the aluminum chloride colorimetric assay. The antioxidant capacities were determine using DPPH free radical scavenging method. Vitamin E content were measured base on reaction between α-tocopherol with ferric chloride. Vitamin C content were measured by iodine method. The result indicated that the changes in these bioactive compounds depended on the germination times. Germination of soybean provided a higher bioactive compounds than that of mung bean. Both TPC in soybean and mung bean increased sharply in the period 0-60h of germination, with 4.61 ± 0.03mg GAE/g dw at 60h for soybean and 3.59 ± 0.03mg GAE/g dw at 48h for mung bean. TFC were found to be 3.26 ± 0.15mg QE/g dw for soybean and 4.03 ± 0.02 mg QE/g dw for mung bean after 60h of germination. Besides, the DPPH values rose continuously during germination and reached a peaks of 76.20% at 60h of germination for soybean and 71.89% at 48h of germination for mung bean. The result also reported that vitamin C content and vitamin E content in two legumes increased during germination and the highest values at 72h (for soybean, 0.25 mg/g dw, 13.43± 0.21 mg/g dw and mung bean 0.19 ± 0.01 mg/g dw, 8.05 ± 0.24 mg/g dw, respectively). As so, the germination process in the period 60-72h affected the greatest enhancement in antioxidant compounds in soybean and mung bean.

Keywords: soybean, mung bean, antioxidant, total polyphenol content, germination.

1. Introduction

Mung bean (*Vigna radiate* L.) and soybean (*Glycine max* L.) are legumes widely consumed in Asia countries (Xue *et al*., 2016; Yin *et al.*, 2014), especially China, Korea, Japan (Dhakal *et al.*, 2009; Gamarnik and Frydman, 1991; Hosken, 2003; Huang *et al*., 2014; Jiang *et al*., 2013; Kim *et al*., 2006; Medic *et al*., 2014; Paucar-Menacho *et al*., 2010a, 2010b; Tang *et al*., 2014; Tiansawang *et al*., 2014; Xu *et al*., 2005). They play an important role in traditional diets of human and feed animal due to their nutritional properties and the beneficial characteristics of their constituent compounds (Fernandez-Orozco

et al., 2008; Kayembe and Jansen van Rensburg, 2013). Legumes are an excellent and inexpensive source of proteins with a high nutritive value, carbohydrate, lipid, vitamins, minerals and essential amino acid profile (Fernandez-Orozco *et al*., 2008; Guo *et al*., 2012b; Huang *et al*., 2014; Khang *et al*., 2016; Tajoddin *et al*., 2011). In soybean and mung bean seeds also contain many bioactive compounds such as polyphenol, flavonoid, vitamin E, vitamin C, phytochemicals (Fernandez-Orozco *et al*., 2008; Limwiwattana *et al*., 2016) which are known as active antioxidant and scavenge free radicals (FR) (Bolanho and Beléia, 2012). However, the functional compounds content in non-germination are very low (Guo *et al*., 2012a). Xiya *et al*., 2014 was reported that both soybean and mung bean seeds do not detect ascorbic acid content (Fernandez-Orozco *et al*., 2008; Huang *et al*., 2014; Xu *et al*., 2005).

Germination is an economical and effective technology to improve nutritive values by prevention of lipid oxidation, enhance digestibility by remove anti-nutritional factor as proteolytic inhibitors, lectin, antitrypsin, raffinose and stachyose in legumes (Fernandez-Orozco *et al*., 2008; Gamarnik and Frydman, 1991; Huang *et al*., 2014; Jiang *et al*., 2013; Kayembe and Jansen van Rensburg, 2013; Kim *et al*., 2013; Li *et al*., 2008; Limwiwattana *et al*., 2016; Paucar-Menacho *et al*., 2010a; Silva *et al*., 2013; Xu *et al*., 2005; Xue *et al*., 2016). Yang *et al*. (2010) and Guo *et al*. (2012) were published L-ascorbic acid content, antioxidant capacities, total polyphenol content and isoflavones content in mung bean and soybean were significant increased as compared to non-germination seeds during the germination process (Cevallos-Casals and Cisneros-Zevallos, 2010; Guo *et al*., 2012a; Paucar-Menacho *et al*., 2010a; Yang *et al*., 2010). Many researches showed that the presence of many bioactive compounds in legumes can help people prevent risk of danger such as heart disease, cancer, obesity, osteoporosis, cardiovascular diseases and coronary risk (Badole and Mahamuni, 2013; Guo *et al*., 2012a; Huang *et al*., 2014; Jooyandeh, 2011; Kim *et al*., 2013; Malenčić *et al*., 2007; Tiwari *et al*., 2011; Tsao, 2010).

