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Yield and quality characteristics of Korean red bean sprouts produced with different time of seed soaking

Ji-Hye Mun[1] · Il-Doo Kim[2] · Sanjeev Kumar Dhungana[3] · Yong-Sung Park[1] · Jeong-Ho Kim[4] · Dong-Hyun Shin[1]

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Abstract This study was conducted to investigate the yield and quality characteristics of red bean sprouts of three cultivars (Arari, Geomguseul, and Chungju) soaked in water for 0, 6, 12 or 24 h. The sprout yields of 'Arari' and 'Geomguseul' on day 7 were highest with the seeds soaked for 12 h. For 'Chungju', the yields from the seeds soaked for 12 and 24 h were not significantly ($p > 0.05$) different. Longer hypocotyls and shorter roots, which are also desirable characteristics of good sprouts, were also found in the sprouts with 12 h of seed soaking. The amounts of total minerals, thiamine, total free amino acids, and total phenols and DPPH radical scavenging potential of sprouts of all cultivars were higher than those of their seeds. This study showed that higher yield and better quality of red bean sprouts could be obtained with the seeds soaked for 12 h.

Keywords Antioxidant activity · Nutritional value · Presoaking time · Red bean sprout · Sprout yield

✉ Dong-Hyun Shin
dhshin@knu.ac.kr

Ji-Hye Mun
jh0007@nate.com

Il-Doo Kim
ildookim@hanmail.net

Sanjeev Kumar Dhungana
sanjeevdhungana@yahoo.com

Yong-Sung Park
pjyjsj3@gmail.com

Jeong-Ho Kim
klab003@gmail.com

[1] School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

[2] International Institute of Research and Development, Kyungpook National University, Daegu 41566, Korea

[3] National Institute of Crop Science, Rural Development Administration, Miryang 50424, Korea

[4] Department of Green Technology Convergence, Konkuk University, Chungcheongbuk-do 27478, Korea

Introduction

Adzuki bean or red bean (*Vinga angularis*) is an important source of protein, starch, mineral elements, and vitamins (Gohara et al., 2016). It is also called as a 'weight-loss bean' because of its low calorie and fat, digestible protein, and enough bioactive compounds contents (Amarowicz et al., 2008; Kitano-Okada et al., 2012). It is widely used in a number of food products, such as paste in pastries, desserts, cake, porridge, adzuki rice, jelly, adzuki milk, ice cream (Lestari et al., 2014). The bean has been reported to contain considerable amounts of flavonoids, including kaempferol (Amarowicz et al., 2008). Kaempferol is useful in tumor necrosis factor-related apoptosis-inducing ligand-based treatments for cancer (Yoshida et al., 2008). It shows hypoglycemic, hepatoprotective, anti-obesity, and anti-inflammatory properties (Kitano-Okada et al., 2012). Since the red bean is rich in thiamine, it could be mixed with cooked-rice to supply thiamine which is greatly lost in the polished rice. Moreover, the red beans are rich in essential amino acids, especially lysine which is the limited amino acid in rice, making them more appropriate in the regions where rice is the main food.

Sprout production is a simple and an inexpensive technique to enhance the nutritional value of seeds. The sprouting of seeds can lead to increase in nutritional quality of proteins (Gulewicz et al., 2008) and decrease in caloric

content (Vidal-Valverde et al., 2002) and anti-nutritional factors like enzyme inhibitors, making the sprouts more beneficial for human health (Luo et al., 2013). The germinated seeds are a potential source of ascorbic acid, riboflavin, choline, thiamine, pantothenic and nicotinic acids, tocopherols and different free amino acids (Sangronis and Machado, 2007). Seed spouting has been identified as a suitable technology to supply nutritional vegetables year-round. In addition, various studies have been reported showing the possibility of enhancing the nutritional value of sprouts and supplying different essential elements to help fight against the nutritional deficiencies (Wei et al., 2013), especially in the poor and developing countries.

Red bean is a popular legume in Asia, especially in China, Japan, and Korea (Tavares et al., 2016). In addition to the application of red beans in different food items (Lestari et al., 2014), they could be used to produce nutritious sprouts as other grains. The dietary and health concerns of people are increasing and consumers' demands have also been diversified these days. Although a large number of studies have been conducted on the quality and nutritional value of sprouts of other grains like soybean, very limited studies have been carried out on the nutritional and functional properties of the red bean sprouts of different cultivars. Considering the nutritional values of red beans and consumers' attitude towards new food materials, this study aimed to investigate the quality characteristics and antioxidant potential of red bean sprouts.

Materials and methods

Seed materials

Red bean (*Vinga angularis*) seeds of three cultivars Arari, Geomguseul, and Chungju were obtained from National Institute of Crop Science in Miryang, Korea. These cultivars were among the popularly grown red beans of the region.

