

## Soaking Soybean Seeds with *Abeliophyllum distichum* Nakai Extract Increased the Yield and Nutritional Value of Soybean Sprouts

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*Abeliophyllum distichum* Nakai, an ornamental plant, contains a wide range of phytochemicals having pharmaceutical properties. The use of different plant-based extracts for the enhancement of yield and/or quality of soybean sprouts, one of the most inexpensive but nutritious food products, is common. The objective of this study was to examine the effect of *A. distichum* flower extract (ADE) on the yield and nutritional value of soybean sprouts. Soybean seeds were soaked in ADE solutions with concentrations (w/v) of 0.5% (ADE-0.5), 1% (ADE-1), 3% (ADE-3), and 5% (ADE-5). The effect of ADE concentration on the yield and different nutrient components varied. The highest soybean sprout yield and vitamin C content were found with ADE-3. The most abundant essential amino acid content was detected in ADE-1, whereas the greatest amounts of total isoflavones and total minerals were determined in ADE-5 and the ADE-untreated control, respectively. Overall results of yield, color, and contents of vitamin C, amino acids, and isoflavones suggest that 1% or 3% of *A. distichum* extract could be an optimum concentration to soak the soybean seeds for higher sprout yield and nutrient content.

### INTRODUCTION

*Abeliophyllum distichum* Nakai is the single species in the *Oleaceae* family [Oh *et al.*, 2003]. *A. distichum*, commonly known as white forsythia, is mainly used as an ornamental deciduous plant. It has recently drawn attention of many researchers due to a wide range of bioactive phytochemicals, such as acteoside, eutigoside B, dendromonilide D, forsythoside B, isoacteoside, isomucronulatol 7-*O*-glucoside, plantamajoside, wighteone, rutin, cornoside, hirsutrin, chlorogenic acid, caffeic acid, gentisic acid, ferulic acid, and quercetin [Ahn & Park, 2013; Choi *et al.*, 2017; Ju *et al.*, 2021; Lee *et al.*, 2021; Li *et al.*, 2013; Oh *et al.*, 2003; Yoo *et al.*, 2021]. So far, these phytochemicals have been reported to exhibit antioxidant activity [Choi *et al.*, 2017], anti-proliferative activity against human colorectal cancer cells [Park *et al.*, 2014], antidiabetic effect [Li *et al.*, 2013], and antihypertensive activity [Oh *et al.*, 2003]. Kwon *et al.* [2014] have determined the nutrient components and evaluated the safety of *A. distichum* leaves and stems to be used as a natural constituent in the preparation of nutritional and functional foods. They

found that *A. distichum* leaves and stems, which contained proteins, lipids, sugars, vitamins, minerals, organic acids, and phenolic compounds, were safe to be used as a food material.

Soybeans are a rich source of proteins, lipids, and several phytochemicals, including phenolics (isoflavones and anthocyanins) and saponins [Brummer *et al.*, 1997; Hubert *et al.*, 2008; Lee *et al.*, 2009; Lee & Cho, 2012]. Germination may further enhance the nutritional value of soybean seeds [Pau-car-Menacho *et al.*, 2010] because it not only modifies the existing nutrients but also produces new compounds [Spanier *et al.*, 2001]. Generally, a week is sufficient to prepare soybean sprouts, which can be grown year-round using simple and inexpensive technologies.

Various studies have shown that the seed soaking and/or treatment with irrigating solutions could enhance the quality and nutritional values of soybean sprouts. The soaking and spraying of seeds with a zinc sulfate solution has been reported to enhance the zinc content [Zou *et al.*, 2014], while in tap water with persimmon fruit powder to increase the yield and the contents of vitamin C, isoflavones, and total phenolics

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of soybean sprouts [Kim *et al.*, 2017]. The use of Pu-erh tea extracts for seed soaking also augmented the yield and nutritional values of soybean sprouts [Kim *et al.*, 2020].

Considering the pharmaceutical properties of *A. distichum* and the use of different plant-based extracts, including persimmon fruit powder [Kim *et al.*, 2017], lacquer stem [Kwak *et al.*, 2017], and Pu-erh tea [Kim *et al.*, 2020] for soybean sprout cultivation, this study aimed to investigate the effect of seed soaking in *A. distichum* extracts on the quality characteristics and yields 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.

