

Effect of lacquer (*Toxicodendron vernicifluum*) extract on yield and nutritional value of soybean sprouts

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Abstract Soybean sprouts are the third-most consumed vegetable in Korea. Several studies on cultivation techniques, including use of medicinal plants extracts, have been performed to enhance the quality and yield of soybean sprouts. The objective of the present study was to investigate the effect of lacquer, a medicinal plant extract, on the yield and nutritional value of soybean sprouts. Linolenic acid content was significantly ($p<0.05$) increased in the sprouts produced by soaking seeds in lacquer extract diluted with equal volume of water. Lacquer extract significantly increased the flavonoid and phenolic contents ($p<0.05$). The content of total free amino acids, including γ -aminobutyric acid, was higher in lacquer extract-treated sprouts than in the untreated control. Results of this study suggest that lacquer extract could be used for enhancing the yield and nutritional values of soybean sprouts.

Keywords: antioxidant activity, lacquer extract, nutritional value, soybean sprout, yield

Introduction

Soybean sprouts are an important perennial vegetable in Korea, China, and Japan. Large-scale production of soybean sprouts for human consumption has been undertaken in Korea (1). Unlike other vegetables, sprouts can be grown in a considerably short time, and are edible without much processing techniques. They are consumed mainly as a side dish and are excellent source of nutrients (2). The demand for soybean sprouts has been increased because of the renewed interest in functional foods (3). Soybean sprouts, soy sauce, and other soy foods have beneficial health effects in heart disease, cancer, and osteoporosis (4-6). Production of sprouts is also gaining more popularity because of its reliable supply.

Many studies on cultivation techniques have been carried out to enhance the quality and yield of soybean sprouts. Soybean seeds and sprouts were exposed to γ -radiation to enhance their microbial safety (7). Seeds were subjected to germination in the presence of zinc sulfate solution to produce biofortified zinc-enriched soybean sprouts (8). In one study, sprouts were grown under different light conditions to determine the isoflavone content (9) while in another, bacterial strains were used to enhance bioactive contents and antioxidant activity of soybean sprouts (10). Brown seaweed, the seersucker kelp was also used to study the growth of soybean sprouts (11). A study showed that watering soybean sprouts with grapefruit seed extract, chitosan,

and phosphate buffer significantly increased yield and inhibited rot of the soybean sprouts (12). *Panax ginseng* extract also improved the quality of soybean sprouts (13).

A research on the effect of lacquer (*Toxicodendron vernicifluum*) extract on soybean sprouts has not yet been studied. Lacquer tree possesses medicinal properties (14). Lacquer consists of urushiol (50-80%), laccase (3-8%), nitrogen-containing substances (1-3%), and water (11-38%) (15). Urushiol causes inflammation and dermal sores on physical contact. However, it also shows antioxidant, anti-cancer, and immunity-enhancement properties. Lacquer polysaccharide and its derivatives show bioactive properties, such as anticoagulant (16,17), antitumor (18), and antileucopenia properties, induced by cyclophosphamide (19). Lacquer polysaccharide may be a promising antioxidant for application in pharmaceuticals (20,21). Plant-based extracts, such as brown seaweed (11), grapefruit seed (12), and ginseng (13), were found to increase the yield and/or quality of soybean sprouts. Since lacquer trees possess numerous medicinal properties, and no report has been published on their effects on yield and nutrient of soybean sprouts until date, the present study aimed to investigate the same.

Materials and Methods

Soybean seeds

Soybean seeds of cultivar 'Sohwang' (22) were obtained from the Foundation of Agricultural Technology Commercialization and Transfer, Seed Production Farm, Andong, Korea. The average seed weight of 100 seeds of the cultivar was 8.5 g.

Lacquer extraction

Lacquer (*Toxicodendron vernicifluum*) stems containing bark and wood were cut into 2-3 mm-thick slices. The slices were

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subjected to drying at 170°C for 5 h. One kilogram of the dried slices was extracted in 10 L of distilled water in a high-pressure extractor (1.5 kg/cm²) at 121°C for 2 h. The extract was filtered through filter paper (Whatman No. 2). The filtered lacquer extract (LE) was further diluted with water into different proportions: 1:0 (v/v), LE-0; 1:1 (v/v), LE-1; 1:2 (v/v), LE-2; 1:5 (v/v), LE-5; and 1:10 (v/v), LE-10.

