Persimmon Fruit Affects Bacterial Growth, Hardness, Vitamin C and Chlorophyll Content of Soybean Sprouts during Storage

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Soybean sprouts have been established as an important year-round vegetable in Korea, China, and Japan for a long time. Very few studies on quality of soybean sprouts during storage have been reported so far although considerably large number of reports have been available for production techniques. Since persimmon fruit powder is reported to have positive influence on yield and nutritional value of soybean sprouts, the objective of the present study was to investigate the effect of persimmon fruit powder on quality of soybean sprouts during storage at room temperature (20±1°C) and at low temperature (4±1°C). Bacterial count was low in treated soybean sprouts as compared to that of untreated control. Vitamin C content was high in treated soybean sprouts but the rate of its depletion was high in untreated control sample. Chlorophyll content was not affected during storage at room temperature. Storage caused decrease in hardness of soybean sprouts and the rate of decrement in hardness was higher at untreated control than in treated samples. This study showed that soybean seeds soaking in water containing persimmon fruit powder could help reduce bacterial infection, resist in depleting vitamin C content, and maintain the texture of soybean sprouts during storage.

Keywords: Hardness, Nutritional value, Persimmon fruit powder, Soybean sprout, Storage.

Soybean is one of the important crops and has been included in Asian cuisine for centuries because of their beneficial health effect[1]. Among various products of soybean, sprouts have been established as an important year-round vegetable in Korea, China, and Japan for a long time. Germination has been found to help improve nutritional potential[2] and reduce the effects of antinutritional factors such as lectins and enzyme inhibitors present in the seeds[3]. Considerable amount of soybean sprouts is produced for human consumption in Korea[4]. They are consumed mainly as side dish and are good source of nutrients and vitamins[5]. Consumers's renewed interests towards functional foods have resulted in increased demand of soybean sprouts[6]. Production of sprouts also may help improve the greater stability of food supply because of its quick growing potentiality.

Nutritive value, texture, and organoleptic characteristics of legumes could be improved[7, 8]; undesirable anti-nutritional factors be reduced; and antioxidant potentials be increased[9,10] by allowing the seeds for germination.

A number of experiments have been conducted aiming to enhance the yield and quality of soybean sprouts by employing different seed treatment and cultivation techniques[11-16]. Few

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studies have been carried out to investigate the effect of storage on quality of seed sprouts. Koo *et al*[17] studied the quality change of cooked soybean sprouts during storage. Zwieca and Gawlik-Dziki[18] investigated the effects of sprouting and postharvest storage under cool temperature conditions on starch content and antioxidant capacity of green pea, lentil and young mung bean sprouts. In another study impact of cold storage on glucosinolate levels in seed-sprouts of broccoli, rocket, white radish, and kohl-rabi was examined[19]. However, very few studies have been reported for effect of different storage temperatures on the quality of uncooked soybean sprouts. Since persimmon fruit powder enhanced yield and nutritional value of soybean sprouts[20] objective of the present study was to investigate the influence of storage conditions on vitamin C and chlorophyll content and hardness of soybean sprouts treated with persimmon fruit powder.

MATERIALS AND METHODS

Soybean seed and persimmon powder

Soybean (*Glycine max* L.) seeds of Sowon, a sprout growing cultivar having 12 g of 100-seed weight, were purchased from a local store in Deagu, Korea. The freeze-dried fruits of persimmon cv. Sangjudungsi were ground into powder (Speed Rotor Mill, Model KT-02A, Shanghai, China) and passed through a 200-mesh sieve.