Sowonkong, one of the common sprout cultivars of soybean (*Glycine max* L.) in Korea, was used to produce sprouts. The seeds were obtained from the Agricultural Research and Extension Services, Gyeongsangbuk-do, Korea. The mean 1000-seed weight of the cultivar was 120 g. Flowers from the 5-year-old *Abeliophyllum distichum* Nakai plants, grown in Youngdong-gun, Chungcheongbuk-do, Korea, were collected for this study.

### Preparation of *Abeliophyllum distichum* extracts

The *A. distichum* flowers were oven-dried (50°C until constant weight). The oven-dried flowers were ground into powder using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea). Initially, 10% (w/v) extract of *A. distichum* (ADE) 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, four different concentrations (0.5, 1, 3, and 5%; w/v) of *A. distichum* extracts (ADEs) were prepared using tap water.

### Cultivation of soybean sprouts

One kilogram of seeds, in three replicates, were washed with tap water, and then the excess water was drained. The seeds were soaked in tap water or four different concentrations (0.5, 1, 3, and 5%; w/v) of ADE for 6 h [Kim *et al.*, 2020]. The sprout samples were named with the concentration of the seed soaking extract used *i.e.*, control, ADE-0.5, ADE-1, ADE-3, and ADE-5 for that soaked in tap water alone, 0.5, 1, 3, and 5% of ADE, respectively. After 6 h of soaking, the seeds were thoroughly rinsed with tap water and put into the bottom-perforated 15-L plastic buckets, and covered with double-layered black landscape fabric to minimize light exposure during sprout growing [Kim *et al.*, 2017]. The germinating seeds and sprouts of all five treatments were periodically sprinkled with tap water for 2 min every 3 h using two hoses of 1-cm diameter. Soybean sprouts were cultivated at 20±1°C for 6 days.

### Measurement of sprout yield and preparation of sprout powders

Sprout yield, assessed as the fresh weight of soybean sprouts, was measured on day 6 by subtracting the weight of the empty bucket from the gross weight of each bucket containing sprouts. The freshly harvested sprouts with cotyledons, hypocotyl, and roots were kept at -70°C for 24 h, followed by freeze-drying. The freeze-dried sprouts were ground into powder using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea) and passed through a 100-mesh sieve [Kim *et al.*, 2017].

### 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. The ascorbic acid (vitamin C) content of sprout powders was determined by an external standard of ascorbic acid with an aqueous solution of 0.025% (w/v) 2,6-dichloroindophenol. The absorption was read at 250 nm using a spectrophotometer (OPTIZEN POP-V, LAB Keen Innovative Solutions, Daejeon, Korea). A calibration standard curve of ascorbic acid was plotted and used to calculate the vitamin C content as milligrams of ascorbic acid per 100 g fresh weight (FW).

### 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 content

The free amino acid content was assayed following the method described by Je *et al.* [2005]. Sprout powder (1.5 g) 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), followed by incubation in ice for 30 min and centrifugation (3913×g, 15 min). The supernatant was filtered through a filter paper (Whatman No. 41) and adjusted to pH 7.0 using a KOH solution (33%, w/v). The precipitate of potassium perchlorate was removed by centrifugation (3913×g, 10 min). Then, the pH of the supernatant was adjusted to 2.2 with hydrochloric acid (10 M), and distilled water was added to make the final volume of 50 mL. Two milliliters of sample aliquot were mixed with 1 mL of a lithium citrate buffer (pH 2.2) to prepare the reaction mixture for the free amino acid determination. The content of free amino acids was analyzed using

an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech Co., Uppsala, Sweden) and expressed as mg per g sprout dry weight (DW).

#### Determination of mineral content

The mineral content of soybean sprouts was analyzed according to the procedures described in a previous report [Skujins, 1998] with some modifications. Sprout powder (0.5 g) was digested in a mixture of 65% HNO<sub>3</sub> (15.0 mL) and 35% H<sub>2</sub>O<sub>2</sub> (2 mL). An equal volume of distilled water was added to dilute the mixture. The mineral content of the samples was estimated using an inductively coupled plasma atomic emission spectrometer (ICP AES, Varian Vista, Victoria, Australia) after calibrating the instrument with a working standard prepared from a commercially available multielement standard solution (Merck, Darmstadt, Germany). The results were expressed as mg per kg sprout DW.