Soybean sprouts cultivation

One kilogram of intact seeds was thoroughly washed with tap water and soaked in different concentrations of LE (3 L) for 6 h. The seeds soaked in water alone were considered as controls. After 6 h of soaking, the LE and water were drained out, and the seeds were kept in 15 L plastic buckets with a perforated base for cultivation of sprouts. The buckets of seeds and sprouts were irrigated with two hoses of 1 cm diameter for 2 min every 3 h. Soybean sprouts were grown at room temperature (20–22°C) for 6 days.

Sprout yield

Sprout yield was measured after 6 days by deducting the weight of the empty bucket from the weight of each bucket containing soybean sprouts. Variations in sprout yield among different treatments were measured on the basis of the yield of the control sample.

Sample preparation

Soybean sprouts were harvested after 6 days, stored at –70°C, and subjected to freeze drying. The freeze-dried sprouts were ground into a powder using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea) and filtered through a 100 mesh (0.149 mm) sieve. The powdered samples were stored at 4°C until analysis. Physicochemical analyses were conducted in triplicate, unless otherwise mentioned.

Color value determination

Color values were measured as L* (lightness), a* (redness, + or greenness, –), b* (yellowness, + or blueness, –) values of sprouts powder using a Chroma Meter (CR-400, Minolta, Tokyo, Japan). A calibration plate (CR-A43, Minolta) was used to standardize the instrument using a D65 illuminant. Color values were measured on 10 spots of powdered samples, and the average value was calculated.

Free amino acid composition

Free amino acids were analyzed following the method given by Aristoy and Toldra (23) with some modifications. One gram of sample was extracted in 20 mL of trichloroacetic acid (TCA, 3% in water) using a shaking incubator (150 rpm, 25°C) for 2 h. The mixture was centrifuged (2,259×g, 15 min) and filtered through a 0.2 µm syringe filter (Waters, Milford, MA, USA). Contents of free amino acids were measured using HPLC (L-8900, Hitachi Ltd., Tokyo, Japan) under following conditions: mobile phase, PF-1,2,3,4,6, PF-RG, R-3, C-1; ninhydrin solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan); amino

acid separator, ion exchange column; column temperature, 50°C; reactor temperature, 135°C; sample injection volume, 20 µL. Amino acid standard solutions, Type ANII and Type B were purchased (Wako Pure Chemical Industries, Ltd.).

Fatty acid composition

Fatty acid profile in soybean sprouts was determined using gas chromatography (GC), using a previously described method (24) with some modifications. Oil was extracted from 0.1 g of sample powder in 1 mL of extraction reagent [chloroform:hexane:methanol (8:5:2, v/v/v)] overnight at room temperature. Derivatization was done by transferring 150 µL of extract to the vials and by adding 75 µL of the methylation reagent [0.25 M methanolic sodium methoxide:petroleum ether:ethyl ether (1:5:2, v/v/v)]. Hexane (775 µL) was added to make up the mixture volume to 1 mL. A gas chromatograph (7890A, Agilent Technology, Palo Alto, CA, USA) fitted with a flame ionization detector was used for fatty acid profiling. GC conditions for fatty acid analysis were as follows: column, 0.25 µm i.d.×30 m DB-FFAP capillary column; oven temperature, 230°C; injection temperature, 210°C; detector temperature, 250°C; carrier gas, N₂ (1.5 mL/min); and injection volume, 1 µL.

Total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method (25). Powdered sample (1 g) was extracted with methanol (10 mL) in a shaking incubator (150 rpm, 25°C) for 24 h. The mixture was centrifuged (2,259×g, 15 min), and the supernatant was filtered through a 0.2 µm syringe filter (Waters). Fifty microliters of methanolic extract were added to 1 mL aqueous solution of 2% sodium carbonate and incubated for 3 min. The mixture was incubated with 50 µL of 1 N Folin-Ciocalteu reagent in the dark for 30 min at room temperature. The absorbance was measured at 750 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fischer Scientific, Vantaa, Finland). The standard calibration curve was plotted using gallic acid.

Flavonoid content

Flavonoid content in soybean sprouts was determined following the method given by Zhishen *et al.* (26) with some modifications. Powdered sample (1 g) was extracted with methanol (10 mL) in a shaking incubator (150 rpm, 25°C) for 24 h. The mixture was centrifuged (2,259×g, 15 min), and the supernatant was filtered through a 0.2 µm syringe filter (Waters). Sample extract (100 µL), methanol (500 µL), 10% AlCl₃ (50 µL), 1 M HCl (50 µL), and dH₂O (300 µL) were mixed and incubated in dark for 30 min. Absorbance reading was measured at 510 nm using a microplate spectrophotometer (Multiskan GO). The standard calibration curve was plotted using quercetin (QE).