Chemicals and reagents

1,1-diphenly-2-picrylhydrazyl (DPPH), Folin–Ciocalteau-reagent, isoflavone standards ($\geq$ 95% purity) dimethyl sulfoxide (DMSO), and pyrogallol were purchased from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO, USA) and amino standards from Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All the other chemicals used in this study were of analytical grades.

Cultivation of red bean sprout

Red bean sprouts were grown by following the method described earlier (Kim et al., 2016) with some modifications. Twenty-five grams of intact seeds in three replications for four soaking treatments were washed and steeped in tap water for 0, 6, 12, and 24 h. After soaking the seeds for the specified periods, they were kept into sprout cultivators (Chungsiru SC-9000A, Shinchang Inc., Osan, Korea) for the sprout cultivation. The seeds and sprouts were sprinkled with tap water for 1 min every 20 min. Red bean sprouts were grown at room temperature (20 $\pm$ 2 °C) for 7 days.

Measurement of growth parameters and preparation of sprout powders

Sprout yields (total weight of red bean sprouts) in each batch of the four soaking time points were recorded every day until 7 days. For the measurement of length and thickness of hypocotyls, 30 sprouts were randomly selected from each batch on day 7.

Since 12 h of seed soaking was identified as the optimum time for the production of red bean sprouts, sample powders for physicochemical studies were prepared with the same. The fresh sprouts were stored at − 70 °C for 24 h and subjected to freeze drying. The freeze-dried sprouts were powdered using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea) and strained using a 100-mesh sieve.

Determination of mineral content

The concentrations of mineral elements in the sample powders were determined using an inductively coupled plasma atomic emission spectrometer (ICP AES; Varian Inc., Victoria, Australia) by following an earlier method (Skujins, 1998). Briefly, the sample powder (0.5 g) was digested in a mixture of 15 mL HNO_3 (65%) and 2 mL H_2O_2 (35%). The mixture was diluted with an equal volume of distilled water. The instrument was calibrated using known standards for each mineral.

Determination of vitamin B1 (thiamine) content

The thiamine contents of red bean sprout powders were determined by following a method described earlier (Eitenmiller and Landen, 2016) with some modifications. A mixture of homogenized samples was prepared using the Thiochrom fluorescence colorimetric method and then added a 5-mM sodium hexanesulfonate as the pretreatment solution, and extracted for 30 min using an ultrasonic shaker. The volume of extracted solution was adjusted to

50 mL, centrifuged at 1660×*g*, and the supernatant was filtered and analyzed by HPLC (Shiseido, Nanospace SI-2, Tokyo, Japan). The chromatographic conditions were set as: column (250 × 4.6 mm and 5 µm particles, Shiseido Co., Tokyo, Japan) which was set at 30 °C. The mobile phase consisted of a solution of sodium hexanesulfonate:methanol (35:65 v/v) pumped at the flow rate of 0.6 mL/min. A 10-µL of injection volume was set and detection was made at 270 nm.

Determination of DPPH free radical scavenging potential

The DPPH free radical scavenging potential was determined following the method described by Blois (1958). Sprout powder was extracted in absolute methanol at room temperature for 6 h. The concentration of sample extract was 10% (w/v). After 6 h of extraction, the mixture of sprout powder and methanol was centrifuged at 1660×*g* for 10 min and then the supernatant was filtered through syringe filter (2 µm). Fresh DPPH solution (0.1%, w/v) was prepared in methanol. An equal volume (100 µL) of sample extract and freshly prepared DPPH solution was mixed in microplate wells and left in dark for 30 min. The absorbance was measured at 517 nm (Multiskan GO Microplate Spectrophotometer, ThermoFischer Scientific, Vantaa, Finland). The DPPH radical scavenging activity was calculated based on the absorbance (A) values of reaction mixtures using the following equation:

$$\text{DPPH radical scavenging potential } (\%) = [1 - (A - A_b)/A_o] \times 100$$

where A is the absorbance of sample and DPPH, and A_b is the absorbance of sample and methanol, and A_o is the absorbance of DPPH and methanol.

Determination of total polyphenol content

Total polyphenols in the sample powders were measured following the Folin–Ciocalteau method (Singleton, 1999). The sample extract was prepared as in the DPPH analysis. Fifty microliters of sample extract was added to 250 µL of 1 N Folin–Ciocalteau reagent. After 1 min, 750 µL of 20% (w/v) aqueous Na_2CO_3 was added to the mixture, and the final volume was made to 5 mL with distilled water. After 2 h of incubation at room temperature in dark, absorbance values of the reaction mixtures were measured at 750 nm using a microplate spectrophotometer (Multiskan GO, ThermoFisher Scientific). Total polyphenols were determined as gallic acid equivalent (µg GAE/g dry sample).

