doi: 10.17221/434/2015-CJFS

Bioactive Compounds and Antioxidant Activity of Mung Bean (*Vigna radiata* L.), Soybean (*Glycine max* L.) and Black Bean (*Phaseolus vulgaris* L.) during the Germination Process

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Abstract

Xue Z., Wang C., Zhai L., Yu W., Chang H., Kou X., Zhou F. (2016): **Bioactive compounds and antioxidant activity of mung bean (*Vigna radiata* L.), soybean (*Glycine max* L.) and black bean (*Phaseolus vulgaris* L.) during the germination process**. Czech J. Food Sci., 34: 68–78.

Bioactive compounds and antioxidant activity of germinated mung bean, soybean and black bean sprouts were investigated to find out the effect of germination and the optimum germination time. We found that vitamin C gradually increased from zero. Compared to seeds, water-soluble protein and total flavonoid content showed a trend of sustained growth while vitamin E, total phenolic content increased at first and then decreased, the maximal value of total bioactive compound content was reached on the fifth day. The analysis of relative contribution revealed that total phenolics and total flavonoids made the highest (44.87–90.31%) contribution to total antioxidant activity. In conclusion, the optimum germination time for sprouts was 3–5 days when total bioactive compound content and antioxidant activities both reached their peak values, which provide a sufficient theoretical basis for dietary processing and lay a solid foundation for continued research.

Keywords: vitamin C; vitamin E; water-soluble protein; total flavonoids; total phenolics

Mung bean (*Vigna radiata* L.), soybean (*Glycine max* L.), and black bean (*Phaseolus vulgaris* L.) are popular food legumes consumed worldwide. However, processing is necessary before consumption due to non-nutritive factors (Vidal-Valverde *et al.* 2002). Germination is an economical and effective way to improve the bioactive compound content of the sprouts (Mbithi-Mwikya *et al.* 2000; Vidal-Valverde *et al.* 2003). Sprouts have substantial nutritional benefits for the human body because of their high concentration of nutrients which can be used readily by the body (Randhir *et al.* 2004).

Vitamin C (Vc) is an excellent antioxidant, especially in a food system to maintain the active state for many bioactive compounds, such as vitamin E (Ve), flavonoids and phenolics (Guo *et al.* 2012). Vitamin E can protect polyunsaturated fatty acids against oxidant damage in cell membranes (Niki 2014). Water-soluble protein (SP) is a critical factor of food quality. During seed germination, storage proteins are mobilised to provide nutrients for seedling growth (Wang *et al.* 2007). Phenolics are located in the lipid-water interface of membranes, so they can scavenge free radicals inside and outside the cell. The flavonoids found in beans have been widely studied due to their protective role against cardiovascular diseases and cancer (Guajardo-Flores *et al.* 2013).

Over the last decade, several studies have been focused on changes in bioactive compounds of beans, when germination increase Vc and Ve content, water-

Supported by the grants from the National Natural Science Foundation of China, Grants No. 31271979 and No. 31201245.

Xiaohong Kou and Fengjuan Zhou contributed equally to this paper.

doi: 10.17221/434/2015-CJFS

soluble protein content, total phenolic content, and antioxidant activity (Wang *et al.* 2007; Fernandez-Orozco *et al.* 2008; Guo *et al.* 2012; Guajardo-Flores *et al.* 2013; Vijaylaxmi 2013; Huang *et al.* 2014; Pajak *et al.* 2014). However, there were no systematic researches on changes in bioactive compounds and antioxidant activities of mung bean, soybean and black bean during the germination process. Besides, no scientific methods were available to evaluate the total bioactive compound content of sprouts in a comprehensive way.

This study was aimed at determining the effects of germination on the bioactive compounds and antioxidant activities of mung bean, soybean and black bean in order to find the optimum conditions of germination, understand the underlying mechanisms of increased antioxidant activity and provide theoretical guidance for further production. TOPSIS (Technique for Order Performance by Similarity to Ideal Solution) was also introduced and tested to assess the total bioactive compounds of different beans, provide data-based foundation for manufacturing companies to optimally select the germination period.

MATERIAL AND METHODS

Material and chemicals. Mung bean, soybean, and black bean were supplied by the Midaocailai Company (Jilin, China). Ascorbic acid (ASA), 2,6-dichloroindophenol (DIP), diethyl ether, tocopherol, phosphoric acid, 1,10-orthopenanthroline, bovine serum albumin (BSA), Coomassie brilliant blue (CBB), gallic acid (GA), rutin, deoxyribose (DR), thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzthiazoline-6)sulfonic acid (ABTS) were all provided by Sigma-Aldrich Co. (St. Louis, USA). Ethylene Diamine Tetraacetic Acid (EDTA) was provided by Solarbio (Beijing, China).

