

Seeds Treatment with Ginger (*Zingiber officinalis* Rosc.) Extract Enhanced the Yield and Nutritional Value of Soybean Sprouts

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Abstract: Soybean sprouts have long been established as the major vegetables in Korea, China, and Japan for a long time. The yield and quality of soybean sprouts are influenced by various factors, including seed-soaking and cultivation conditions. This study was carried out to investigate the effects of ginger extracts on the production and nutrients content of soybean sprouts. Soybean seeds soaked in three concentrations (0.5%, 1%, and 3%, w/v) of ginger extract and tap water for 6h were named GE-0.5, GE-1, GE-3, and control, respectively. The highest soybean sprout yield was found GE-1, followed by GE-3 and GE-0.5, which had 11.5%, 0.9%, and 7.4% increments, respectively, compared to the control. The contents of vitamin C and total polyphenol were higher in many of the ginger extract-treated soybean sprouts than in the control. The results indicated that pro-soaking soybean seeds before cultivation in low concentrations (0.5~1%, w/v) of ginger extract could be helpful to enhance the yield and nutritional value of soybean sprouts.

Keywords: Nutrient, Antioxidant Potential, Ginger Extract, Soybean Sprouts

Introduction

Soybean (*Glycine max* L.) is an economically important crop with versatile end uses (Brumm et al. 1990; Hurburgh et al. 1994); serving as an oil seed crop, feed for livestock, food for humans, and biofuel feedstock (Masuda and Goldsmith 2009). Soybean are also good sources of nutrients; protein, fat, carbohydrates, and other phytochemicals that are essential for good health. Germination enhances the nutritional values such as vitamins, phytosterols, tocopherols, and isoflavones (Shi et al. 2010; Gu et al. 2017). On the other hand, germination can remove or reduce several unwanted food constituents or their activities present in soybean seeds, including trypsin, chymotrypsin, lipoxygenase activity, phytic acid, and oligosaccharides (Shi et al. 2010; Bau et al. 1997; Júnior and Ida, 2015). Soybean sprouts have long been established in Korea as the major vegetables. Soybean sprouts could help increase food security because it can be grown in a considerably short time, even without using advanced technologies. Soybean sprouts are an inexpensive food sources to supply dietary functional materials (Kim et al. 2017) and can also be used in producing cosmetic products with anti-aging and skin whitening effects (Lai et al. 2012). Production of sprouts also may help improve the greater stability of food supply. Nutritive value, texture and organoleptic characteristics of legumes by the way of the seed germination could improved and undesirable anti-nutritional factors be reduced and antioxidant potentials be increased (Frias et al. 2005; Granito et al. 2005; Vidal-Valverde et al. 2002; Doblado et al. 2007). A number of experiments have

been conducted to enhance the yield and quality of soybean sprouts by employing different seed treatment and cultivation techniques (Choi et al. 2000; Choi et al. 2003; Lee et al. 2007; Algar et al. 2013; Yun et al. 2013; Zou et al. 2014).

A few studies show that the use of different plant products such as persimmon fruit powder (Kim et al. 2017), lacquer stem (Kwak et al. 2017), and brown seaweed (Chaikina et al. 2009) can significantly enhance the nutrients and phytochemicals (Choi et al. 2018; Choi et al. 2019; Lintschinger et al. 2000; Mckenzie et al. 2010). Ginger is a common spice used worldwide, whether for a variety of food items or as a folk medicine. Ginger is receiving increased attention due to its health benefits for a variety of dietary supplement, antioxidative, antitumor and free radical scavenging activities (White 2007).

So far, no reports on the effect of ginger extracts on soybean sprouts have been published. This study was conducted to investigate the effect of ginger extracts on the yield and nutritional values of soybean sprouts.

Materials and Methods

Chemicals and experimental materials

The following chemicals: metaphosphoric acid, 2,6-dichloroindophenol, indophenol dye, and isoflavone standards were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) and amino acid standards were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the chemicals used in this study



were of analytical grade. Soybean (*Glycin max* L.) cv. Sowonkong (Park et al. 2000), obtained from the Agricultural Research and Extension Services, Gyeongsangbuk-do, Korea, was considered to produce sprouts. The mean 100-seed weight of the cultivar was 12g.