Determination of total flavonoids content

The total flavonoid content was evaluated as described by Michalska et al. (2007) with some modifications. In brief, 100-µL sample extracts (as for the DPPH and total polyphenols assay) were mixed with 50 µL of 5% (w/v) aqueous $NaNO_2$. The mixture was incubated at room temperature for 6 min. Subsequently, 300 µL of 10% (w/v) aqueous $AlCl_3$ was added and incubated for another 5 min. After the incubation, 1 mL of 1 M NaOH was added to the mixture, vortexed, and the absorbance was measured at 510 nm using a microplate spectrophotometer (Multiskan GO, ThermoFisher Scientific). The amount of total flavonoids contained in the sample powder was calculated based on the standard curve plotted using quercetin and the amount was expressed as quercetin equivalent (QE).

Amino acid analysis

Amino acid composition of sprout powder was analyzed using an automatic amino acid analyzer (Biochrom-20, Pharacia Biotech Co., Stockholm, Sweden) by following the procedure of Je et al. (2005) with some modifications. Sample powder (1 g) was hydrolyzed using 6 N HCl (10 mL) in a sealed-vacuum ampoule at 110 °C for 24 h. The hydrochloric acid in the hydrolyzed sample was removed using a rotary evaporator, and the final volume was made of 5 mL with 0.2 M sodium citrate buffer (pH 2.2). The sample was passed through a C18 Sep Pak (Waters Co., Milford, MA, USA) cartridge and filtered through a 0.22 µm membrane filter (Millipore, Billerica, MA, USA).

For determination of free amino acids, 1.5 g of sample powder was homogenized (12,000 rpm, 2 min) with 10 mL of ice-cold 6% (v/v) perchloric acid in an ice bath using an ACE homogenizer (Nissei AM-7, Nihonseikei Kaisha Ltd., Tokyo, Japan) and then incubated for 30 min in ice before centrifuging at 4600×*g* for 15 min. The supernatant was filtered through a filter paper (Whatman No. 41). The filtrate pH (7.0) was adjusted using KOH solution (33%, w/v), and centrifuged at 4600×*g* for 10 min to remove the precipitate of potassium perchlorate. pH of the supernatant was adjusted to 2.2 with a 10 M HCl and the final volume was brought to 50 mL with distilled water. A sample aliquot (2 mL) was mixed with 1 mL of lithium citrate buffer (pH 2.2). Free amino acids were then analyzed using the same amino acid analyzer.

Statistical analysis

Data were subjected to analysis of variance using SAS 9.4 (SAS Institute, Cary, NC, USA). The significant differences between means at $p < 0.05$ were identified using

Tukey test. Average values are three replications are reported unless otherwise mentioned.

Results and discussion

Growth characteristics

The sprout yields and growth characteristics of three cultivars significantly varied with the duration of seed soaking (Tables 1, 2). The increase in sprout yield of 'Arari' (65%) and 'Geomguseul' (90%) on day 7 was significantly higher with the seeds soaked for 12 h as compared to the seeds soaked for 0 h. However, the increase in sprout yield in 'Chungju' was different from the other two cultivars. There were no significant differences between the sprout yields obtained from the seeds soaked for 0 and 6 h; however, 32 and 41% yield increments were found in 'Chungju' with the seeds soaked for 12 and 24 h, respectively.

The mean hypocotyl length and thickness and root length of the sprouts of three cultivars produced after seed soaking for different durations were significantly different except for the root length and hypocotyl thickness of RB-G and root length of RB-C (Table 2). The length of sprout hypocotyls in three cultivars was, generally, increased with the duration of seed soaking. RB-C (13.93 cm) produced the longest hypocotyl followed by RB-G (12.06 cm) and RB-A (11.33 cm). However, the hypocotyl thickness and root length of RB-G were the highest among the sprouts of three cultivars.

Generally, a widely practiced seed soaking time for sprout production is 24 h. However, in RB-A and RB-G, 12 h of soaking produced significantly higher amount of sprouts than the other soaking times. Therefore, the optimum seed soaking time for the sprout production of these two cultivars was found 12 h. The optimum seed soaking time for 'Chungju' was 12 or 24 h and which could be opted depending on the sprout growing conditions. Moreover, relatively longer hypocotyls and shorter roots, which are some of the desirable characteristics of good sprouts, were found with 12 h of seed soaking in all three cultivars.

The variation in sprout yield among cultivars might be due to the genetic variation which significantly affects the sprout yield, including whole sprout length, root length, and hypocotyl length (Jeong et al., 2007). Seed soaking time may vary with cultivars which also affects the quality of sprouts (Devi et al., 2015).

Mineral content

The mineral content of red bean seeds and sprouts of three cultivars produced after soaking for 12 h were significantly different on day 7 (Table 3). The most abundant mineral found in the seeds and sprouts was K. The seeds of RB-C (12,414 mg/kg) had significantly lower amount of K compared to the seeds of other two cultivars (12,905–13,136 mg/kg). However, K contents in the sprouts of three cultivars were not significantly different. Mg and Ca contents of the sprouts of RB-G (1440.62 and 2242.8 mg/kg) was significantly higher than that of RB-A (1366.56 and 2098.70 mg/kg) and RB-C (1349.27 and 1930.7 mg/kg), respectively. Both the seeds and sprouts of RB-A showed significantly higher Mn, Fe, and Zn contents than in the other two cultivars. The total mineral contents of sprouts were higher than those of seeds of the red bean cultivars. The amount of total mineral contents of seeds of the red bean cultivars were in order of RB-A > RB-G > RB-C, whereas that of sprouts were slightly different (RB-G > RB-A > RB-C). The results showed that a higher level of mineral in the red beans can be obtained with sprouts than the red bean seeds.