Seed germination. Bean seeds were cleaned and soaked in water for 1 h at room temperature before being placed into a semi-automatic germination machine (DYJ-S6365; Guangzhou Xiaoxiong Electrical Equipment Company, Foshan, China) with 50 beans per vessel. The germination process proceeded at 25°C in the dark for 6 days. Sprouts were rinsed with running water every 1 hour. Germination rate was calculated as a germinated percentage of seeds:

$$\text{Germination rate (\%)} = (\text{number of seeds germinated}/\text{total number of seeds at beginning}) \times 100$$

Determination of vitamin C. The vitamin C content was determined by titration against 2,6-dichlorophenolindophenol (Thimmaiah 1999). The sample (5 g) was blended with 1 g/kg oxalic acid solution and homogenised for 3 minutes. After the mixture was centrifuged at 1200 *g* for 5 minutes, The extract solution (10 ml) were filtered for titration. The samples were titrated against the ascorbic acid standard solution (1 mg/ml) calibrated with 2,6-dichlorophenolindophenol solution until the colour turned pink for 15 seconds. The Vc content was expressed as mg/g fresh weight (FW).

Determination of vitamin E. The vitamin E was extracted according to He and Zhang (1997). The pulverised sample (5 g) was blended with 20 ml of 95% ethanol for 12 h and then added 1 ml of saturated KOH for saponification (30°C, 15 min). The solution was separated with 20 ml distilled water and 20 ml diethyl ether. The content of Ve (μg/g FW) was measured based on a colorimetric assay by adding 0.5 ml/1 10-phenanthroline, 0.5 ml 1.0 mM $FeCl_3$, 0.5 ml 40 mM phosphoric acid to 4 ml of diluted extraction solution and the absorbance at 510 nm was measured (Huang *et al.* 2011).

Determination of water-soluble protein content. The fresh material (1 g) was homogenised with 5 ml of distilled water and subsequently centrifuged at 2550 *g* for 10 min at 4°C; the suspension was removed to measure the soluble protein content (SPC in mg/g FW) of all sample extracts that was estimated colorimetrically with protein assay reagent by the Bradford method (Bradford 1976).

Preparation of bean sprout extracts. The samples (25 g) were extracted with 80 g/kg acetone (50 ml) for 5 min (Julkunen-Tiitto 1985; Eberhardt *et al.* 2000; Liu *et al.* 2002; Liu & Sun 2003). The acetone extracts were evaporated using a rotary evaporator at 45°C until the weight decreased to less than 10% of the initial weight and then vacuum freeze dried to obtain crude extracts. The extracts were reconstituted to a final volume of 25 ml at the time of analysis. Extractions were performed in 3 replications for each individual sample. The extracts were prepared for the determination of total phenolics, total flavonoids and antioxidant capacity (Xu & Chang 2007).

Determination of total phenolic content. The amount of total phenolics (TPC) was analysed based on a modified Folin-Ciocalteu colorimetric method (Singleton *et al.* 1999). It was determined from the regression equation of the calibration curve of gallic acid (concentrations ranging from 50 μg/ml

doi: 10.17221/434/2015-CJFS

to 500 µg/ml) and expressed as mg gallic acid equivalents per g FW (mg GAE/g FW). Briefly, 125 µl of standard gallic acid solution or distilled water diluted extracts were mixed with 125 µl of Folin-Ciocalteu reagents and 1.25 ml of 7 g sodium carbonate solution/kg was then added. After incubation at room temperature for 30 min, the absorbance was measured at 760 nm.

Determination of total flavonoid content. The total flavonoid content (TFC) was determined based on a colorimetric assay method (Zhishen *et al.* 1999) with slight modifications. Briefly, distilled water diluted extract was mixed with 0.75 ml of 5 g $NaNO_2$/kg. After incubation for 5 min, 0.5 ml of 10 g $Al(NO_3)_3$/kg was added and let stand for 6 min before mixing with 4 ml of 5 g NaOH/kg. Then the mixture was diluted to 25 ml and the absorption readings were taken at 510 nm. The total flavonoid content was determined using a calibration curve with rutin (10, 20, 30, 40, 50, and 60 mg/l) as the standard and expressed as mg of rutin equivalents per g FW (mg RE/g FW).

Antioxidant capacity

Total reducing power. The total reducing power (TRP) of the extracts was determined by a previously described method (Oyaizu 1988) with slight modifications. 1 ml of extracts was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 10 g $C_6N_6FeK_3$/l. After the mixtures were incubated at 50°C for 20 min, 2.5 ml of 10 g trichloroacetic acid/kg was added followed by centrifugation at 1650 *g* for 10 minutes. The supernatant (5 ml) was mixed with 5 ml of distilled water and 1 ml of 1 g ferric chloride/l to measure the absorbance at 700 nm. An increase in the absorbance of reaction mixture indicated the reducing power of samples.

Hydroxyl radical scavenging capacity. The hydroxyl radical scavenging capacity (HOSC) of the extracts was measured according to the method previously described by Halliwell *et al.* (1987) with slight modifications. The final reaction mixture contained 100 ml of sample solution, 1 mM $FeCl_3$, 1.04 mM EDTA, 10 mM H_2O_2, 2 mM L-vitamin C, and 60 mM deoxyribose in potassium phosphate buffer (pH 7.4). The mixture was incubated at 37°C for 1 h and put in water bath for 15 min after 1 ml of 250 ml HCl/l and 1 ml of 10 g TBA/kg were added. The absorbance was read at 532 nm against a blank containing phosphate buffer. The percentage antioxidant activity (AA) was calculated according to the equation:

$$AA\ (\%) = \{[1 - (A_i - A_j)]/A_0\} \times 100$$

where: A_i – absorbance of the sample; A_j – absorbance of the sample control; A_0 – absorbance of the control

Relative DPPH radical scavenging capacity (RDSC). The RDSC was determined according to the method of Brand-Williams *et al.* (1995) with slight modifications. The sprout solution (1 ml) was mixed with 1 ml of 0.1 mM DPPH in 95 g ethanol/kg and let stand in the dark for 20 minutes. Ethanol was used instead of DPPH in the DPPH control. Distilled water was used instead of the sample in the sample control. The absorbance was measured at 517 nm and converted to AA (%).