Preparation of ginger (*Zingiber officinals* Rosc.) extracts

The ginger was oven-dried (60°C until constant weight). The oven-dried ginger was ground into powder using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea). Initially, 10% (w/v) extract of ginger was prepared with 70% (v/v) ethanol by extracting at room temperature using a shaking incubator (250 rpm) for 24 h. The ethanol was removed at 60°C using a rotary evaporator (RV 10D, IKA, China). The concentrated extracts were freeze-dried. Later, three different concentrations (0.5, 1, and 3%; w/v) of ginger (*Zingiber officinals* Rosc.) were prepared using tap water.

Cultivation of soybean sprouts

One kilogram (1kg) of seeds, in three replicates, were washed with tap water, and soaked in tap water of three different concentrations (0.5, 1, and 3%; w/v) of ginger extracts for 6h. The sprout samples were named control, GE-0.5, GE-1, and GE-3 for that soaked in tap water alone, 0.5%, 1%, and 3% ginger extract, respectively.

After soaking for 6h, the seeds were thoroughly rinsed with tap water and put into 15L plastic buckets. The sprouts were sprinkled with tap water for 2min every 2h using two hoses of 1cm diameter. Soybean sprouts were cultivated at 20±1°C for 6 days.

Measurement of sprout yield and preparation of sprout powders

Sprout yields (fresh weight of soybean sprouts) in each batch were measured at 6 d. The yield of sprouts was calculated by deducting the weight of the empty bucket from the weight of each bucket containing sprouts. Sprout powders prepared from the sprouts with cotyledons, hypocotyl, and roots were used for different physicochemical assays as described earlier. The freshly harvested whole sprouts were kept at –70°C for 24h before freeze-drying. The freeze-dried sprout samples were ground into powder using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea) and filtered using a 100-mesh sieve.

Determination of vitamin C content

The vitamin C content in the sprouts was measured following the method of AOAC (1990). One gram of sprout powder was mixed with 7.5 mL of 3% (w/v) metaphosphoric acid and homogenized (AM-8, Nihonseike Kaisha, Tokyo, Japan). The mixture was

filtered through 0.45 µm membrane filter (Millipore, Bedford, MA, USA) and made to the final volume of 12 mL. A half volume (6 mL) of the mixture was titrated with 0.025% (w/v) 2,6-dichloroindophenol. The vitamin C present in the mixture is oxidized, and the indophenol dye was reduced to a colorless compound. Triplicate measurements were considered for statistical analysis.

Color parameters measurement

Hunter's color of soybean sprout powder was measured following the procedures described earlier (Kim et al. 2014). The L (lightness), a (redness), and b (yellowness) values were determined using a Chroma Meter (CR-300, Minolta Corp., Tokyo, Japan). The instrument was calibrated using a calibration plate (Minolta Corp.; YCIE=94.5, XCIE=0.3160, YCIE=0.330) and a standard plate (Hunter Associates Laboratory Inc., Reston, VA, USA; L=97.51, a=-0.18, b=1.67).

Determination of free amino acid contents

The free amino acid profile was determined following the method of Je et al. (2005) with some modifications. Sprout powder (1g) was hydrolyzed with 6N hydrochloric acid (10mL) in a sealed-vacuum ampoule at 110°C for 24h. The mixture was passed through a C-18 Sep Pak (Waters Co., Milford, MA, USA) cartridge and filtered through a 0.22µm membrane filter (Millipore, Billerica, MA, USA). The amino acids were determined using an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech Co., Uppsala, Sweden).