Nowadays, the information about the effect of germination process on the changes of legumes functional compounds of legumes was relatively restricted. Therefore, the objective of this study was analyzing the effects of germination on the antioxidant capacities of soybean and mung bean.

2. Materials and Method

Plant Materials: The Mung bean (*Vigna radiate* L., CS-208) were purchased from Nong Thanh manufacture factory, An Giang province, Vietnam. Soybean (*Glycine max* L., MTĐ 760 variety) were supplied by Crop Sciences, College of Agriculture &Applied Biology, Can Tho University.

Chemicals: Folin-Ciocalteu reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were provided by Sigma-Aldrich Co. Aluminum chloride ($AlCl_3$), iodine solution, α,α'-dipyridyl, tocopherol, ferric chloride ($FeCl_3$), ethanol, acetone, methanol, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), oxalic acid, stach, hydrochloric acid (HCl), ascorbic acid were purchased from Xilong Chemical, China.

Germination process: Bean seeds were cleaned with sodium hypochlorite (100mg/kg) for 10 min (Paucar-Menacho *et al*., 2010a), soaked in gibberellic acid (GA3; 0.1mg.L^{-1}) for 12h. Then seeds were germinated under dark conditions at 25±1^{o}C. Samples were removed at the following progress points of germination: 0, 12, 24, 36, 48, 60 and 72h.

Sample extraction: A mass of 0.5g of defatted bean powder was extracted three time with acetone concentration 69% (v/v), extraction temperature 42^{o}C and time at 184 minutes, soybean to solvent ratio of 1:8 (w/v) (Lien *et al*., 2015).

Determination of total polyphenol content (TPC): TPC were determined by the Folin – Ciocalteu method (Jiang *et al*., 2013) by using gallic acid (GA) as the external standard. The assay contained Folin –

Ciocalteu reagent (200 µL), sample extract (50 µL), Na_2CO_3 7.5% (500 µL) and distilled water (1750 µL) was vortexed and kept in the dark in room temperature. The absorbance was measured at 765 nm after 2h.

Determination of total flavonoid content (TFC):TFC was measured base on by the aluminum chloride colorimetric assay (Cai *et al*., 2010). The mixture contained distilled water (3000 µL), 5% $NaNO_2$ (150 µL), an aliquot (500 µL) of extract from sample and 10% $AlCl_3$ (300 µL). After 45 min of incubation at ambient temperature, the absorbance of the mixture was measured at 415 nm by using spectrophotometer.

Determination of vitamin C: This method used iodine was as a titrant and was added during the titration. Vitamin C was oxidized to form dehydroascorbic acid while the iodine solution was reduced to iodide ions. When all vitamin C has been oxidized. The excess iodine solution will react with starch indicator to form blue-black color as endpoint of the titration (Azrin and Kanafe, 2009).

Determination of vitamin E: The vitamin E content as α-tocopherol was conducted as described Emmerie and Engel, (1938) with several modifications. The formation of a red complex of the Fe^{2+} with α,α'-dipyridyl based on the reduction in Fe^{3+} to Fe^{2+} by the tocopherols and the absorbance was measured at 520 nm (Emmerie and Engel, 1938; Karthikeyan *et al*., 2010, 2012; Martinek, 1964; Rutkowski and Grzegorczyk, 2007).

Determination of the Antioxidant Activity: The scavenging activity of DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) was determined according to Anshu *et al*. (2011) (Singh *et al*., 2011). This spectrophotometric method used stable DPPH radical as a reagent. To 0.8 mL an aliquot of extract from sample was mixed with 0.8 mL of DPPH 1mM in methanol. By the similar way, adding 0.8 mL acetone instead of sample in the control sample. Then the mixtures were shaken and kept in the dark for 15 min at room temperature. The absorbance value at 517nm. The DPPH radical scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = $[(A_o - A_s)/A_o].100$

Where A_o is the absorbance of the control and A_s is the absorbance of the sample.