ABTS radical scavenging capacity (ARSC). The ARSC was determined according to Re *et al.* (1999) with some modifications. Briefly, $ABTS^+$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate. In the assay, samples were mixed with PBS (pH 7.4) diluted $ABTS^+$ (absorbance of 0.70 ± 0.02 at 734 nm) at a ratio of 1 : 100 (v/v), and the absorbance reading (734 nm) was taken after 6 min incubation in the dark. The AA (%) was calculated. The relative activity was calculated using a trolox calibration curve and the results were converted to TEAC (trolox equivalent antioxidant capacity) value for further relative contribution of antioxidants analysis.

Comprehensive evaluations of total bioactive compounds. TOPSIS was proposed by Hwang and Yoon (1981). The basic principle of TOPSIS is that chosen alternatives should have the shortest distance from the ideal solution and the farthest distance from the negative ideal solution. There were six steps to do.

Step 1: Establish a decision matrix for ranking:

$$X = \begin{pmatrix} x11 & x12 & \cdots & x1n \\ x21 & x22 & \cdots & x2n \\ \vdots & \vdots & \vdots & \vdots \\ xm1 & xm2 & \cdots & xmn \end{pmatrix}, i = 1, 2, \cdots, m; j = 1, 2, \cdots, n$$

where: x_{ij} – rating of alternative A_i (m) with respect to criterion C_j (n) evaluated

Step 2: Calculate the normalised decision matrix r_{ij}:

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{m} {x_{ij}}^2}},\ i = 1, 2 \ldots, m; j = 1, 2, \ldots, n$$

Step 3: Construct the weighted normalised decision matrix by multiplying the normaliaed decision matrix by its associated weights. The weighted normaliaed value v_{ij} is calculated as follows:

$$v_{ij} = w_i \times r_{ij},\ \sum_{i=1}^{m} w_i = 1$$

where: w_i – weight of j^{th} criterion

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Step 4: Determine the positive and negative ideal solution:

$$A^+ = \{v_1^+, v_2^+, ..., v_n^+\} = \{(\max_j v_{ij} \,|i \in I), (\min_j v_{ij} | i \in J)\}$$
$$A^- = \{v_1^-, v_2^-, ..., v_n^-\} = \{(\min_j v_{ij} \,|i \in I), (\max_j v_{ij} | i \in J)\}$$

where: I – associated with the benefit criteria; J – associated with the cost criteria

Step 5: Measure the distance from the positive and negative ideal solution using two Euclidean distances.

$$d_j^+ = \sqrt{\sum_{i=1}^{n} (v_{ij} - v_i^+)^2}\,; d_j^- = \sqrt{\sum_{i=1}^{n} (v_{ij} - v_i^-)^2}$$

where: d_j^+ – distance of alternative A_j from the positive and negative ideal solutions

Step 6: Calculate the relative closeness to the ideal solution and compare R_j values to rank the alternatives

$$R_j = d_j^-/(d_j^+ + d_j^-),\ R_j = [0,1]$$

where: R_j – relative closeness

Relative contribution of antioxidant compounds. In order to carry out the calculation, the following TEAC values of individual compounds were taken: 0.99 μM/g for Vc, 0.97 μM/g for Ve, 3.01 mM for gallic acid, and 2.4 mM for rutin (Rice-Evans 1996). The theoretical antioxidant activity was calculated as follows:

$$TAA_{calculated} = \Sigma_i\, TEAC_i \times Conc_i$$

where: i – one kind of antioxidant compound; $Conc_i$ – concentration of the compound (μmol/g or mM/l)

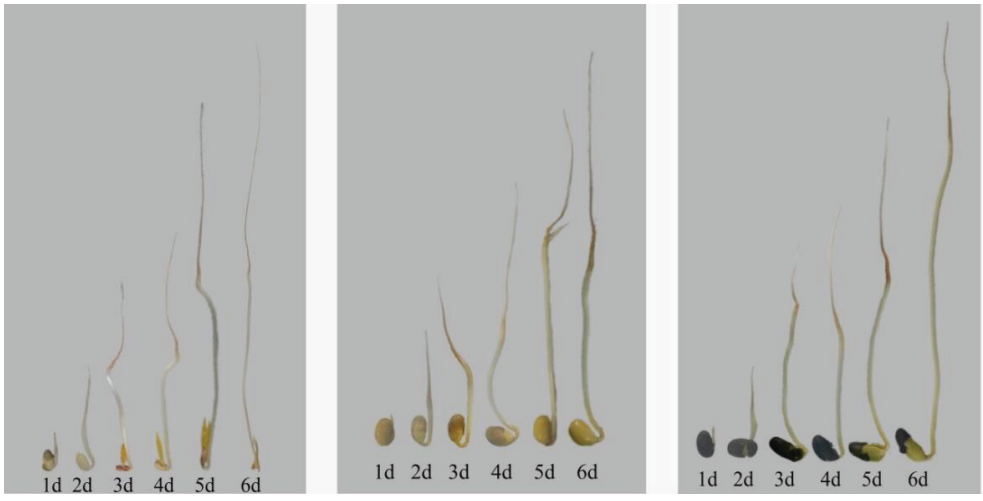
The relative contribution rate (%) of individual compound was calculated by the following equation:

$$r_i\,(\%) = (TEAC_i/TEAC_{EVALUATED}) \times 100$$

Statistical analysis. All of the extractions and analyses were performed in triplicate and all data were expressed as the mean ± SD for each sample. The differences between the bean sprout samples were analysed by one-way analysis of variance (ANOVA) followed by a two-tailed *t*-test. Pearson correlation tests were conducted to determine the correlations between the data. All of the statistical calculations were performed using the SPSS statistical software Version 17.0.

RESULTS AND DISCUSSION

Germination of bean seeds. Morphological changes of seeds and sprouts during germination are shown in Figure 1. All of the bean seeds sprouted after one-day germination, and the sprouts gradually became thicker and longer during the subsequent days. No visible morphological differences were found between mung bean, soybean, and black bean sprouts. Concerning the rate of germination depending on the time (Day 1–6) (Figure 2A), mung bean showed a different pattern from soybean and black bean, increased quickly and slowed, which was consistent with a previous study (Huang *et al.* 2014). Mung bean showed a similar value of germination rate to soybean from Day 2 to 6, whereas a higher rate (65.0%) than in soybean (42.0%) occurred on the first day. Black bean showed the lowest germination rate compared to mung bean and soybean during the entire germination time, even the highest germination rate (72.0%) was lower by 26.5% than in the others



Mung bean Soybean Black bean

Figure 1. The morphological features of three types of bean sprouts during germination periods

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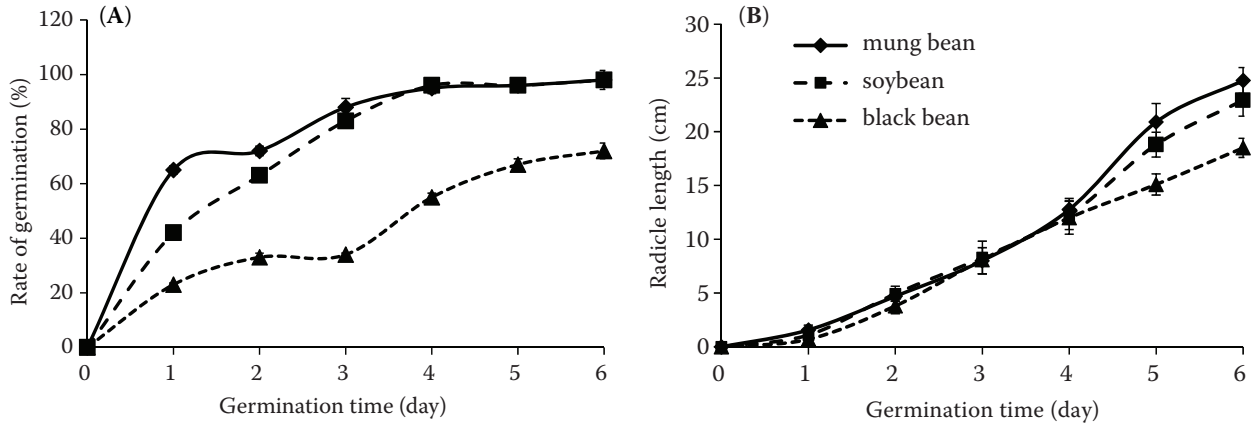

Figure 2. The germination rate and radicle length of mung bean, soybean, and black bean at different germination times

(98.0%). The radicle length of sprouts of mung bean, soybean, and black bean gradually increased and reached 24.77, 22.93, and 18.50 cm, respectively, on Day 6 (Figure 2B). This trend may be positively related to the germination rate, the lower rate of black bean ended up with a shorter radicle length of sprout after 6 days of germination.

Changes of bioactive compounds. The Vc content was not detected in bean seeds (Figure 3A), which was in agreement with Fernandez-Orozco *et al.* (2008) and Huang *et al.* (2014), who found out that the Vc content was zero in mung bean and soybean seeds. The Vc content increased in a time-dependent pattern and reached a peak on Day 6, amounting to 1.97, 1.94, and 1.93 mg/g FW for mung bean, soybean, and black bean, respectively.

Ve is an essential lipid-soluble antioxidant, well-known for its potential beneficial antioxidant effects on various types of human cancer including prostate, breast and colon (Constantinous *et al.* 2008; Miyazawa *et al.* 2009). Kim *et al.* (2013) found that as germination progressed, the total Ve content of soy germs increased rapidly by 32.4% (34.99 mg/100 g) after 24-h germination. A similar pattern of changes in Ve was detected in all types of bean seeds, even though there were slight differences when mung bean and black bean showed higher and lower Ve content, respectively, than soybean during the entire period (Figure 3B). Ve increased gradually on Days 0–4 and then decreased on Days 5 and 6. The highest value for mung bean, soybean, and black bean sprouts on Day 4 was 46.56, 67.97, and 59.39 μg/g FW, which was 4.10, 2.43, and 2.54 times more than in ungerminated beans. The results coincided with the previous study of germinated soybean and mung bean (Fernandz-Orozco *et al.* 2006; Fernandz-Orozco *et al.* 2008; Shi *et al.* 2010).