#### Determination of isoflavone content

Two hundred micrograms of sprout powder were extracted with 6 mL of methanol (80%, v/v) using an ultrasonic-assisted method at 40°C for 30 min, followed by centrifugation (3913×g, 15 min) and filtration of the supernatant through a membrane filter (0.45 μm, Millipore, Bedford, MA, USA). The filtrate was used for the isoflavone analysis using a high-performance liquid chromatography (HPLC) system with a UV detector (Prostar 230, Varian Co., Palo Alto, CA, USA) by following a previously described method [Jiao et al., 2016]. A Nova-Pak C18 reversed-phased column (150×3.9 mm, 4 μm particle size) and Adsorbosphere C18 direct-connect guard column (Waters Co., Milford, MA, USA) were used. The flow rate of the mobile phase was 1 mL/min and the gradient elution of solvents A and B (solvent A – aqueous acetic acid (0.1%, v/v) and solvent B – acetic acid in acetonitrile (0.1%, v/v)) was used as follows: 13–35% of B for 52 min. The oven temperature was set to 35°C. The injection volume was 20 μL. The eluted isoflavones were detected at 260 nm. Each peak was identified by the retention time and the characteristic UV spectrum in comparison with the corresponding standards. The isoflavone content was calculated using the calibration curve of an internal standard 2,4,4'-trihydroxydeoxybenzoin (THB) and expressed as mg of isoflavone per kg of freeze-dried soybean sprouts (mg/kg DW).

#### Statistical analysis

Analysis of variance was carried out using SAS 9.4 software (SAS Institute, Cary, NC, USA) to compare the treatments. Three batches of soybean sprouts were produced for each treatment. Two replicates for free amino acid and three replicates for the other analyses were carried out. The significant differences among the treatment means were identified at 5% probability using Tukey's test.

## RESULTS AND DISCUSSION

#### Yield of sprouts and their moisture and vitamin C contents

Vitamin C is an important nutrient with a strong antioxidative capacity. The yield and vitamin C content of soybean

TABLE 1. Yield of soybean sprouts grown after seed soaking in different concentrations (0.5%–5%, w/v) of *Abeliophyllum distichum* extracts (ADE) and their moisture and vitamin C contents.

Sample*	Total fresh weight (g)	Moisture (g/100 g)	Vitamin C (mg/100 g fresh weight)
Control	5425±32 <sup>a</sup> (100.0%)	87.31±0.03 <sup>a</sup>	16.07±0.31 <sup>c</sup>
ADE-0.5	5718±21 <sup>c</sup> (105.4%)	87.21±0.21 <sup>a</sup>	16.59±0.52 <sup>bc</sup>
ADE-1	5930±30 <sup>b</sup> (109.3%)	88.00±1.12 <sup>a</sup>	16.99±0.32 <sup>b</sup>
ADE-3	6031±40 <sup>a</sup> (111.2%)	87.42±0.08 <sup>a</sup>	18.20±0.20 <sup>a</sup>
ADE-5	5768±45 <sup>c</sup> (106.3%)	88.02±1.01 <sup>a</sup>	16.81±0.21 <sup>b</sup>

\* The values at ADE correspond to its concentrations in the seed soaking solution. Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same column are significantly different ( $p < 0.05$ ). Values, after total fresh weight, in the brackets indicate the sprout yield difference in comparison to the control (100%).

sprouts were significantly affected by ADE treatment; however, the moisture content remained unaffected (Table 1). A significantly ( $p < 0.05$ ) high yield increment was obtained in ADE-3 (11.2%), followed by ADE-1 (9.3%) compared to that of the control. ADE-0.5 (5.4%) and ADE-5 (6.3%) had the least yield increment and these values did not differ significantly ( $p \geq 0.05$ ). ADE-3, which showed the utmost yield, had the greatest vitamin C content of 18.20 mg/100 g FW. In turn, the contents of vitamin C of ADE-0.5 (16.59 mg/100 g FW) and the control (16.07 mg/100 g FW) were not significantly ( $p \geq 0.05$ ) different.