Determination of DPPH Radical Scavenging Activity

The DPPH radical-scavenging potential of sprouts was measured according to the method developed by Blois(1958). One gram sprout powder was extracted with 10 mL of absolute methanol using a shaking incubator (150rpm, 25°C) for 8h, followed by centrifugation at room temperature (3,000rpm, 15 min) and filtration through a 0.2-µm syringe filter (Waters Co., Milford, MA, USA). One hundred microliters of sample extract and 0.1% (w/v) methanolic solution of DPPH each were mixed in microplate wells and then incubated at room temperature for 30 min under dark conditions. The absorbance value of reaction mixtures was measured at 517 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland).

Determination of Total Polyphenol Content

The total polyphenol content (TPC) of samples was measured according to the Folin–Ciocalteu method (Singleton et al. 1999). The sample extracts (50 µL) and 2% (w/v) aqueous Na₂CO₃ (1000 µL) were

mixed in microtubes and kept at room temperature for 3 min. Then, 1 N Folin–Ciocalteu reagent (50 µL) was put into the mixture and incubated at room temperature for 30 min under dark condition. The absorbance value of reaction mixtures was read at 750 nm using a microplate spectrophotometer (Multiskan GO; Thermo Fisher Scientific). Gallic acid (GA) was used as the standard to plot the calibration curve and the TPC content of sprout samples was determined as GA equivalent (GAE).

Statistical analysis

Data were subjected to analysis of variance using SAS 9.4 (SAS Institute, Cary, NC, USA). The significant differences the treatment means were analyzed using Tukey test ($p < 0.05$). Average values two or triplicate measurements were considered for

statistical analysis.

Results and Discussion

Yield of sprouts and their moisture and vitamin C contents

Ginger extracts treatment significantly influenced the yield and vitamin C of soybean sprouts; however, the moisture contents of the sprouts were not affected. The orders of soybean yield were found GE-1(11.5%) > GE-3(9.0%) > GE-0.5(7.4%), compared to the control (Table 1).

The vitamin C content was also significantly the highest for GE-1(17.92mg/100g FW). These results indicated that both GE-3(high concentrations of ginger extracts) and GE-0.5(low concentrations of ginger extracts) samples do not favor the sprout yield increase and vitamin C content.

Table 1. Effect of different concentrations of ginger extracts treatment on the yield, moisture content, and vitamin C content of soybean sprouts cultivated for 6 days

Sample ¹⁾	Total weight (g)	Moisture (%)	Vitamin C (mg/100g Fresh weight)
Control	5412±25 ^{d2)} (100.0%)	86.92±0.62 ^a	16.10±0.20 ^c
GE-0.5	5812±20 ^c (107.4%)	87.12±1.00 ^a	16.51±0.12 ^b
GE-1	6037±28 ^a (111.5%)	87.61±1.01 ^a	17.92±0.35 ^a
GE-3	5900±31 ^b (109.0%)	87.51±0.72 ^a	16.99±0.61 ^b

¹⁾ Control, soybean seeds soaked in tap water for 8h; GE-0.5, soybean seeds soaked in tap water containing 0.5% (w/v) ginger extracts for 8h; GE-1, soybean seeds soaked in tap water containing 1.0% (w/v) ginger extracts for 8h; GE-3, soybean seeds soaked in tap water containing 3.0% (w/v) ginger extracts for 8h.

²⁾ Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same column indicate significant difference ($p < 0.05$, Tukey test).

Color parameters of soybean sprouts

Although the L*(Lightness) value was unaffected, a*(Redness) and b*(Yellowness) values of soybean sprout color were significantly ($p < 0.05$) influenced by seed-soaking in ginger extract treatment during

sprout growth (Table 2). The significantly highest redness value was found for control(0.98), GE-0.5(0.92), GE-3(0.87), and GE-1(0.86), respectively. GE-1(22.51) and GE-3(23.69) had significantly highest yellow values.