Part of energy for seed germination and development is provided by the mobilisation of storage proteins which will be hydrolysed with proteolytic enzymes and converted into soluble state after germination (Rahman *et al.* 2007; Tian *et al.* 2010; Ribeiro *et al.* 2011). The SPC changes of three kinds of beans are shown in Figure 3C. After 6 days germination, the SPC

Table 1. TOPSIS analysis of total bioactive compounds of bean sprouts

Day	Mung bean			Soybean			Black bean		
	d_j^+	d_j^-	R_j	d_j^+	d_j^-	R_j	d_j^+	d_j^-	R_j
0	0.9949	0.0004	0.0004	0.7905	0.0007	0.0009	0.8288	0.0432	0.0495
1	0.8907	0.1398	0.1357	0.6893	0.1180	0.1461	0.8024	0.0383	0.0455
2	0.7990	0.3135	0.2818	0.4991	0.2975	0.3734	0.7520	0.0993	0.1167
3	0.6048	0.4284	0.4146	0.2950	0.5033	0.6304	0.6683	0.2480	0.2707
4	0.3256	0.7803	0.7056	0.1530	0.6848	0.8174	0.3876	0.5302	0.5777
5	0.1778	0.8555	0.8279	0.0641	0.7541	0.9216	0.2063	0.6741	0.7657
6	0.1845	0.9057	0.8308	0.1181	0.7455	0.8633	0.2243	0.7667	0.7736

d_j^+ – positive ideal solution of Euclidean distance; d_j^- – negative ideal solution of Euclidean distance; R_j – closeness coefficient

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was around 1.80 mg/g FW, even though mung beans showed the much lower SPC (0.76 mg/g FW) than soybeans (1.74 mg/g FW), and black beans (1.58 mg/g FW) at the beginning. The SPC of mung bean showed a significant increase on Days 0–2 and the maximum value was 1.89 mg/g FW, which was 2.49 times more than in seeds, while the SPC of soybeans and black beans fluctuated between 1.5 and 2.0 mg/g FW. The SPC in our study was relatively lower than that reported before (Vijaylaxmi 2013), which can be caused by the difference in germination conditions and varieties. Zhang *et al.* (2014) studied 192 collections of soybeans from the whole world and found that there were large genetic variations in protein content and SPC, and also provided evidence why soybean varieties have high protein content but low SPC.

Phenolics are important bioactive compounds that have plentiful benefits for the human body. There are some studies reporting that germination is an efficient process to increase the TPC of beans (Randhir *et al.* 2004; Fernandz-Orozco *et al.* 2006; Fernandz-Orozco *et al.* 2008; Dueñas *et al.* 2015). The changes of TPC are presented in Figure 3D. During the germination process, the TPC of soybean was higher than that of the other two types of beans from the second day to the fifth day and reached a peak on the fourth day (6.67 mg GAE/g), which was almost 1.51 fold of the seeds. The highest TPC in black bean was 6.74 mg GAE/g on Day 6, which was almost 1.54 fold of the ungerminated beans. The fastest growth of mung bean occurred on the fifth day and achieved a maximum content of