Although the availability of plant growth regulators in ADE was not measured, it can be stated from the previous reports on the plant-based extracts, including persimmon fruit powder [Kim et al., 2017], lacquer stem [Kwak et al., 2017], and Pu-erh tea [Kim et al., 2020] that some growth-promoting substances could be present in ADE and that might have played a role in changes observed in the yield and nutritional value of the soybean sprouts. Wang et al. [2016] reported that the supplementation of soybean sprout growth with calcium increased both the sprout yield and vitamin C content, and claimed these effects could be caused by the Ca-induced increased content of plant hormones, like indoleacetic acid and gibberellin. A high calcium content was found in *A. distichum* [Kwon et al., 2014], which seems to be important for increasing the yield and vitamin C content of the ADE-treated soybean sprouts. We could not exactly explain the reasons for the higher vitamin C content in ADE-3 than in ADE-5. There might be some kinds of stress in soybean sprouts due to a higher concentration of ADE. The elevated yield and nutrient content might be due to the absorption of *A. distichum* phytochemicals [Ahn & Park, 2013; Choi et al., 2017; Ju et al., 2021; Lee et al., 2021; Li et al., 2013; Kwon et al., 2014; Oh et al., 2003; Yoo et al., 2021] during seed-soaking [Lintschinger et al., 2000]. Wang et al. [2016] hypothesized that the regulation of enzyme activity promoted growth and increased the nutritional value of soybean by calcium would involve hormones.

TABLE 2. Hunter's color parameters of 6-day-old soybean sprouts grown after seed soaking in different concentrations (0.5%–5%, w/v) of *Abelophyllum distichum* extracts (ADE).

Sample*	Lightness (L)	Redness (a)	Yellowness (b)
Control	77.56±0.86 <sup>a</sup>	1.51±0.06 <sup>a</sup>	21.35±0.33 <sup>b</sup>
ADE-0.5	77.50±0.66 <sup>a</sup>	0.25±0.03 <sup>d</sup>	22.00±0.29 <sup>a</sup>
ADE-1	77.75±0.92 <sup>a</sup>	0.74±0.16 <sup>c</sup>	22.15±0.28 <sup>a</sup>
ADE-3	77.49±0.61 <sup>a</sup>	0.81±0.04 <sup>b</sup>	21.33±0.09 <sup>b</sup>
ADE-5	77.68±0.42 <sup>a</sup>	0.94±0.11 <sup>b</sup>	21.46±0.05 <sup>b</sup>

\* The values at ADE correspond to its concentrations in the seed soaking solution. Lightness (100, white; 0, black); redness (–, green; +, red); yellowness (–, blue; +, yellow). Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same column are significantly different ( $p < 0.05$ ).

### Color parameters of soybean sprouts

Redness and yellowness values of soybean sprout color were significantly ( $p < 0.05$ ) influenced by seed-soaking in ADE during sprout growth; however, lightness values remained unaffected ( $p \geq 0.05$ ) (Table 2). The redness value of all the ADE-treated samples was significantly ( $p < 0.05$ ) lower compared to the control. The yellowness values of ADE-0.5 and ADE-1 were increased by ADE treatment. The lowest redness value was obtained in ADE-0.5 (0.25), which was one of the samples with the greatest yellowness value.

Although the reason behind the color variations in soybean sprouts was not well known, ADE treatment affected the color appearance of soybean sprouts. Treatment of soybean seeds with different plant extracts could alter the color parameters of soybean sprouts [Kim *et al.*, 2017, 2020; Kwak *et al.*, 2017]. The color of a food product is an influencing factor to determine the alacrity of consumers to pay for the product [Udomkun *et al.*, 2018]. The soaking of soybean seeds with ADE enhanced the yellowness of the sprouts, which is one of their looked-for characteristics [Park *et al.*, 1995].