Statistical Analysis: All data were statistically analysed using Portable Statgraphics Centurion software (Version 15.1). Every experiment was carried out in triplicate. Mean comparisons were performed using Analysis of variance (ANOVA) to determine the significant differences ($p<0.05$).

3. Results and Discussion

The changes in bioactive compound of soybean and mung bean are presented in **Table 1**. There are significant differences in antioxidant composition from soybean and mung bean during germination.

Total Polyphenol Content

The changes of TPC are showed in **Table 1**. In raw seeds, the TPC in soybean 2.29mg GAE/g dw and 1.25 mg GAE/g dw in mung bean. During the germination process, the TPC of soybean was higher than that of the mung bean, which increased gradually on times 0-60h germination and the maximum value was 4.61 mg GAE /g dw after 60 h germination, which was almost 2 fold of seeds. However, there are a slight decreased in 72h geminated soybean (4.25 mg GAE/g dw). A similar pattern of changes in TPC in mung bean, even though there were slight differences when germination mung bean showed lower content. The highest TPC in mung bean sprout was 3.58 mg GAE/g dw after 48h germination. Noticeably, there were no difference between 48h and 60h germination. Xue *et al*. (2016) showed a significant increase of TPC in soybean and mung bean during the germination process (Xue *et al*., 2016).

In addition, Guo *et al*. (2012) reported a similar result that germination enhance TPC in mung bean (approximately 310mg GAE/100g dw on day 2 after germination) (Guo *et al*., 2012a).

Total flavonoids content

The TFC of soybean and mung bean were raised rapidly during germination. The fastest growth of mung bean occurred after 12h germination and the highest content was 4.04 mg QE/g dw at 48 h germination, which was almost 2.9 – fold of the seeds. However, it then showed a slight decline after 60h (4.03 mg GAE/g dw) and 72 h (3.99 mg QE/g dw) germination. Comparing with mung bean, TFC in soybean was lower than and a regular increased in the period 0-60h germination, reached a peaks of 60h germination, at around 3.26 mg QE/g dw – which was 3.2 times more than seeds. On the next period, there were a remarkable fell about 2.42 mg QE/g DW. There were several studies presented the similar result that germination is an efficient process to improve the TFC of soybean and mung bean (Guo *et al*., 2012a; Xue *et al*., 2016).

Antioxidant activity

Table 1 illustrated that the DPPH free radical scavenging capacities of soybean and mung bean rose linear during germination. Antioxidant activity increased continuously with germination time and the highest antioxidant activity fell into 72h germination, approximately 76% of soybean and 71% of mung bean, which was almost 1.1- fold increase of antioxidant activity of raw seeds. In addition, The DPPH values in soybean were relative higher than that of mung bean. One reason for this result is that bioactive compounds content in soybean such as were overwhelmingly higher than that of mung bean. Moreover, TPC, TFC, vitamin C are powerful antioxidant, which were improved significantly during germination.

Table 1. Effect of germination on the TPC, TFC and DPPH of soybean (*Glycine max* L.) and mung bean (*Vigna radiate* L.)

Germination time (hours)	Soybean			Mung bean		
	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (%)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (%)
Raw	2.29^{f} ±0.12	1.02^{e} ±0.02	62.45^{e} ±0.92	1.25^{f} ±0.03	1.38^{f} ±0.02	59.97^{f} ±0.09
0	3.15^{e} ±0.03	2.03^{d} ±0.03	70.93^{d} ±0.28	1.61^{e} ±0.03	1.44^{e} ±0.01	59.88^{f} ±0.08
12	3.31^{d} ±0.08	2.41^{d} ±0.14	72.15^{c} ±0.53	2.51^{d} ±0.01	3.73^{d} ±0.01	63.46^{e} ±0.05
24	3.68^{c} ±0.02	2.64^{c} ±0.13	74.26^{b} ±0.67	2.72^{c} ±0.02	3.84^{c} ±0.03	64.45^{d} ±0.05
36	3.73^{c} ±0.04	3.02^{b} ±0.03	74.63^{b} ±0.44	2.72^{c} ±0.02	3.87^{c} ±0.01	64.67^{c} ±0.13
48	4.54^{a} ±0.03	3.11^{b} ±0.03	74.84^{b} ±0	3.59^{a} ±0.03	4.04^{a} ±0.01	71.89^{a} ±0.05
60	4.61^{a} ±0.03	3.26^{a} ±0.15	76.21^{a} ±0.34	3.58^{a} ±0.03	4.03^{a} ±0.02	71.77^{a} ±0.18
72	4.25^{b} ±0.03	2.42^{d} ±0.08	75.97^{a} ±0.26	3.42^{b} ±0.04	3.99^{b} ±0.02	71.12^{b} ±0.14