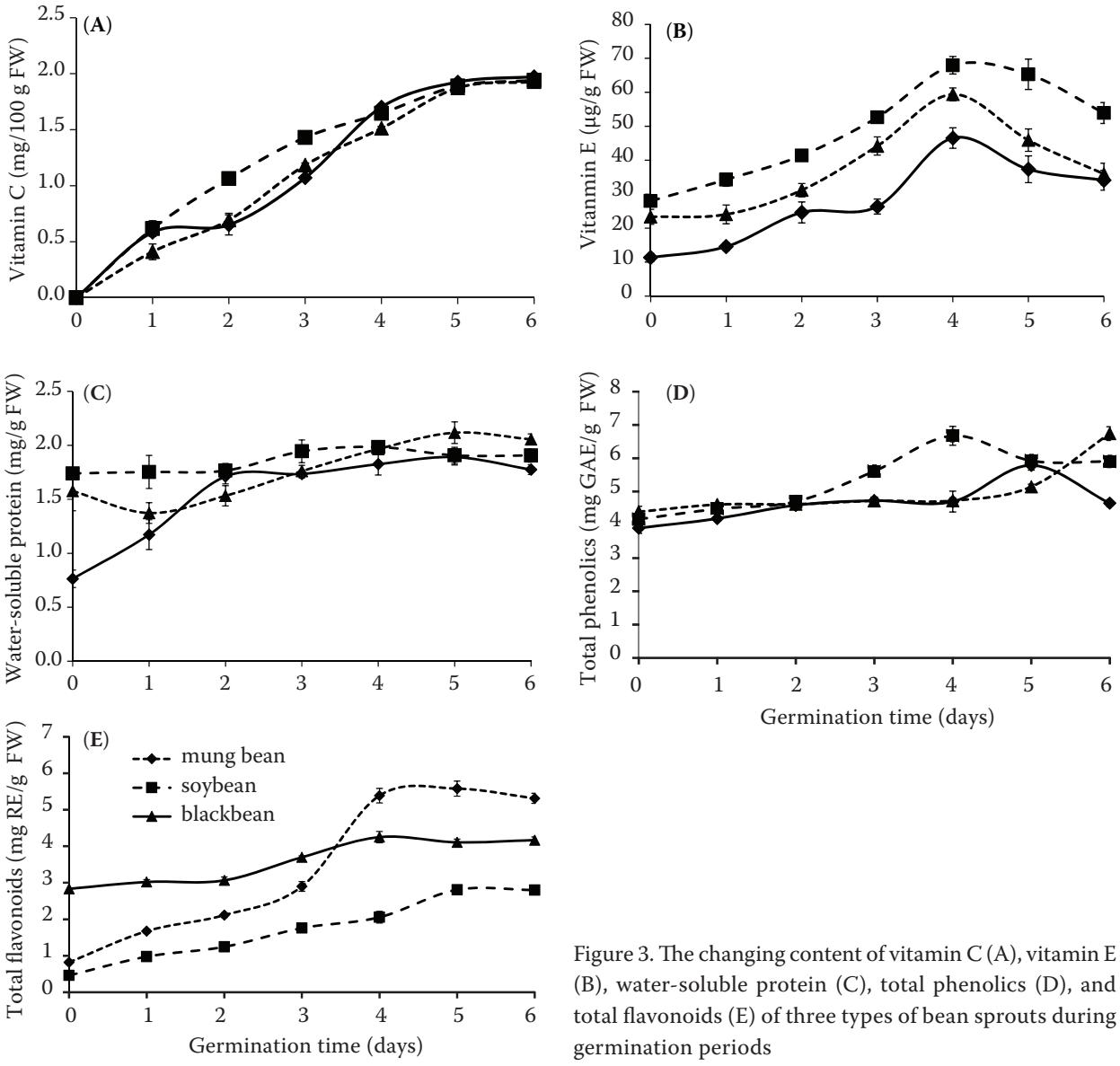

Figure 3. The changing content of vitamin C (A), vitamin E (B), water-soluble protein (C), total phenolics (D), and total flavonoids (E) of three types of bean sprouts during germination periods

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Table 2. Correlation coefficient (*r*) of three types of sprouts

		TPC	TFC	TRP	HOSC	RDSC	ABTS
Mung bean	TPC	1.000	0.758*	0.378	0.646	0.637	0.956**
	TFC		1.000	0.102	0.708	0.840*	0.723
	TRP			1.000	0.722	0.407	0.597
	HOSC				1.000	0.905**	0.800*
	RDSC					1.000	0.740
	ABTS						1.000
Soybean	TPC	1.000	0.837*	0.714	0.755*	0.760*	0.699
	TFC		1.000	0.502	0.497	0.639	0.573
	TRP			1.000	0.832*	0.827*	0.983**
	HOSC				1.000	0.903**	0.771*
	RDSC					1.000	0.840*
	ABTS						1.000
Black bean	TPC	1.000	0.596	0.472	–0.010	0.112	0.585
	TFC		1.000	0.785*	0.652	0.669	0.751
	TRP			1.000	0.794*	0.857*	0.917**
	HOSC				1.000	0.922**	0.733
	RDSC					1.000	0.840*
	ABTS						1.000

TPC – total phenolics; TFC – total flavonoid content; TRP – total reducing power; HOSC – hydroxyl radical scavenging capacity; RDSC – relative DPPH radical scavenging capacity; ABTS – 2,2'-azino-bis(3-ethylbenzthiazoline-6)sulfonic acid; correlations between the obtained data were run using the standard Pearson correlation; *$P < 0.05$; **$P < 0.01$

5.79 mg GAE/g, almost 1.20 fold of the seeds. Guo *et al.* (2012) reported that the TPC of mung bean dramatically increased to 996.4 mg GAE/100 g DW (4.5 fold of the seeds) during 9-day germination. Huang *et al.* (2014) found that the TPC range of germinated mung bean and soybean was 0.41–1.2 and 0.32–0.44 mg GAE/g DW during 5-day germination. The contents of TPC were different depending on the species of beans and the extraction method. Zhang *et al.* (2013) studied ten commercial samples of mung bean and the highest TPC of acetone-water (1 : 1, v/v) extracts was 5.07 mg GAE/g.

Germination not only transforms beans into a healthier food for consumption (López *et al.* 2013), but also improves their flavonoid content (Díaz-Batalla *et al.* 2006). The TFC of sprouts was increased significantly during germination (Figure 3E). Mung bean had the fastest growth rate on the third day and reached the highest value of 5.58 mg RE/g FW on the fifth day. Compared with mung bean, the TFC of black bean had a flat growth rate and reached the highest content of 4.25 mg RE/g FW. However, the TFC of soybean was relatively lower than that of the other two types of beans and had a peak content