### Free amino acid content

The free amino acid compositions of soybean sprouts grown after seed-soaking in tap water and in different concentrations of ADE are shown in Table 3. The essential amino acid content of ADE-0.5 (14.47 mg/g DW) and ADE-1 (14.92 mg/g DW) was higher than that of the control (13.99 mg/g DW). However, the soaking of soybean seeds with higher concentrations of *A. distichum* extracts *i.e.*, ADE-3 (11.08 mg/g DW) and ADE-5 (8.41 mg/g DW), reduced the content of essential amino acids compared to the control. On the other hand, although contents of some of the individual amino acids, such as proline, were increased in the soybean sprouts, the contents of non-essential and total amino acids decreased upon the seed treatment with *A. distichum* extracts. The total content of other free amino acids also decreased upon the ADE treatment; however, the contents of L- $\alpha$ -amino adipic acid and L- $\alpha$ -amino-*n*-butyric acids were found to increase.

Similar results of a higher content of essential amino acids, like leucine, isoleucine, lysine, methionine, and valine,

were found in the soybean sprouts treated with Pu-erh tea extracts [Kim *et al.*, 2020]. Seed soaking in ADE reduced the non-essential and other amino acids of soybean sprouts compared to the control except for the content of a few amino acids. It can be speculated that calcium, which is present in *A. distichum* [Kwon *et al.*, 2014], might have induced conversion among some amino acids [Wang *et al.*, 2016]. Additionally, there might be some kinds of stress at higher ADE concentrations resulting in reduced contents of some of the amino acids. ADE treatment has increased the content of a few amino acids in the soybean sprouts, making them more nutritive. Fortification of food products with certain nutrients to make them more nutritious is not uncommon. Soybean sprouts were fortified with zinc sulfate to increase zinc availability [Zou *et al.*, 2014]. Fortification and consumption of wheat flour with lysine significantly improved the sensitive indicators of nutritional status of a studied population [Husain *et al.*, 2004]. Functional non-essential amino acids, like proline, the content of which was significantly high in ADE-1 (Table 3), play a key role in the metabolic pathways associated with maintenance, growth, reproduction, and immunity [Wu, 2009].

### Mineral content

Like the total amino acid content, the total mineral content of soybean sprouts decreased as a result of seed-soaking in ADE solutions; however, contents of some of the individual mineral elements increased upon the ADE treatment (Table 4). Among the eight minerals measured, K (11322–14913 mg/kg DW) was the most abundant, while Cu (16.3–36.3 mg/kg DW), followed by Mn (38.2–38.8 mg/kg DW) was the least abundant mineral in the sprout samples. Content of four minerals (Ca, Fe, Na, and Zn) were higher in at least one of the ADE-treated soybean sprouts, whereas contents of two minerals (Cu and K) were higher in the control. The Mg content of the control, ADE-0.5, and ADE-1 did not differ significantly ( $p \geq 0.05$ ). The Mn content of the control did not differ significantly ( $p \geq 0.05$ ) from those of the ADE-treated soybean sprouts.

The higher mineral content in the ADE-treated soybean sprouts compared to the control might be due to the mineral-rich ADE [Kwon *et al.*, 2014]. Similar results of higher mineral content were found in previous studies with zinc sulfate-treated soybean sprouts [Xu *et al.*, 2012; Zou *et al.*, 2014], zinc sulfate-fortified germinated brown rice [Wei *et al.*, 2012], selenium-applied cereal sprouts [Lintschinger *et al.*, 2000], iron-fortified soybean sprouts [Kujawska *et al.*, 2016], and Pu-erh tea-treated soybean sprouts [Kim *et al.*, 2020]. Fe, Zn, and Ca, which increased in soybean sprouts upon the ADE treatment, are some of the most commonly lacking minerals in human diets [White & Broadley, 2009].

### Isoflavone content

Isoflavones are considered a type of bioactive dietary supplement that elicits a number of health benefits. The contents of total isoflavones and individual isoflavones, except genistein and glycitein, increased significantly ( $p < 0.05$ ) in soybean sprouts with ADE treatments (Table 5). Daidzin (332–362 mg/kg DW), followed by genistin (292–335 mg/kg DW),



TABLE 3. Free amino acid composition (mg/g dry weight) of 6-day-old soybean sprouts grown after seed soaking in different concentrations (0.5%–5%, w/v) of *Abeliophyllum distichum* extracts (ADE).