DPPH radical scavenging activity

The free radical scavenging activity of soybean sprouts was measured following the protocols described by Blois (27) with some modifications. One gram of powdered sample was extracted in 10 mL methanol in a shaking incubator (150 rpm, 25°C) for

24 h. The mixture was centrifuged ($2,259\times g$, 15 min) at room temperature, and the supernatant was filtered through a $0.2\ \mu\text{m}$ syringe filter (Waters). Freshly prepared DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in methanol (99.9%) was used for the experiment. A mixture of equal volumes of sample extract and freshly prepared 0.1% DPPH solution was incubated in the dark for 30 min. The absorbance was measured at 517 nm using a microplate spectrophotometer (Multiskan GO). Equal proportions of methanol and sample extract were mixed to measure the absorbance of control. The DPPH radical scavenging activity was calculated from the absorbance (A) using the following equation:

$$\text{DPPH scavenging ability (\%)} = [1 - (A_i - A_j) / A_c] \times 100$$

where, A_i is absorbance of extract and DPPH, A_j is absorbance of extract and methanol, and A_c is absorbance of DPPH and methanol.

Statistical analyses

Data were analyzed using SAS 9.3 (SAS Institute, Cary, NC, USA) statistical package to generate analysis of variance (ANOVA) and significant differences among treatment means were identified using Tukey test at 5% probability level.

Results and Discussion

Yield

LE treatment increased the sprout yield after 6 days, as compared with that of the control. The average sprout yields of control, LE-0, LE-1, LE-2, LE-5, and LE-10, were 4.94, 5.13, 5.37, 5.12, 5.19, and 5.22 kg, respectively. All the LE treated sprouts showed higher yields than those of the controls. The results of this experiment showed that better yield of soybean sprouts could be achieved with LE diluted with equal volume of water.

Color value of soybean sprout

The 'L' value is a measure of lightness, from completely opaque (0) to completely transparent (100), the 'a' value is a measure of redness ('-a' greenness), and the 'b' value is a measure of yellowness ('-b' blueness). The lightness value of LE-1 (82.64) was significantly higher than those of LE-2 (81.49) and LE-5 (81.83). However, the value was not significantly different from those of the control, LE-0, and LE-10. Redness value of LE-2 (-4.72) was the highest, whereas yellowness value was the lowest for LE-1 (Table 1).

Free amino acid composition

The amount of amino acids is one of the key factors determining the nutritional values of fruits, vegetables, and foods (28). Twenty eight free amino acids were detected in the soybean sprouts (Table 2), and twenty five free amino acids were found in all the treatments. However, glutamic acid, histidine, and citrulline were not detected in the control. The content of γ -amino-n-butyric acid (GABA) was the highest for

Table 1. Hunter's color values of soybean sprout powder

Sample ¹⁾	Color value ²⁾		
	Lightness (L)	Redness (a)	Yellowness (b)
Control	82.23±0.09 ^{ab3)}	-5.46±0.03 ^c	32.22±0.14 ^{ab}
LE-0	82.06±0.27 ^{abc}	-5.12±0.02 ^c	31.75±0.21 ^b
LE-1	82.64±0.05 ^a	-4.93±0.02 ^b	31.23±0.12 ^c
LE-2	81.49±0.27 ^c	-4.72±0.03 ^a	32.02±0.23 ^{ab}
LE-5	81.83±0.12 ^{bc}	-5.11±0.02 ^c	32.39±0.15 ^a
LE-10	82.32±0.27 ^{ab}	-5.22±0.03 ^d	32.48±0.16 ^a

¹⁾Control: soybean seed soaked in tap water; soybean seed soaked in the ratio of lacquer extract and water (v/v) with 1:0 (LE-0), 1:1 (LE-1), 1:2 (LE-2), 1:5 (LE-5), 1:10 (LE-10).

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

³⁾Different superscripts followed by mean±standard error values in the same column are significantly different by Tukey test (n=10, $p<0.05$).

LE-2 (263.46 $\mu\text{g/g}$), followed by LE-10 (236.99 $\mu\text{g/g}$), LE-1 (200.995 $\mu\text{g/g}$), LE-0 (177.814 $\mu\text{g/g}$), and LE-5 (155.99 $\mu\text{g/g}$). The control sample (153.49 $\mu\text{g/g}$) showed the lowest amount of GABA. Central nervous system inhibitory neurotransmitters, such as GABA and glycine, are associated with learning, memory, stroke, and neurodegenerative diseases. They mediate signals between neurons that inhibit neutral amino acids, thus relieving anxiety, sedation, in addition to anticonvulsant and muscle relaxation functions (29-31). Food with a high GABA content, also known as brain food, is capable of enhancing blood cholesterol, suppressing triglyceride blood pressure, improving cerebral blood flow, functioning as an antioxidant and diuretic, improving conditions such as insomnia, depression, and anxiety, and stabilizing nerves and pain (32). GABA reduces stress, helps brain development, reduces high blood pressure and body fat, and prevents dementia.