Table 3. Relative contribution rate (%) to antioxidant activity

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mung bean	TPC	57.56	82.94	64.71	55.76	60.13	52.82	63.53
	TFC	8.87	7.37	6.62	7.60	15.33	11.32	14.89
Soybean	TPC	53.34	48.84	42.38	47.88	54.69	54.66	63.06
	TFC	3.78	2.36	2.49	3.33	3.74	5.76	6.64
Black bean	TPC	51.64	49.22	43.48	42.69	41.74	49.87	55.63
	TFC	6.17	7.18	6.41	7.43	8.35	8.85	7.64

doi: 10.17221/434/2015-CJFS

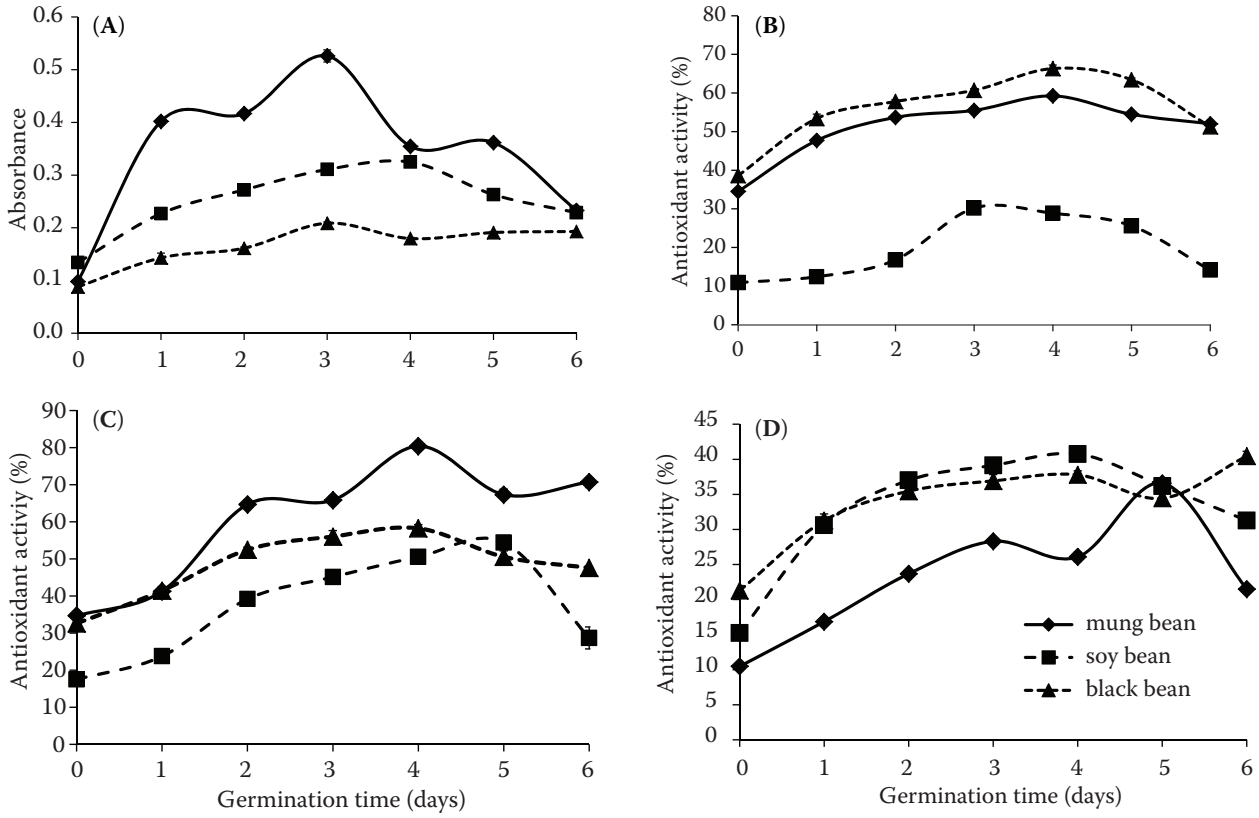

Figure 4. The total reducing power (**A**), hydroxyl radical scavenging capacity (**B**), relative DPPH radical scavenging capacity (**C**), and ABTS radical scavenging capacity (**D**) of three types of bean sprouts during germination periods

of 2.81 mg RE/g FW on the fifth day. This tendency was similar like in Kim *et al.* (2012), who found that the TFC of germinated mung bean was almost three times higher compared to seed. Pajak *et al.* (2014) reported that the TFC of 5 days germinated mung bean was 13.7 mg QE/g, which was about a 50% increase compared to ungerminated beans.

Comprehensive evaluation. The TOPSIS analysis revealed the rank R_j of the total bioactive compound content for mung bean, soybean, and black bean seeds during germination (Table 1): black bean > soybean > mung bean. As the germination is progressing, the total bioactive component content of bean sprouts increases. On the same day of germination period, soybean had the highest bioactive component content compared to the other two types of bean, and black bean had the lowest one. The results demonstrated that the TOPSIS method can be efficiently utilised for the assessment of the total bioactive compound content of different bean, thus providing a data-based foundation for manufacturing companies to optimally select the germination period.