(Mean ± SD, The values showing different superscripts within a row are significant different at p=0.05)

Vitamin C content

The concentrations of vitamin C in soybean and mung bean during germination periods are presented in **table 2**. It can be seen that vitamin C content increased in a time-dependent patter and reached a peak on 72h, amounting to 13.43 mg/g dw for mg/ soybean and 8.05 g dw for mung bean, respectively. Xue *et al*. (2016) found that vitamin C of soy bean was higher than that of mung bean during germination times. This result also reported concentrations of vitamin C were significant improved by the germination process (Xue *et al*., 2016). Another studies also given the similar evidences (Fernandez-Orozco *et al*., 2008; Guo *et al*., 2012a; Huang *et al*., 2014; Suryanti *et al*., 2015; Xu *et al*., 2005). Xu *et al*. (2005) was

explained that concentration of vitamin C in soybean increased during germination was due to the reactivation of its biosynthesis. GLDH (L-Galactono-γ-lactone dehydrogenase) is one of the key enzymes in ascorbic acid biosynthesis increased significantly during germination. It play an important role in the oxidation of L-galatono-1,4-lactone to ascorbic acid (Xu *et al*., 2005).

Table 2. Effect of germination on the vitamin C, vitamin E of soybean (*Glycine max* L.) and mung bean (*Vigna radiate* L.)

Germination time (hours)	Soybean		Mung bean	
	Vitamin E (mg/g)	Vitamin C (mg/g)	Vitamin E (mg/g)	Vitamin C (mg/g)
Raw	0.08^{f} ±0.01	4.81^{f} ±0	0.01^{d} ±0.01	2.99^{f} ±0.24
0	0.01^{e} ±0.01	7.54^{e} ±0.21	0.08^{c} ±0.01	4.22^{e} ±0.22
12	0.11^{d} ±0.01	9.04^{d} ±0.21	0.08^{c} ±0.01	5.01^{d} ±0.22
24	0.15^{c} ±0.01	9.68^{d} ±0.56	0.10^{c} ±0.02	5.15^{d} ±0.22
36	0.21^{b} ±0.01	10.86^{c} ±0.22	0.15^{b} ±0.01	5.38^{cd} ±0.21
48	0.26^{a} ±0.01	12.01^{b} ±0.85	0.18^{a} ±0.01	5.63^{c} ±0.21
60	0.26^{a} ±0.01	12.54^{b} ±0	0.18^{a} ±0.01	6.28^{b} ±0.22
72	0.25^{a} ±0	13.43^{a} ±0.21	0.19^{a} ±0.13	8.05^{a} ±0.24

(Mean ± SD, The values showing different superscripts within a row are significant different at p=0.05)

Vitamin E content

The changes in the vitamin E content in soybean and mung bean during germination are illustrates in **Table 2**. The vitamin E content increased in a time-dependent way. In case of soybean, the vitamin E content rise sharply from 0.08mg/g dw in seeds to the highest value of 0.26 mg/g dw at 60h of germination, respectively. This was followed by no significant fall after 72h germination. Regarding mung bean, there are a gradually rise in vitamin E content during germination process and a reached the peak of about 0.19 mg/g dw after 72 h of germination. By comparison, vitamin E in soybean is overwhelmingly greater than that of mung bean during germination. Fernandez-Orozco *et al.* (2008) found that germination brought about a sharp rise in the content of vitamin E in soybean (1.59 mg/100 dw in raw and 1.98mg/100g dw after 3 days, respectively) and mung bean (1.05 mg/100g dw in raw and 1.09 mg/100g dw after 3 days, respectively) (Fernandez-Orozco *et al*., 2008). Another study also reported the similar result, Kim *et al.* (2013) researched on nutritional evaluation of germinated soy germ and reported that vitamin E content was lower than about 12.36 mg/100g dw at 24h germination (Kim *et al*., 2013). Kim *et al* (2013) also reported that soy germ contains a relatively higher bioactive compounds as total tocopherol. As germination progressed, the length of soy germ interact with total tocopherol content. The result indicate that germination promotes the total tocopherol content of soy germ by the way the length of germ significant increased during germination (Kim *et al*., 2013).