Amino acid	Control	ADE-0.5	ADE-1	ADE-3	ADE-5
Essential amino acids					
L-Histidine	3.34±0.1 <sup>a</sup>	3.30±0.4 <sup>a</sup>	3.26±0.2 <sup>b</sup>	2.21±0.1 <sup>c</sup>	1.66±0.1 <sup>d</sup>
L-Isoleucine	1.25±0.2 <sup>c</sup>	1.30±0.1 <sup>b</sup>	1.39±0.1 <sup>a</sup>	1.11±0.1 <sup>d</sup>	0.87±0.1 <sup>c</sup>
L-Leucine	0.95±0.1 <sup>b</sup>	0.98±0.2 <sup>b</sup>	1.06±0.2 <sup>a</sup>	0.77±0.2 <sup>c</sup>	0.57±0.1 <sup>d</sup>
L-Lysine	2.44±0.3 <sup>c</sup>	2.55±0.2 <sup>b</sup>	2.66±0.1 <sup>a</sup>	2.01±0.1 <sup>d</sup>	1.25±0.1 <sup>c</sup>
L-Methionine	0.33±0.1 <sup>a</sup>	0.35±0.2 <sup>a</sup>	0.34±0.2 <sup>a</sup>	0.25±0.1 <sup>b</sup>	0.16±0.2 <sup>c</sup>
L-Phenylalanine	1.75±0.1 <sup>b</sup>	1.87±0.1 <sup>a</sup>	1.86±0.1 <sup>a</sup>	1.11±0.2 <sup>c</sup>	1.08±0.1 <sup>c</sup>
L-Threonine	1.66±0.1 <sup>b</sup>	1.82±0.2 <sup>a</sup>	1.79±0.1 <sup>a</sup>	1.51±0.2 <sup>c</sup>	1.09±0.1 <sup>d</sup>
L-Valine	2.27±0.1 <sup>c</sup>	2.30±0.1 <sup>b</sup>	2.56±0.2 <sup>a</sup>	2.11±0.1 <sup>d</sup>	1.73±0.2 <sup>c</sup>
Sub-Total	13.99	14.47	14.92	11.08	8.41
Non-essential amino acids					
Glycine	0.32±0.2 <sup>a</sup>	0.31±0.1 <sup>a</sup>	0.32±0.1 <sup>a</sup>	0.21±0.2 <sup>b</sup>	0.19±0.1 <sup>b</sup>
L-Alanine	3.07±0.1 <sup>c</sup>	3.51±0.3 <sup>b</sup>	3.78±0.2 <sup>a</sup>	3.01±0.1 <sup>d</sup>	2.01±0.2 <sup>c</sup>
L-Arginine	17.4±1.2 <sup>a</sup>	15.21±0.9 <sup>b</sup>	12.78±0.8 <sup>c</sup>	10.12±1.2 <sup>d</sup>	6.28±0.5 <sup>c</sup>
L-Aspartic acid	2.54±0.2 <sup>a</sup>	2.50±0.1 <sup>b</sup>	2.54±0.1 <sup>a</sup>	2.01±0.1 <sup>c</sup>	1.82±0.1 <sup>d</sup>
L-Serine	3.49±0.1 <sup>b</sup>	0.35±0.2 <sup>b</sup>	3.58±0.2 <sup>a</sup>	2.01±0.1 <sup>c</sup>	1.98±0.1 <sup>d</sup>
L-Tyrosine	0.35±0.2 <sup>a</sup>	0.34±0.2 <sup>a</sup>	0.35±0.1 <sup>a</sup>	0.22±0.1 <sup>b</sup>	0.16±0.3 <sup>c</sup>
Proline	1.69±0.1 <sup>b</sup>	1.72±0.2 <sup>b</sup>	1.83±0.2 <sup>a</sup>	1.12±0.2 <sup>c</sup>	0.82±0.1 <sup>d</sup>
Sub-total	28.86	23.94	25.18	18.70	13.26
Other free amino acids					
Aminoisobutyric acid	0.26±0.1 <sup>a</sup>	0.27±0.1 <sup>a</sup>	0.26±0.1 <sup>a</sup>	0.20±0.1 <sup>b</sup>	0.10±0.1 <sup>c</sup>
Ethanolamine	0.57±0.2 <sup>a</sup>	0.50±0.2 <sup>b</sup>	0.45±0.3 <sup>c</sup>	0.21±0.3 <sup>d</sup>	0.19±0.1 <sup>d</sup>
L- $\alpha$ -Aminoadipic acid	0.47±0.2 <sup>b</sup>	0.50±0.2 <sup>b</sup>	0.56±0.3 <sup>a</sup>	0.38±0.3 <sup>c</sup>	0.28±0.2 <sup>d</sup>
L- $\alpha$ -Amino- <i>n</i> -butyric acid	0.14±0.1 <sup>b</sup>	0.15±0.1 <sup>a</sup>	0.15±0.1 <sup>a</sup>	0.11±0.1 <sup>c</sup>	0.09±0.2 <sup>c</sup>
$\beta$ -Alanine	0.52±0.2 <sup>a</sup>	0.51±0.1 <sup>a</sup>	0.53±0.2 <sup>a</sup>	0.42±0.1 <sup>b</sup>	0.39±0.1 <sup>b</sup>
$\gamma$ -Amino- <i>n</i> -butyric acid	1.96±0.2 <sup>a</sup>	1.16±0.2 <sup>b</sup>	0.93±0.2 <sup>c</sup>	0.66±0.2 <sup>d</sup>	0.45±0.1 <sup>c</sup>
Sub-total	3.92	3.09	2.88	1.98	1.50
Total	46.77	41.50	42.98	31.76	23.17