The amounts of several mineral elements found in the seeds were increased in the sprouts. The higher amount of minerals in the red bean sprouts than in the seeds might be due to the physiological changes in the seeds during germination which enhances the nutritive values (Ghavidel and Prakash, 2007). Mineral elements Mg, K, and Ca are reported to have beneficial health effects against hypertension (Houston and Harper, 2008). Fe plays roles in oxygen transport, energy metabolism, mitochondrial respiration, DNA synthesis, and cellular growth and differentiation (Ganz, 2013). Zn is associated with growth, development, differentiation, DNA synthesis, RNA transcription, and cellular apoptosis (MacDiarmid et al., 2000).

Thiamine (vitamin B1) content

A significant difference in the thiamine content in seeds and sprouts of three red bean cultivars were observed (Table 4). RB-G seeds and sprouts contained significantly high amounts of thiamine compared to those of the other two cultivars. However, an interesting point was that the increment in the amount of thiamine content from seed to sprout greatly varied in the three cultivars. RB-G which had the highest amount of thiamine content in seeds and sprouts showed 1.66 times increment from seeds (385.99 mg/kg sample) to the sprouts (639.01 mg/kg). However, the seeds (143.43 mg/kg) of RB-C which contained the lowest amount of thiamine produced 4.33 times higher thiamine in the sprouts (620.85 mg/kg). There were no significant difference in the thiamine contents in the seeds and sprouts of RB-A and RB-C.

There is little information available about the effect of germination on the thiamine content of red beans. A decrease in thiamine content in rapeseeds after germination has been reported (Zieliński et al., 2006). However, the

Table 1 Changes in sprout yields of three red bean cultivars until 7 days produced with 0, 6, 12 or 24 h of seed soaking

Cultivar[1]	Seed soaking time (h)	Red bean sprout yield (g/batch) until 7 days						
		1	2	3	4	5	6	7
RB-A	0	41.97 ± 1.25[2]c	48.73 ± 2.81b	48.80 ± 3.25d	55.92 ± 2.88c	58.22 ± 3.85d	62.54 ± 3.11d	63.20 ± 3.16d (100%)[3]
	6	46.01 ± 2.20b	52.08 ± 2.25b	57.40 ± 3.10c	62.16 ± 3.15c	69.60 ± 4.88c	73.84 ± 3.57c	77.43 ± 4.84c (123%)
	12	52.74 ± 1.89a	59.48 ± 3.16a	66.00 ± 4.10a	73.20 ± 3.85a	81.35 ± 6.10a	88.22 ± 5.13a	95.01 ± 6.18a (165%)
	24	52.02 ± 1.66a	58.43 ± 2.88a	61.97 ± 3.88b	69.62 ± 3.19b	76.16 ± 5.55b	82.96 ± 4.87b	90.26 ± 3.67b (156%)
	Mean	48.19 ± 5.13	54.68 ± 5.14	58.54 ± 7.38	65.23 ± 7.72	71.33 ± 9.98	76.89 ± 11.26	81.48 ± 14.27
RB-G	0	35.28 ± 2.11c	44.46 ± 1.62c	47.94 ± 2.90c	50.53 ± 2.88c	53.27 ± 2.21d	56.29 ± 1.38d	57.74 ± 3.46d (100%)
	6	47.90 ± 0.88b	49.55 ± 2.11b	54.56 ± 3.20b	61.04 ± 3.11b	67.34 ± 3.54c	73.77 ± 2.81c	84.94 ± 4.54c (147%)
	12	52.95 ± 2.10a	53.70 ± 2.15a	61.73 ± 3.88a	70.50 ± 2.95a	80.61 ± 3.88a	92.37 ± 3.47a	109.76 ± 4.88a (190%)
	24	47.83 ± 1.81b	49.28 ± 1.89b	55.81 ± 2.85b	63.81 ± 3.75b	73.12 ± 2.18b	82.40 ± 3.07b	97.33 ± 3.86b (169%)
	Mean	45.99 ± 7.53	49.25 ± 3.78	55.01 ± 5.66	61.47 ± 8.30	68.59 ± 11.57	76.21 ± 15.30	87.44 ± 22.24
RB-C	0	48.64 ± 1.88c	63.96 ± 3.88c	71.04 ± 3.90b	86.57 ± 4.38b	95.50 ± 5.50c	100.81 ± 5.47c	109.89 ± 5.87b (100%)
	6	58.81 ± 1.10b	68.93 ± 2.10b	76.33 ± 4.10b	90.46 ± 4.10a	100.96 ± 6.10b	110.35 ± 6.54b	120.93 ± 5.66b (110%)
	12	60.98 ± 3.13a	71.49 ± 4.13a	80.35 ± 6.88a	92.08 ± 6.28a	103.76 ± 4.88b	116.50 ± 5.89b	133.85 ± 6.91a (132%)
	24	63.16 ± 2.10a	75.09 ± 1.83a	85.65 ± 5.90a	96.83 ± 4.10a	112.13 ± 3.79a	124.80 ± 4.87a	139.38 ± 5.87a (141%)
	Mean	57.90 ± 6.42	69.87 ± 4.68	78.34 ± 6.19	91.49 ± 4.25	103.09 ± 6.94	113.12 ± 10.12	126.01 ± 13.24