Table 2. Hunter's color values of soybean sprouts grown after different concentration of ginger extracts treatment

Sample ¹⁾	Color value ²⁾		
	L*(Lightness)	a*(Redness)	b*(Yellowness)
Control	74.22±0.81 ^{a3)}	0.98±0.04 ^a	20.92±0.28 ^b
GE-0.5	74.98±0.49 ^a	0.92±0.02 ^b	20.81±0.39 ^b
GE-1	75.00±0.91 ^a	0.86±0.03 ^c	22.51±0.31 ^a
GE-3	74.12±0.78 ^a	0.87±0.02 ^c	23.69±0.59 ^a

¹⁾ Samples are defined in Table 1.

²⁾ L*, lightness (100, white; 0, black); a*, redness (-, green; +, red); b*, yellowness (-, blue; +, yellow).

³⁾ Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same column are significantly different ($p < 0.05$, Tukey test).

Free amino acid content

As the physicochemical characteristics, the essential and total amino acid contents in the soybean sprouts treated with ginger extracts were increased (Table 3).

In the case of non-essential amino acid, all concentrations (0.5%, 1%, and 3%) of ginger extract treatment (28.66~32.28 mg/g DW) increased the amino acid content compared to the control (26.59

mg/g DW).

A total 8 essential, 8 non-essential, and 19 other free amino acids were detected in all samples. The amounts of all the essential and non-essential amino acids were significantly higher in the ginger extracts-treated soybean sprouts than in the non-treated control. Nine free amino acids were not detectable in the sprout samples.

Table 3. Free amino acid composition (mg/g of dry weight) of soybean sprouts cultivated after different concentrations of ginger extracts treatment

Amino acid	Sample ¹⁾			
	Control	GE-0.5	GE-1	GE-3
Essential Amino Acids				
L-Threonine	1.21±0.01 ^{d2)}	1.64±0.01 ^b	1.52±0.01 ^c	1.71±0.01 ^a
L-Valine	2.30±0.02 ^d	2.81±0.02 ^b	2.61±0.02 ^c	2.92±0.02 ^a
L-Methionine	0.32±0.01 ^d	0.38±0.01 ^c	0.61±0.01 ^b	0.77±0.01 ^a
L-Isoleucine	0.81±0.02 ^b	1.71±0.02 ^c	1.71±0.02 ^c	1.90±0.02 ^a
L-Leucine	1.92±0.01 ^b	2.00±0.02 ^a	1.11±0.01 ^c	1.11±0.01 ^c
L-Phenylalanine	1.33±0.02 ^c	1.96±0.01 ^a	1.89±0.02 ^b	1.90±0.01 ^b
L-Lysine	2.71±0.02 ^c	2.80±0.01 ^b	2.70±0.01 ^c	2.87±0.02 ^a
L-Histidine	2.08±0.01 ^d	3.32±0.02 ^b	3.15±0.02 ^c	3.51±0.01 ^a
Sub-total	12.68	16.62	15.30	16.69
Non-essential Amino Acid				
L-Aspartic acid	0.81±0.01 ^b	0.93±0.01 ^b	1.11±0.01 ^a	1.13±0.02 ^a
L-Serine	2.69±0.02 ^d	3.40±0.02 ^c	3.66±0.02 ^a	3.61±0.01 ^b
L-Glutamic acid	5.11±0.01 ^d	5.80±0.03 ^b	5.69±0.07 ^c	6.16±0.05 ^a
Glycine	0.33±0.02 ^b	0.37±0.01 ^a	0.33±0.01 ^b	0.33±0.01 ^b
L-Alanine	1.69±0.01 ^d	2.66±0.02 ^a	2.08±0.02 ^c	2.30±0.02 ^b
L-Throsine	2.35±0.02 ^c	0.25±0.01 ^c	0.39±0.02 ^a	0.31±0.01 ^b
L-Arginine	14.69±0.15 ^b	14.11±0.13 ^c	18.02±0.05 ^a	13.79±0.09 ^d
Proline	1.02±0.01 ^b	1.19±0.02 ^a	1.00±0.01 ^b	1.03±0.02 ^b
Sub-total	26.59	28.71	32.28	28.66
Other Amino Acid				
O-Phospho-L-serine	0.17±0.02 ^a	0.16±0.02 ^a	0.18±0.02 ^a	0.18±0.02 ^a
Taurine	ND ³⁾	ND	ND	ND
O-Phospho ethanol amine	ND	ND	ND	ND
Urea	2.12±0.02 ^d	2.60±0.02 ^a	2.31±0.02 ^b	2.20±0.01 ^c
L-Sarcosine	ND	ND	ND	ND
L- α -Amino-n-butyric acid	0.32±0.02 ^b	0.34±0.03 ^a	0.31±0.03 ^b	0.31±0.02 ^b
L-Cystine	ND	ND	ND	ND
Cystathionine	ND	ND	ND	ND
β -Alnaine	0.39±0.02 ^b	0.45±0.02 ^a	0.39±0.02 ^b	0.46±0.02 ^a
D,L- β -Amino isobutyric acid	0.15±0.02 ^a	0.19±0.02 ^a	0.19±0.02 ^a	0.18±0.02 ^a
γ -Amino-n-butyric acid	0.53±0.03 ^a	0.69±0.02 ^a	0.71±0.02 ^a	0.69±0.02 ^a
Ethanolamin	0.20±0.02 ^a	0.20±0.02 ^a	0.21±0.02 ^a	0.20±0.02 ^a
Hydroxylysine	ND	ND	ND	ND
L-Ornithine	0.05±0.01 ^b	0.20±0.02 ^a	0.21±0.02 ^a	0.19±0.02 ^a
1-Methyl-L-histidine	ND	ND	ND	ND
3-Methyl-L-histidine	ND	ND	ND	ND
L-Anserine	ND	ND	ND	ND
L-Carnosine	ND	ND	ND	ND
Hydroxy proline	0.18±0.03 ^b	0.20±0.04 ^b	0.16±0.03 ^b	0.23±0.02 ^a
Sub-total	4.11	5.08	4.67	4.64
Total Free Amino Acid	43.38	50.41	52.25	49.99