Cultivation and storage of soybean sprouts

Soybean sprouts were grown following the method described by Kim *et al*. [20]. Seeds were soaked in water containing four different amounts of persimmon fruit powder and one untreated control for 6 h. The treatments were named as PP-1 (seeds soaked in water containing 0.5% (v/w) powder), PP-2 (seeds soaked in water containing 1% (v/w) powder), PP-3 (seeds soaked in water containing 2.5 % (v/w) powder), and PP-4 (seeds soaked in water containing 5% (v/w) powder). Soybean sprouts were grown at room temperature (20±1°C) for 6 d. The spoiled sprouts were removed out and the good ones were put into transparent polyethylene (PE) zipper bags and stored at room temperature (20±1°C) or low temperature (4±1°C). Ten PE zipper bags containing 300 g of soybean sprouts each from all the five treatment groups were sealed and stored at different temperatures until 5 d. Samples for physicochemical studies were prepared by freeze-drying. The freeze-dried soybean sprouts were ground into powder using an electric grinder ((HMF 3450S, Hanil Co., Seoul, Korea) and passed through a 100-mesh sieve. The strained samples were packed into airtight sample bottles and stored at -20°C until analysis.

Bacterial count of soybean sprouts

Ten gram of sample was mixed with 100 mL of 0.1% peptone solution and vortexed for 3 min. Portions (0.1 mL) of appropriate dilutions were spread in triplicate on petrifilm aerobic count plates (3M Microbiology, St. Paul, MN, USA). Bacterial growth was estimated by plating out on petrifilm and counting after 36 h of incubation at 32°C[21].

Determination of hardness

Hardness was measured using rheometer (COMPAC-100, Sun Scientific Co. Ltd., Tokyo, Japan) under the following operational conditions: test type, mastication; adaptor type, circle; adaptor area, 0.20 cm^2; and table speed, 60 mm/min. Sprouts from 10 PE bags were randomly measured for hardness test and average value of triplicate measurements were reported.

Vitamin C analysis

Vitamin C content was determined following the standard methods[22] and reported as mg/100 g fresh weight (fw). Twenty grams of sample was blended in 30 mL of 3% metaphosphoric acid solution and homogenized (AM-8, Nihonseike Kaisha, Tokyo, Japan). The homogenate was extracted in 50 mL after filtration. Twenty-five milliliter of the extract was then titrated with 0.025% of 2,6-dichloroindophenol. Triplicate measurements were considered for statistical analysis.

Determination of chlorophyll content

Chlorophyll content in the soybean sprouts were determined using the spectrophotometric method as described by Vernon[23]. Soybean sprout powder (0.3 g) was extracted in 62.5 mL of 80% acetone, filtered, and the filtrate was made to 125 mL with 80% acetone. The absorbance was read using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). Triplicate measurements were considered for statistical analysis.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and differences between means at $p<0.05$ were analyzed using the Tukey test. The statistics package version 4.0 (Analytical Software, Tucson, AZ, USA) was used for statistical analysis. Data are reported as average±SD.

RESULTS

Vitamin C of soybean sprouts during storage at 4 and 20°C

Vitamin C contents of control, PP-1, PP-2, PP-3, and PP-4 were 14.0±1.2, 16.32±0.21, 16.21±0.34, 17.36±0.15, and 17.28±0.21 mg/100 g fw, respectively on 0 d of sprout harvest. Changes in residual rate of vitamin C of soybean sprouts during storage at room temperature (20±1°C) and low temperature (4±1°C) have been shown in Table 1.

During storage at 4°C the residual rates of vitamin C content on 1 d was not significantly different whereas those on 3 and 5 d were significantly different among treatments (Table 1). On 3 d highest residual rates were observed for PP-4 and PP-3 followed by PP-2 and PP-1. Similarly, highest residual rates were found on PP-3 and PP-4 followed by PP-2 and PP-1 on 5 d of storage. The lowest residual rates on 3 and 5 d were observed in untreated control sample. Residual rates of vitamin C content in the sprout samples stored at 20°C also showed similar trends as in those stored at 4°C that is, samples treated with higher amount of persimmon fruit powder during seed soaking contained higher amount of vitamin C on 1 and 3 d (Table 1). Analysis on 5 d was not carried out due to deteriorated quality of sprouts stored at room temperature.

Chlorophyll content of soybean sprouts during storage at 4 and 20°C

Chlorophyll content of soybean sprouts was found to be increased with storage time (Table 2). The chlorophyll contents of sprout samples stored at room temperature were not significantly different on 3 d of storage, however were different in those stored at low temperature. The sprouts obtained from persimmon fruit powder treated seeds generally contained higher chlorophyll than in untreated sample, however the differences were not consistent for all samples and storage periods.