Antioxidant activity. Legumes are gaining increasing attention around the world due to the beneficial effects on human health, for example antioxidants of legumes play an important role in limiting the effects of cellular and molecular damage by reducing reactive oxygen species (Luthria & Pastor-Corrales 2006; Rebello *et al.* 2014). Legume germination has also been suggested as a powerful strategy to increase total antioxidant activity (Fernandz-Orozco *et al.* 2006). The antioxidant activity of different samples evaluated by TRP, HOSC, RDSC, and ARSC is reported in Figure 4. It is evident that germinated seeds show a good antioxidant potential in terms of TRP, HOSC, RDSC, and ARSC values. For example, the TRP, RDSC, and ARSC values of mung bean sprouts were significantly increased by 430.0, 131.8, and 249.4% on Day 3, 4, and 5, respectively. The highest values of soybean and black bean were increased by 146.2/133.3% (TRP), 211.0/78.6% (RDSC), and 167.6/90.1% (ARSC), while changes of antioxidant activity varied between and within species. Guo *et al.* (2012) reported that the antioxidant activity of mung bean sprouts was 6 times higher than in the seeds. Pajak *et al.* (2014) observed a more than 10-fold increase of antioxidant activity against ABTS and DPPH radicals in mung bean after 5-day germina-

doi: 10.17221/434/2015-CJFS

tion. However, Guajardo-Flores *et al.* (2013) found that the germination did not affect the antioxidant activity (oxygen radical absorbance capability) of black bean extracts.

The data also clearly show that during germination mung bean, soybean, and black bean sprouts behave differently in all these assays used. For instance, mung bean sprouts showed higher TRP and RDSC values and lower ARSC value than soybean and black bean, similar HOSC to black bean. Soybean exhibited the lowest HOSC value when compared to mung and black beans during germination. This could be because of antioxidant components being responsible differently for different radical scavenging activities. Interestingly, the antioxidant activity of seeds was generally found to increase at first and then to decrease during the germination period, the highest antioxidant activity occurred in 3–5 days of germination. Consequently, the optimum germination time for reaching high antioxidant activity in mung bean, soybean, and black bean is between 3 and 5 days.

Correlation analysis. Pajak *et al*. (2014) reported that the increment of antioxidant activity during the germination of mung bean seems to be related with changes in the content of antioxidants, such as vitamins and polyphenols. The correlation coefficient between TPC and TFC as antioxidants and antioxidant capacity are shown in Table 2. TPC correlated with ARSC significantly (R^2 = 0.956, P < 0.01) in mung bean, which was consistent with Zhang *et al.* (2013), who proved the significant correlation between TPC and HOSC (R^2 = 0.755, P < 0.05) and RDSC (R^2 = 0.760, P < 0.05) of soybean during germination. However, TPC was not significantly correlated with the antioxidant activity of black bean. Huang *et al.* (2014) also proved that the antioxidant activity was not correlated with TPC of germinated mung bean and soybean. A possible reason could be explained by Randhir and Shetty (2007), who found out that the antioxidant activity might be determined not only by the total phenolic content but also by the qualitative characteristics of phenolics.

Flavonoids were proved to have a significant correlation with antioxidant activity by Hossain and Rahman (2011) and Kou *et al.* (2015). The same result was observed in our study, TFC was significantly correlated with RDSC (R^2 = 0.840, P < 0.05) of mung bean and TRP (R^2 = 0.785, P < 0.05) of black bean. However, there is no obvious correlation between TFC and antioxidant activity in soybean, which might be caused by the antioxidants depending not only on the concentration, but also on the structure and the interaction between the flavonoids.

Relative contribution to total antioxidant activity. Based on the data in the present work, it was possible to calculate the relative contribution of TPC and TFC as antioxidants to the antioxidant activity of germinated seeds. The TEAC value was converted from the results of ABTS, which represent the total antioxidant activity of samples.

In the germination process of mung bean, it was found that TPC (52.82–82.94%) and TFC (6.62–14.89%) contributed the most to the total antioxidant activity. The contribution of TPC decreased sharply on the second day, and then it slightly varied between 55.76 and 64.71%. As to soybean and black bean, there was a similar tendency of contribution like in mung bean. The highest contribution rate of soybean TPC occurred on Day 6 (63.60%), at the same time the peak contribution rate of black bean was 55.63%. As the germination progressed, the sum of relative contributions of TPC and TFC reached a maximum of 90.31, 69.7, and 63.27% corresponding to mung bean, soybean, and black bean, indicating that TPC and TFC contributed the highest percentage of total antioxidant activity, which was verified in the same report by Fernandz-Orozco *et al.* (2008).

CONCLUSION

The germination promotes the bioactive compound content of mung bean, soybean, and black bean. Vc increased from zero, SPC and TFC changed significantly and exhibited un upward trend while Ve and TPC increased first and then decreased. The antioxidant activities of the three types of beans varied according to different test methods (TRP, HOSC, ARSC, and RDSC) and their highest value occurred in 3–5 days of germination. The analysis of the relative contribution to antioxidant activity revealed that TPC and TFC made the highest contribution. In addition, the comprehensive evaluation of bioactive compounds showed that soybean sprouts has higher content of total bioactive compounds than mung bean and black bean. On the whole, this study indicates the germinated seeds are valuable sources of natural bioactive compounds and antioxidants, the best germination period of mung bean, soybean, and black bean was 3–5 days when both the bioactive compound content and antioxidant activity reached their maximum values.

doi: 10.17221/434/2015-CJFS

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Received: 2015–09–08
Accepted after corrections: 2016–01–20

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