¹⁾ Samples are defined in Table 1.

²⁾ Values are expressed as mean \pm standard deviation of two replicates. Values followed by different letters in the same row are significantly different ($p < 0.05$, Tukey test).

³⁾ Non-detectable.

DPPH and total polyphenol contents

Free radical scavenging potentials of soybean sprouts treated ginger extracts were measured through DPPH and total polyphenol contents.

Ginger extracts treatment significantly increased the antioxidant potential of soybean sprouts samples (Table 4). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential was highest in GE-1(86.29%), followed by GE-3(86.20%). The lowest DPPH inhibition was investigated with the control(79.41%).

GE-1 sample had the highest total polyphenol content. The order of total polyphenol content in soybean sprouts were GE-1(422.02 µg GAE/g) > GE-5(415.23 µg GAE/g) > GE-0.5(410.15 µg GAE/g) > control(322.10 µg GAE/g), respectively.

Table 4. 1,1-Diphenyl-2-picrylhydrozyl(DPPH), total polyphenol contents of soybean sprouts cultivated after different concentrations of ginger extracts treatment

Sample ¹⁾	DPPH (% Inhibition)	Total polyphenol (µg GAE ²⁾ /g)
Control	79.41±1.81 ^{c3)}	322.10±2.20 ^d
GE-0.5	82.11±1.69 ^b	410.15±2.11 ^c
GE-1	86.29±0.35 ^a	422.02±1.62 ^a
GE-3	86.20±1.10 ^a	415.23±1.82 ^b

¹⁾ Samples are defined in Table 1.

²⁾ GAE : gallic acid equivalent.

³⁾ Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same row indicate significant difference (p<0.05).

Conclusion

The effects of ginger extract treatment on the quality characteristics and antioxidant activities of soybean sprouts. The quality characteristics and antioxidant activities of many of the ginger extracts-treated soybean sprouts were higher than those of the control. Overall, soaking of soybean seeds in lower concentrations (1~3%, w/v) of ginger extracts could be an effective and efficient way to improve the quality characteristics and antioxidant activities of soybean sprouts.

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