Instrumental hardness of soybean sprouts during storage at 4 and 20°C

Hardness of the soybean sprouts was reduced for all samples during storage at room as well as low temperatures (Table 3). The hardness value was significantly high for treated samples as compared to untreated control until 5 d of storage. Samples stored at room temperature for 3 d showed lower hardness than those of stored at low temperature for 5 d. Results showed that the texture

Table 1. Changes in residual rate of vitamin C (% of the day of sprout harvest) of soybean sprouts during storage at room temperature (20±1°C) and low temperature (4±1°C)

Sample[1)]	Storage (day)[3)]				
	Room temperature		Low temperature		
	1	3	1	3	5
Control	78.5±0.3[2)]c	48.8±0.3d	99.1±0.1a	81.9±0.3c	38.1±0.3c
PP-1	80.3±0.2b	50.2±0.1c	98.3±0.2a	85.2±0.2b	38.7±0.2c
PP-2	84.3±0.2a	55.3±0.2b	98.5±0.1a	85.7±0.3b	40.2±0.2b
PP-3	84.0±0.3a	58.8±0.3a	99.2±0.1a	87.6±0.2a	45.7±0.2a
PP-4	83.2±0.4a	59.2±0.2a	98.1±0.2a	88.1±0.1a	45.5±0.3a

[1)]Control, soybeans soaked in water for 6 h; PP-1, soybean soaked in water with 0.5% persimmon powder for 6 h; PP-2, soybeans soaked in water with 1% persimmon powder for 6 h; PP-3, soybeans soaked in water with 2.5% persimmon powder for 6 h; PP-4, soybeans soaked in water with 5% powder for 6 h.
[2)]Quoted values are means of triplicate experiments. Values followed by different letters within a column indicate significant difference ($p<0.05$).
[3)]Vitamin C contents (mg/100g-fresh weight) of soybean sprouts were 14.0±1.2 mg for control, 16.32±0.21 mg for PP-1, 16.21±0.34 mg for PP-2, 17.36±0.15 mg for PP-3, 17.28±0.21 mg for PP-4.

of soybean sprouts could be preserved at room as well as low temperatures for relatively longer period if treated with persimmon fruit powder.

Bacterial growth during storage at 4 and 20°C

Bacterial growth was significantly affected by the treatment and storage (Table 4). Until 3 d of storage at room or low temperature, the bacterial count was significantly high in untreated control sample as compared to treated ones. The bacterial counts were not significantly different among various samples on 5 d of storage at low temperature. The bacterial counts in soybean sprouts were found to be reduced with the increased amount of persimmon fruit powder added during seed soaking.

DISCUSSION

Results of vitamin C showed that significantly high level of its content could be obtained with persimmon fruit powder treatment. Although the reason for higher vitamin C content in the persimmon fruit treated soybean sprouts was not well understood, persimmon fruits are rich in it[24,25]. The residual rate decreased with increased storage temperature and duration. Koo *et al.*[17] found decreasing ascorbic acid content of blanched soybean sprouts during storage at 3 and 10°C. Waje *et al*[26] also observed that the vitamin C content in broccoli sprouts was reduced during storage at 4 and 8°C.

Table 2. Changes in chlorophyll content (mg%-fresh weight) of soybean sprouts during storage at room temperature (20±1°C) and low temperature (4±1°C)

Sample[1)]	Storage (day)			
		Room temperature	Low temperature	
	0	3	3	5
Control	1.8±0.1[2)]a	3.6±0.1a	2.8±0.1b	3.1±0.1b
PP-1	1.7±0.0a	3.5±0.1a	2.8±0.1b	3.2±0.1ab
PP-2	1.8±0.0a	3.6±0.1a	3.1±0.2ab	3.3±0.1ab
PP-3	1.7±0.1a	3.5±0.1a	3.3±0.1a	3.4±0.1a
PP-4	1.8±0.0a	3.4±0.1a	3.2±0.1a	3.3±0.2ab

[1)]Control, soybeans soaked in water for 6 h; PP-1, soybean soaked in water with 0.5% persimmon powder for 6 h; PP-2, soybeans soaked in water with 1% persimmon powder for 6 h; PP-3, soybeans soaked in water with 2.5% persimmon powder for 6 h; PP-4, soybeans soaked in water with 5% powder for 6 h.
[2)]Quoted values are means of duplicate experiments. Values followed by different letters within a column indicate significant difference (p<0.05).