Nevertheless, the antioxidant composition may depend on the extraction method, seed's quality and environmental conditions such as temperature, humidity (Silva *et al*., 2013; Xue *et al*., 2016).

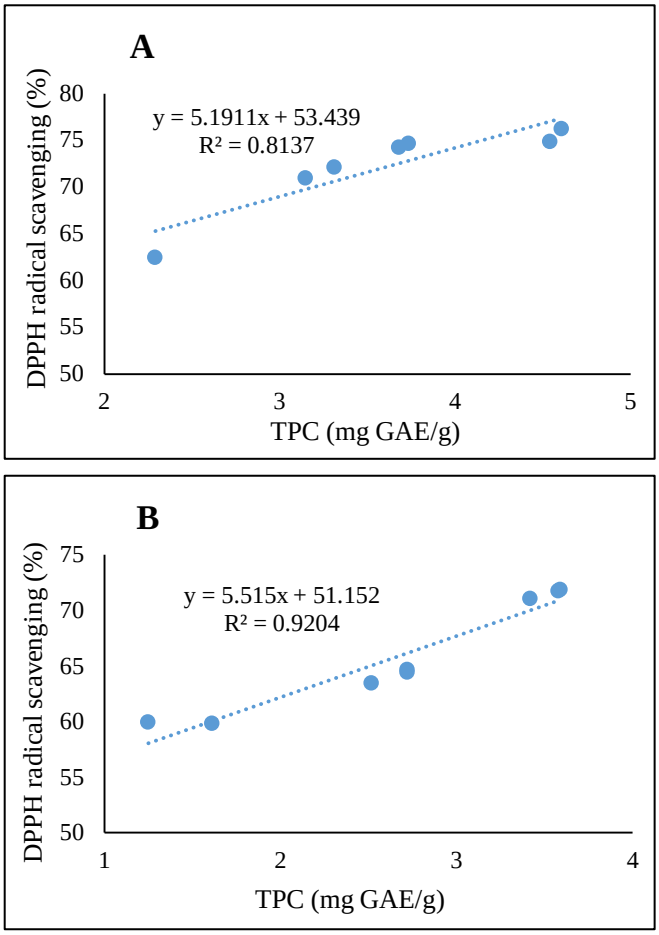

Figure 1. Correlation between DPPH free radical scavenging and TPC in soybean (**A**) and mung bean (**B**).

Total polyphenol contents and DPPH free-radical scavenging increased during germination process and DPPH free-radical scavenging has a highest value at 72h of germination, at 75.97% in soybean and 71.12% in mung bean, respectively. The correlation coefficient between TPC and DPPH free-radical scavenging was fine linear with R^2 = 0.81 for soybean (**Fig.1A**) and R^2 = 0.92 for mung bean (**Fig.1B**). These results indicate a high correlation between TPC and DPPH free-radical scavenging in legumes and TPC play an important role to contribute to the radical scavenging capacity of these legumes extracts. Several studies have also been reported similar result for some legumes (Boué *et al*., 2008; Fidrianny *et al*., 2014, 2015).

4. Conclusions

The result demonstrated that germination process improve significantly TPC, TFC, DPPH, vitamin E content, vitamin C content in soybean and mung bean after 60-72h. TPC ranged from 2.29 ± 0.03 mg GAE/g dw in raw seed to 4.61 ± 0.03 mg GAE/g dw at 60h of germination on soybean and from 1.25 ± 0.03 mg GAE/g dw in raw seeds to 3.59 ± 0.03 mg GAE/g dw at 48h of germination in mung bean, respectively. Both TFC in soybean and mung bean increased about 3 fold, compared to raw seeds. The antioxidant activity increased by 22% of soybean and 20% of mung bean after 48h of germination. Also, vitamin C content and vitamin E content increased in a time-dependent in germination and the highest value fell into 72h of germination. As so, germination is a good way for improving nutritional quality of legumes.

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