[1]RB-A, 'Arari'; RB-G, 'Geomguseul'; RB-C, 'Chungju'

[2]Values are mean ± standard deviation of three replications and the different letters in the same column for each cultivar are significantly different at $p < 0.05$

[3]Percentage, for sprout weight, in parenthesis denotes the variation in sprout yields in the respect to sample cultivated at 0 day

Table 2 Length and thickness of hypocotyls and roots of red bean sprouts cultivated for 7 days after seed soaked for 0, 6, 12 or 24 h

Part	Seed soaking time (h)	Cultivar[1]		
		RB-A	RB-G	RB-C
Hypocotyl				
Length (cm)	0	7.55 ± 1.44[2]c	8.14 ± 1.39c	12.18 ± 1.40b
	6	11.55 ± 1.11b	11.68 ± 0.62b	12.93 ± 1.20b
	12	12.79 ± 0.73ab	13.63 ± 0.72a	15.22 ± 1.09a
	24	13.43 ± 1.11a	14.78 ± 0.98a	15.39 ± 1.37a
	Mean	11.33 ± 2.64	12.06 ± 2.91	13.93 ± 1.62
Thickness (mm)	0	1.36 ± 0.12b	1.70 ± 0.24a	1.34 ± 0.11b
	6	1.57 ± 0.22a	1.81 ± 0.20a	1.82 ± 0.25a
	12	1.54 ± 0.10a	1.93 ± 0.24a	1.66 ± 0.17a
	24	1.46 ± 0.27a	1.77 ± 0.26a	1.53 ± 0.11ab
	Mean	1.48 ± 0.09	1.80 ± 0.10	1.59 ± 0.20
Root				
Length (cm)	0	7.60 ± 1.95b	9.93 ± 1.89a	8.50 ± 1.87a
	6	9.13 ± 2.83ab	10.06 ± 2.51a	7.79 ± 2.46a
	12	7.60 ± 2.13b	11.01 ± 1.54a	8.71 ± 2.27a
	24	11.10 ± 1.49a	11.71 ± 2.36a	11.80 ± 2.07a
	Mean	8.86 ± 1.66	10.68 ± 0.84	9.20 ± 1.78

[1]RB-A, 'Arari'; RB-G, 'Geomguseul'; RB-C, 'Chungju'

[2]Values are mean ± standard deviation of three replications and the different letters in the same column for the same cultivar and sprout part are significantly different at $p < 0.05$

Table 3 Mineral contents (mg/kg) of red bean seeds and sprouts cultivated for 7 days after seed soaked for 12 h

Element	Cultivar[1]					
	RB-A		RB-G		RB-C	
	Seed	Sprout	Seed	Sprout	Seed	Sprout
Na	36.20 ± 0.8[2]b	165.86 ± 2.7b	40.87 ± 0.7a	212.87 ± 5.5a	17.05 ± 0.7c	206.19 ± 5.2a
Mg	1158.65 ± 23.8a	1366.56 ± 28.6b	1137.54 ± 28.0a	1440.62 ± 27.7a	1021.39 ± 21.3b	1349.27 ± 22.1b
K	13,136 ± 197a	14,595 ± 195a	12,905 ± 224a	14,629 ± 338a	12,414 ± 219b	14,704 ± 291a
Ca	786.82 ± 24.6b	2098.7 ± 48.9b	826.47 ± 31.5a	2242.8 ± 46.6a	851.19 ± 25.3a	1930.7 ± 84.0b
Mn	12.65 ± 0.1a	14.21 ± 0.1a	8.94 ± 0.1b	9.79 ± 0.0b	9.05 ± 0.0b	10.71 ± 0.1b
Cu	9.08 ± 0.0b	11.56 ± 0.0a	12.35 ± 0.1a	11.03 ± 0.1a	8.62 ± 0.0b	7.25 ± 0.0b
Zn	51.26 ± 0.1a	63.19 ± 0.3a	47.23 ± 0.4b	40.90 ± 0.2b	26.77 ± 0.2c	28.80 ± 0.3c
Fe	90.13 ± 0.7a	113.67 ± 0.6a	57.44 ± 0.2c	63.31 ± 0.2b	54.35 ± 0.1b	62.03 ± 0.0b
Mo	8.90 ± 0.0c	11.40 ± 0.1b	16.14 ± 0.1a	21.03 ± 0.2a	14.64 ± 0.1b	19.79 ± 0.3a
Total	15,289.69	18,440.15	15,051.98	18,671.35	14,417.06	18,318.74