Total free amino acid content was the maximum in LE-10 (7,300.18 $\mu\text{g/g}$), followed by LE-2 (6,777.64 $\mu\text{g/g}$), and LE-1 (6,037.60 $\mu\text{g/g}$), whereas the control showed the lowest value (4,107.78 $\mu\text{g/g}$). The use of lacquer extracts during seed soaking showed an increase in the free amino acid contents of soybean sprouts.

Fatty acid composition

Fatty acid composition of soybean sprouts was not significantly altered on using lacquer extract, except for the linolenic content in LE-1 (11.62%) (Table 3). Results of this experiment suggest that fatty acids content of soybean sprouts is not highly influenced by lacquer extract during seed soaking.

Total phenolic contents

Total phenolic contents of the control, LE-0, LE-1, LE-2, LE-5, and LE-10 were 447.94, 580.69, 593.13, 632, 521.44, and 551.56 $\mu\text{g/g}$, respectively (Table 4). Total phenolic contents of LE-1 and LE-2 were significantly higher than those of the other samples. The control showed the lowest value for total phenolic contents. Total phenolic contents were high in moderate dilutions of lacquer extracts, and were lower in both concentrated

Table 2. Free amino acid ($\mu\text{g/g}$ of dry weight) composition of soybean sprouts

Amino acid	Sample ¹⁾					
	Control	LE-0	LE-1	LE-2	LE-5	LE-10
Phosphoserine	51.11	64.30	64.19	73.16	55.71	79.78
Phosphoethanolamine	39.07	51.16	51.89	73.44	41.32	82.86
Urea	24.16	27.08	32.24	41.54	25.69	43.30
Aspartic acid	242.71	296.33	321.71	345.88	274.86	349.51
Threonine	315.43	385.18	403.03	442.24	348.33	472.74
Serine	530.83	619.94	602.11	703.12	572.04	763.43
Glutamic acid	nd ²⁾	738.63	848.33	852.68	733.33	1110.72
α -Aminoasipic acid	108.69	137.19	146.77	159.70	123.26	177.49
Glycine	51.79	52.42	46.49	56.21	49.90	62.68
Alanine	395.94	408.22	348.22	440.98	376.78	486.38
Citrulline	nd	29.10	28.43	31.96	26.59	36.85
α -Amino-n-butyric acid	43.17	45.80	46.34	53.76	43.53	55.23
Valine	561.48	688.89	732.82	825.56	629.29	859.35
Methionine	29.52	40.36	48.69	70.49	45.71	68.83
Isoleucine	244.31	293.97	312.99	383.14	264.57	381.96
Leucine	95.53	121.67	140.71	160.83	110.55	162.01
Tyrosine	20.05	29.11	27.26	38.47	24.90	39.85
β -Alanine	90.07	97.06	97.06	102.69	80.66	110.56
β -Aminoisobutyric acid	56.67	56.25	77.28	71.49	39.23	71.25
γ -Amino-n-butyric acid	153.49	177.81	201.00	263.46	155.99	236.99
Ethanol amine	51.58	58.39	72.82	80.19	54.25	77.65
Ammonia	41.93	42.66	49.95	51.41	43.90	49.92
Lysine	3.15	4.34	5.21	6.33	3.93	6.15
1-Methylhistidine	177.92	224.49	239.75	269.26	203.45	276.54
Histidine	nd	nd	24.14	29.52	18.85	28.39
3-Methylhistidine	686.62	875.44	966.69	1042.41	801.54	1100.23
Hydroxy proline	16.83	19.09	32.22	30.09	20.21	22.45
Proline	75.73	79.14	69.31	77.65	76.93	87.08
Total	4107.78	5664.02	6037.60	6777.64	5245.31	7300.18

¹⁾A description of each sample is listed in Table 1.

²⁾nd: not detected.