The values at ADE correspond to its concentrations in the seed soaking solution. Values are expressed as mean  $\pm$  standard deviation of two replicates. Values followed by different letters in the same row are significantly different ( $p < 0.05$ ).

was the most abundant isoflavone in the sprout samples. Interestingly, the amount of these two isoflavones increased with the concentration of ADE used to soak the seeds. Glycitein, which was the least abundant isoflavone in the sprout samples, was not affected by the ADE treatment.

Although the mechanism behind the isoflavone variation due to the ADE treatment was not clear, presumably, the enhanced phenyl-alanine and isoflavone synthetase activities due to the effect of minerals [Jung *et al.*, 2000; Wang *et al.*, 2016] might have increased the isoflavone content in sprouts grown after seed-soaking in ADE solutions. During seed germination, carbohydrates are consumed rapidly, and water-soluble

metabolites are removed, which increases the isoflavone content [Kim *et al.*, 2013]. Variation in isoflavone content of germinated soybean seeds could be due to the conversion of other flavonoids to isoflavones and isoflavones to other flavonoids [Zhu *et al.*, 2005]. The flavonoid content of ADE [Kwon *et al.*, 2014; Lee *et al.*, 2020] might have played a role in increasing the isoflavone content in the ADE-treated soybean sprouts. Moreover, a high calcium content of ADE might also have contributed to an increase in the isoflavone content in the ADE-treated soybean sprouts as in calcium-treated soybean sprouts [Wang *et al.*, 2016]. Similar results of a high isoflavone content were also found in soybean sprouts grown

TABLE 4. Mineral content (mg/kg dry weight) of 6-day-old soybean sprouts grown after seed soaking in different concentrations (0.5%–5%, w/v) of *Abeliophyllum distichum* extracts (ADE).