[1]RB-A,'Arari'; RB-G, 'Geomguseul'; RB-C, 'Chungju'

[2]Values are mean ± standard deviation of two replications and the different letters in the same row for same component (seed or sprout) column are significantly different at $p < 0.05$

results of the present study was in agreement with a report in which thiamine content in the sprouts of wheat, soybean, and alfalfa were increased by about 2.6, 4, and 10 times than in the seeds (Plaza et al., 2003). Thiamine plays different body's functions, such as in immune and anti-inflammatory functions and gene regulation (Manzetti et al., 2014). Thiamine deficiency causes Wernicke's encephalopathy, an acute neuropsychiatric syndrome (Manzo et al., 1994). The present study showed that red bean sprouts could be a potential source of dietary thiamine.

Table 4 DPPH free radical scavenging activity and total polyphenol, flavonoid, and thiamine contents of red bean seeds and sprouts cultivated for 7 days after seed soaked for 12 h

Item	Cultivar[1]					
	RB-A		RB-G		RB-C	
	Seed	Sprout	Seed	Sprout	Seed	Sprout
DPPH (% inhibition)	77.22 ± 1.8[2]a	89.35 ± 0.1a	73.86 ± 3.0b	85.82 ± 1.3b	70.32 ± 0.6b	81.20 ± 1.2c
Total polyphenols (μg GAE/g)[3]	100.56 ± 41.3b	487.37 ± 73.4a	191.52 ± 25.5a	445.15 ± 71.8a	132.56 ± 14.5b	465.63 ± 24.1a
Total flavonoids (mg QE[4]/100 g)	1355 ± 73a	1452 ± 15a	1394 ± 51a	1256 ± 156b	1222 ± 79b	1106 ± 74c
Thiamine (mg/kg)	177.18 ± 23.9b	554.89 ± 59.8b	385.99 ± 41.3a	639.01 ± 39.2a	143.43 ± 35.1b	620.85 ± 29.1b

[1]RB-A,'Arari'; RB-G,'Geomguseul'; RB-C,'Chungju'

[2]Values are mean ± standard deviation of three replications and the different letters in the same row for same components (seed or sprout) are significantly different at $p < 0.05$

[3]*GAE* gallic acid equivalents

[4]*QE* quercetin equivalents

Antioxidant potential

The total polyphenol and flavonoid contents and DPPH radical scavenging activity were considered to evaluate the antioxidant potentials of the sprouts. The DPPH free radical scavenging potentials and total polyphenolic content in the sprouts of all three cultivars were remarkably higher than those in their seeds (Table 4). However, the seeds of RB-G and RB-C contained higher total flavonoid contents than in their sprouts. The DPPH free radical scavenging potential of the seeds and sprouts of RB-A (77.22 and 89.35%) was significantly higher than those of RB-G (73.86 and 85.82%) and RB-C (70.32 and 81.20%), respectively.

The higher total polyphenol content in the sprouts than in the seeds might be due to the physiological and metabolic changes occurred during the seed germination which increase the phenolic content and antioxidant activity of peanut (Adhikari et al., 2018). There are various enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, vitamin C, vitamin E, polyphenols, and carotenoids. The antioxidant potential of foods is a complex outcome which is affected by various factors including the partitioning properties of particular antioxidants, the oxidation conditions, and the physical state of the oxidizable substrate (Frankle and Meyer, 2000). Therefore, a visible increment in the amount of a particular antioxidant, including total polyphenol content, might not always contribute to higher antioxidant potentials as found in RB-G seeds and RB-A sprouts. The total polyphenol content of RB-G seeds was highest among three cultivars but its DPPH free radical scavenging potentials was less than that of RB-A seeds. Similarly, the DPPH value of RB-A sprout was highest among the sprouts of three cultivars but their total polyphenol contents were not significantly different. The variations in the antioxidant potentials of different samples may be the results of the contributions of several phytochemicals, including polyphenols, flavonoids, vitamins, and carotenoids.