Table 3. Fatty acid composition (% of total oil) of soybean sprouts

Sample ¹⁾	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Control	12.70 \pm 0.06 ^{a2)}	3.91 \pm 0.16 ^a	21.22 \pm 0.57 ^a	51.52 \pm 0.28 ^a	10.64 \pm 0.20 ^b
LE-0	12.78 \pm 0.04 ^a	4.14 \pm 0.15 ^a	21.27 \pm 0.76 ^a	51.37 \pm 0.52 ^a	10.45 \pm 0.21 ^b
LE-1	12.96 \pm 0.25 ^a	4.05 \pm 0.13 ^a	20.95 \pm 0.44 ^a	51.77 \pm 0.46 ^a	11.62 \pm 0.29 ^a
LE-2	12.74 \pm 0.05 ^a	3.87 \pm 0.11 ^a	20.82 \pm 0.28 ^a	51.65 \pm 0.16 ^a	10.92 \pm 0.06 ^b
LE-5	12.73 \pm 0.10 ^a	3.84 \pm 0.06 ^a	21.02 \pm 0.21 ^a	51.42 \pm 0.17 ^a	10.99 \pm 0.17 ^b
LE-10	12.82 \pm 0.6 ^a	3.90 \pm 0.05 ^a	21.43 \pm 0.29 ^a	51.22 \pm 0.24 ^a	10.64 \pm 0.07 ^b

¹⁾A description of each sample is listed in Table 1.

²⁾Different superscripts followed by mean \pm standard error values in the same column are significantly different by Tukey test ($n=3$, $p<0.05$).

(LE-0) and highly diluted lacquer extracts (LE-5 and LE-10).

The high total phenolic contents in the soybean sprouts soaked with moderately diluted lacquer extract might be due to the oxidative stress caused by the chemicals present in the lacquer extracts (20,21) and/or higher antioxidant potentials of lacquer (33,34). It is evident from this experiment that lacquer extract enhances total phenolic content of soybean sprouts during seed soaking.

Flavonoid content

The total flavonoid content in various sprout samples showed varying results in the range of 1,035.25-1,320.75 μg QE/g (Table 4). Soybean sprouts grown with LE-2 (1,320.75 $\mu\text{g/g}$) extract had the highest flavonoid content, followed by LE-5 (1,238.13 $\mu\text{g/g}$), LE-1 (1,128.13 $\mu\text{g/g}$), and the lowest (1,035.25 $\mu\text{g/g}$) content in the control. The high flavonoid content in the soybean sprouts soaked with moderately diluted lacquer extract might be

Table 4. Total phenolic and flavonoid contents and DPPH radical scavenging activity of soybean sprouts

Sample ¹⁾	Polyphenolic content (µg GAE ²⁾ /g)	Total flavonoid content (µg QE ³⁾ /g)	DPPH radical scavenging capacity (%)
Control	447.38±9.44 ^d	1035.25±46.25 ^c	95.13±0.40 ^{a4)}
LE-0	580.69±8.31 ^{abc}	1039.50±1.75 ^c	94.69±0.40 ^a
LE-1	593.13±15.13 ^{ab}	1128.13±11.63 ^{bc}	94.98±0.18 ^a
LE-2	632.00±16.5 ^a	1320.75±1.00 ^a	94.88±0.15 ^a
LE-5	521.44±10.31 ^c	1238.13±2.63 ^{ab}	94.91±0.25 ^a
LE-10	551.56±7.31 ^{bc}	1043.13±17.38 ^c	94.95±0.13 ^a

¹⁾A description of each sample is listed in Table 1.

²⁾Gallic acid equivalent.

³⁾Quercetin equivalent.

⁴⁾Different superscripts followed by mean±standard error values in the same column are significantly different by Tukey test ($n=3$, $p<0.05$).

due to the effects of antioxidative properties of the lacquer extracts (20,21) and/or higher antioxidant potentials of lacquer itself (33,34). These results support the hypotheses that lacquer extract during seed soaking enhances the antioxidative potential of soybean sprouts.

DPPH radical scavenging activity

The DPPH radical scavenging activity of soybean sprouts treated with different concentrations of lacquer extract during seed soaking did not differ significantly (Table 4).

Conclusion

Several aspects of soybean sprouts, such as yield, nutritional value, total phenolic content, and total flavonoid content were enhanced with the use of lacquer extract during seed soaking. Fatty acid content was not significantly affected, except for the linolenic acid content in sprouts from seeds soaked in equal volume of lacquer extract and water. Flavonoid and total phenolic contents increased significantly with the use of lacquer extract. However, the effect of DPPH radical scavenging potential was not different in the presence of lacquer extract. The levels of total free amino acids, including GABA content, were higher in the sprouts treated with lacquer extract than in the control. Based on these results, LE-1 and LE-2 were found to be optimum concentrations of lacquer for yield and nutrient improvement. Thus, lacquer extract could be used in enhancing the physicochemical properties of soybean sprouts.

Conflict of interests

The authors declare no conflicts of interest.

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