Element	Control	ADE-0.5	ADE-1	ADE-3	ADE-5
Ca	2897±11 <sup>b</sup>	2782±10 <sup>c</sup>	2522±12 <sup>d</sup>	2533±8 <sup>d</sup>	3210±7 <sup>a</sup>
Cu	36.3±1.2 <sup>a</sup>	29.6±1.6 <sup>b</sup>	25.3±1.4 <sup>c</sup>	20.2±1.6 <sup>d</sup>	16.3±1.2 <sup>c</sup>
Fe	60.3±0.9 <sup>c</sup>	72.1±0.8 <sup>d</sup>	89.3±1.2 <sup>c</sup>	112.0±1.6 <sup>b</sup>	131.4±1.5 <sup>a</sup>
K	14,913±21 <sup>a</sup>	13,222±1 <sup>b</sup>	12,213±30 <sup>c</sup>	12,002±40 <sup>c</sup>	11,322±35 <sup>d</sup>
Mg	1611±9 <sup>a</sup>	1601±8 <sup>a</sup>	1599±12 <sup>ab</sup>	1580±10 <sup>b</sup>	1548±1 <sup>c</sup>
Mn	38.2±0.7 <sup>a</sup>	38.7±0.8 <sup>a</sup>	38.2±2.1 <sup>a</sup>	38.4±2.9 <sup>a</sup>	38.8±0.9 <sup>a</sup>
Na	477±4 <sup>c</sup>	531±4 <sup>d</sup>	582±5 <sup>c</sup>	612±9 <sup>b</sup>	633±9 <sup>a</sup>
Zn	46.6±1.2 <sup>c</sup>	47.2±1.0 <sup>c</sup>	49.3±1.1 <sup>b</sup>	53.3±1.7 <sup>a</sup>	55.2±0.8 <sup>a</sup>
Total	20,079.4	18,323.6	17,118.1	16,950.9	16,954.7

The values at ADE correspond to its concentrations in the seed soaking solution. Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same row are significantly different ( $p < 0.05$ ).

TABLE 5. Isoflavone content (mg/kg dry weight) of 6-day-old soybean sprouts grown after seed soaking in different concentrations (0.5%–5%, w/v) of *Abeliophyllum distichum* extracts (ADE).

Sample*	Daidzin	Daidzein	Genistin	Genistein	Glycitin	Glycitein	Total
Control	332±10 <sup>d</sup>	16±3 <sup>c</sup>	292±4 <sup>d</sup>	35±3 <sup>a</sup>	84±4 <sup>c</sup>	10±1 <sup>a</sup>	769
ADE-0.5	342±11 <sup>c</sup>	20±2 <sup>b</sup>	300±3 <sup>c</sup>	23±1 <sup>b</sup>	90±3 <sup>b</sup>	11±2 <sup>a</sup>	786
ADE-1	351±10 <sup>b</sup>	22±2 <sup>b</sup>	303±8 <sup>c</sup>	21±2 <sup>bc</sup>	90±3 <sup>b</sup>	11±2 <sup>a</sup>	798
ADE-3	356±6 <sup>b</sup>	20±3 <sup>b</sup>	325±3 <sup>b</sup>	20±2 <sup>c</sup>	95±2 <sup>a</sup>	10±1 <sup>a</sup>	826
ADE-5	362±1 <sup>a</sup>	25±1 <sup>a</sup>	335±4 <sup>a</sup>	18±1 <sup>d</sup>	99±2 <sup>a</sup>	10±2 <sup>a</sup>	849

\* The values at ADE correspond to its concentrations in the seed soaking solution. Values are expressed as mean ± standard deviation of three replicates. Values followed by different letters in the same column are significantly different ( $p < 0.05$ ).

after the seed soaking with persimmon fruit powder and Puerh tea extracts [Kim *et al.*, 2017, 2020]. Soy isoflavones are found to be beneficial against a number of health disorders. Their coupled intake with vitamin D has been reported to mitigate the irritable bowel disease in female patients [Jalili *et al.*, 2016]. In addition, they have been shown to provide protection against breast and prostate cancers, osteoporosis, cardiovascular diseases, and diabetic conditions, and also to assuage menopause-related symptoms [Abdelrazek *et al.*, 2019; Sathyapalan *et al.*, 2018].

## CONCLUSIONS

The effects of seed soaking with different concentrations of the *A. distichum* extract on the growth and nutritional value of soybean sprouts were examined. The yield of soybean sprouts increased by up to 11.2% compared to the control. The vitamin C content also significantly improved with 1 to 5% of ADE treatment. Although the total free amino acid content was lower in the ADE-treated sprouts compared to the control, the amount of essential amino acid increased upon the 0.5 and 1% ADE treatments. The contents of minerals: Ca, Fe, Na, and Zn, were higher in at least one of the ADE-treated soybean sprouts. The isoflavone content

in all the ADE-treated soybean sprouts was higher than that of the control. Overall results suggest that soybean seed soaking with 1 or 3% of *A. distichum* extract could be a good option to improve the sprout yield and quality despite a reduction in some nutrient components.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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