Free amino acid composition

Out of the 36 free amino acids analyzed, a total of 32 were detected at least in the seeds and/or sprouts (Table 5). A total of 8, 8, and 20 were essential, non-essential, and other free amino acids, respectively. The total free amino acid contents of sprouts were 3.77 (RB-A) to 5.22 (RB-C) times higher than that of the seeds. The highest amount of total free amino acids among three cultivars' seeds was found in RB-A (7836.49 μg/g dw) followed by RB-G (7489.91 533.75 μg/g dw) and RB-C (7206.26 μg/g dw). However, the total free amino acid content in the sprouts was in the order of RB-C > RB-G > RB-A. The content of essential amino acids in the sprouts showed 26.37 to 48.23 times increments as compared to in their seeds. The highest amount of essential amino acids was detected in the sprouts of RB-C (13,584.20 μg/g dw) followed by RB-G (13,191.48 μg/g dw) and RB-A (9486.97 μg/g dw). Similarly, the ratio of essential to non-essential amino acids was the highest for RB-C (1.03) followed by RB-G (0.95) and RB-A (0.82) sprouts. The ratio for the seeds ranged from 0.06 to 0.08. The content of γ-Amino-*n*-butyric acid (GABA) in the seeds was 39.66, 26.04, and 46.83 μg/g and in the sprouts was 1051.36, 589.14, and 1311.60 μg/g for RB-A, RB-G and RB-C, respectively. The GABA content in the sprouts was increased by more than 22-fold compared to in their seeds.

Table 5 Free amino acid composition (μg/g) of red bean seeds and sprouts cultivated for 7 days after seed soaked for 12 h

Amino acid	Cultivar[a]					
	RB-A		RB-G		RB-C	
	Seed	Sprout	Seed	Sprout	Seed	Sprout
Essential amino acid						
L-Threonine	47.23 ± 3.11[b]	952.63 ± 46.03	45.49 ± 2.89	1181.66 ± 53.66	44.55 ± 2.91	1164.96 ± 87.13
L-Valine	68.07 ± 6.05	2337.25 ± 174.07	53.73 ± 4.33	2970.12 ± 1.45	66.19 ± 5.22	3196.67 ± 177.31
L-Methionine	15.18 ± 1.03	352.94 ± 28.05	12.94 ± 1.06	490.49 ± 30.05	12.64 ± 1.07	491.09 ± 38.04
L-Isoleucine	34.45 ± 1.06	1424.02 ± 113.94	27.21 ± 1.43	1968.00 ± 143.01	31.65 ± 2.11	2011.53 ± 107.53
L-Leucine	20.44 ± 1.08	1386.14 ± 108.92	17.60 ± 0.88	1923.02 ± 151.18	21.36 ± 1.91	1851.61 ± 100.32
L-Phenylalanine	99.74 ± 6.07	1603.64 ± 88.13	50.17 ± 3.99	2708.63 ± 161.89	83.14 ± 6.00	2728.58 ± 183.07
L-Lysine	74.68 ± 6.02	1430.35 ± 99.03	66.37 ± 5.06	1949.56 ± 139.06	67.63 ± 4.04	2139.76 ± 127.05
L-Histidine	209.60 ± 11.02	2384.04 ± 153.56	217.92 ± 8.73	2781.18 ± 164.03	216.96 ± 13.04	3084.54 ± 204.89
Sub-total	359.79	9486.97	273.51	13,191.48	327.16	13,584.2
Non-essential amino acid						
L-Asparitic acid	636.69 ± 23.66	1378.48 ± 132.70	626.02 ± 49.42	1882.95 ± 0.29	610.29 ± 6.55	1775.71 ± 150.63
L-Serine	38.01 ± 2.01	1700.02 ± 129.93	23.05 ± 3.79	2065.54 ± 173.99	38.87 ± 3.22	2027.32 ± 165.71
L-Glutamic acid	1660.58 ± 83.11	2363.79 ± 201.38	1337.45 ± 117.99	2341.80 ± 211.50	1650.17 ± 138.79	2320.94 ± 183.24
Glycine	48.19 ± 1.62	191.83 ± 28.70	37.65 ± 2.39	240.98 ± 13.46	42.57 ± 4.97	234.12 ± 24.13
L-Alanine	140.53 ± 6.56	1087.94 ± 75.88	104.59 ± 7.73	1005.38 ± 2.01	117.80 ± 11.90	1049.72 ± 73.13
L-Tyrosine	44.79 ± 0.63	989.60 ± 55.55	50.44 ± 4.73	1466.62 ± 108.49	47.38 ± 4.58	1374.51 ± 119.80
L-Arginine	2208.41 ± 121.93	3928.33 ± 264.77	2338.84 ± 150.81	4874.59 ± 301.92	1745.27 ± 124.60	4440.77 ± 297.67
Proline	111.76 ± 8.33	654.26 ± 92.78	50.68 ± 3.03	750.52 ± 81.85	116.32 ± 8.09	836.24 ± 100.59
Sub-total	4777.2	11,639.99	4518.04	13,877.86	4252.35	13,223.09
Other amino acid						
O-Phospho-L-serine	60.79 ± 6.80	102.73 ± 5.11	62.26 ± 4.38	95.11 ± 1.92	57.03 ± 1.86	116.68 ± 2.21
Taurine	92.85 ± 5.01	196.48 ± 8.68	142.52 ± 11.46	207.89 ± 18.54	143.12 ± 10.94	306.99 ± 21.55
O-Phospho ethanol amine	83.24 ± 3.69	266.34 ± 21.46	ND[c]	271.26 ± 1.80	ND	330.99 ± 1.64
L-Sarcosine	49.64 ± 2.87	ND	28.70 ± 1.66	ND	35.41 ± 3.59	ND
L-α-Amino asipic acid	151.39 ± 8.81	437.96 ± 37.98	138.28 ± 8.00	470.70 ± 33.32	142.80 ± 11.86	472.49 ± 24.99
L-Citrulline	ND3)	ND	ND	ND	ND	ND
L-α-Amino-*n*-butyric acid	83.54 ± 5.64	ND	48.94 ± 3.84	ND	68.56 ± 5.83	ND
L-Cystine	268.53 ± 12.64	316.65 ± 31.54	339.43 ± 24.65	331.83 ± 21.46	349.92 ± 34.69	411.94 ± 40.13
Cystathionine	ND	7.40 ± 1.11	20.27 ± 0.73	ND	17.75 ± 1.75	ND
β-Alanine	44.96 ± 3.99	94.57 ± 4.58	48.72 ± 2.48	114.42 ± 4.87	42.14 ± 3.87	130.24 ± 9.18
D,L-β-Amino isobutyric acid	28.88 ± 1.94	30.58 ± 2.76	24.08 ± 1.99	25.94 ± 2.10	24.89 ± 1.88	30.26 ± 1.11
γ-Amino-*n*-butyric acid	39.66	1051.36	26.04	589.14	46.83	1311.60
Ethanolamin	40.66 ± 3.13	196.88 ± 16.54	31.91 ± 3.78	222.37 ± 19.42	36.92 ± 4.54	278.70 ± 25.00
Hydroxylysine	ND	ND	ND	ND	ND	ND
L-Ornithine	25.98 ± 2.63	20.36 ± 2.21	18.88 ± 1.49	21.03 ± 2.92	18.30 ± 1.85	26.36 ± 2.33
1-Methyl-L-histidine	ND	34.78 ± 1.87	ND	50.28 ± 3.44	ND	ND
3-Methyl-L-histidine	1.86 ± 0.52	ND	1.37 ± 0.16	4.73 ± 0.91	1.99 ± 0.36	5.52 ± 1.19
L-Anserine	ND	ND	ND	ND	ND	ND
L-Carnosine	ND	ND	ND	ND	ND	ND

Table 5 continued

Amino acid	Cultivar[a]					
	RB-A		RB-G		RB-C	
	Seed	Sprout	Seed	Sprout	Seed	Sprout
Hydroxy proline	136.54 ± 12.18	96.18 ± 3.87	118.32 ± 7.64	74.30 ± 5.10	179.32 ± 10.13	90.91 ± 6.46
Sub-total	1111.52	2852.27	1049.72	2479.00	1164.98	3512.68
Total free amino acids	7836.49	29,567.08	7489.91	36,070.99	7206.26	37,609.09

[a]RB-A, 'Arari'; RB-G, 'Geomguseul'; RB-C, 'Chungju'

[b]Values are expressed as mean ± standard deviation of two replications

[c]*ND* non-detected

Foods containing high ratios of essential to non-essential amino acids are regarded as ideal foods from the protein deposition point of view (Reeds, 2000). Glutamic acid is a precursor for GABA synthesis in plant tissues (Nikmaram et al., 2017). GABA and glycine are considered to be related to learning and memory; against stroke and neurodegenerative diseases; relieving anxiety, sedation, anticonvulsant; and muscle relaxation functions (Mody et al., 1994; Oh and Oh, 2004). The foods with high GABA content are also known as brain foods and considered to play roles in different bioactive functions, including regulating blood cholesterol and pressure, decreasing insomnia and depression, and relieving pain (Dhakal et al., 2012). GABA is also found to function against diabetes (Reeds, 2000). The results of this study showed that amino acid content can remarkably be enhanced by germinating the red bean seeds.

Conclusively, the yield, nutritional value, and antioxidant potential of the sprouts of three red bean cultivars produced after soaking their seeds for 0, 6, 12, and 24 h were investigated. The sprout yield on day 7 in cultivars Arari and Geomguseul was highest with the seeds soaked for 12 h among the other seed soaking times. In case of 'Chungju', the sprout yields obtained from the seeds soaked for 12 and 24 h were not significantly different. For these reasons, 12 h of soaking before keeping the seeds for sprout growing was found optimum. Therefore, the nutrient contents and antioxidant potentials were evaluated for the sprouts obtained from 12 h of seed soaking. The total minerals, thiamine, total free amino acids, and total phenols, and DPPH radical scavenging potential of sprouts of all three cultivars were remarkably higher than those of the seeds. In the contexts of consumers increasing preferences toward functional foods of diversified origins, red bean sprouts which possess high nutritional values and functional properties offer a good potential to be used as a healthy food.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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