Table 3. Changes in instrumental hardness (Kg_f) of soybean sprouts during storage at room temperature (20±1°C) and low temperature (4±1°C)

Sample[1)]	Storage (day)			
		Room temperature	Low temperature	
	0	3	3	5
Control	1.32±0.03[2)]c	0.33±0.02b	0.88±0.01b	0.66±0.02c
PP-1	1.34±0.04c	0.36±0.02b	0.92±0.02a	0.71±0.02ab
PP-2	1.47±0.03b	0.42±0.03a	0.94±0.01a	0.72±0.02ab
PP-3	1.55±0.02a	0.47±0.02a	0.96±0.03a	0.75±0.02ab
PP-4	1.56±0.03a	0.45±0.02a	0.97±0.03a	0.76±0.02a

[1)]Control, soybeans soaked in water for 6 h; PP-1, soybean soaked in water with 0.5% persimmon powder for 6 h; PP-2, soybeans soaked in water with 1% persimmon powder for 6 h; PP-3, soybeans soaked in water with 2.5% persimmon powder for 6 h; PP-4, soybeans soaked in water with 5% powder for 6 h.
[2)]Quoted values are means of triplicate experiments. Values followed by different letters within a column indicate significant difference (p<0.05).

Table 4. Changes in bacterial counts of soybean sprouts during storage at room temperature (20±1°C) and low temperature (4±1°C)

Sample[1)]	Storage (day)				
	Room temperature		Low temperature		
	1	3	1	3	5
Control	6.01±0.05[2)]a	9.78±0.06a	5.34±0.04b	6.77±0.03a	8.89±0.04a
PP-1	6.00±0.06a	9.70±0.02a	5.55±0.02a	6.81±0.04a	8.80±0.06a
PP-2	5.59±0.07b	9.69±0.04b	5.40±0.03b	6.50±0.03b	8.85±0.03a
PP-3	5.02±0.03c	9.50±0.06c	5.03±0.04c	6.03±0.03c	8.83±0.05a
PP-4	5.10±0.02b	9.57±0.07c	5.01±0.03c	6.00±0.05c	8.81±0.06a

[1)]Control, soybeans soaked in water for 6 h; PP-1, soybean soaked in water with 0.5% persimmon powder for 6 h; PP-2, soybeans soaked in water with 1% persimmon powder for 6 h; PP-3, soybeans soaked in water with 2.5% persimmon powder for 6 h; PP-4, soybeans soaked in water with 5% powder for 6 h.
[2)]Quoted values are means of triplicate experiments. Values followed by different letters within a column indicate significant difference ($p<0.05$).

Lee *et al.*[27] found increased greenness of soybean sprouts during storage. The increment in chlorophyll content in the sprouts might be due to exposure to light during storage and moreover due to chlorophyll protein, CP668, which might be produced by a catalytic action of chlorophyllase[28]. Chlorophyll shows beneficial health effect as it could play primary roles in chemoprotection and anti-carcinogenic effects[29,30].

The results of instrumental hardness in the present experiment also agreed to those of Lee *et al*[27]. They also found the instrumental hardness of the soybean sprouts to be decreased during storage. The reduced hardness during storage might be as a result of loosened starch structure, which probably creates a large space within the matrix and increases the susceptibility to enzymatic attack[31].

The lower values for bacterial counts in the persimmon fruit powder treated soybean sprouts might be due to antibacterial compounds present in persimmon fruit. Gallic acid, methylgallate, ellagic acid, kaempferol, quercetin,myricetin, myricetin 3-O-beta-glucuronide, and myricetin-3-O-alpha-rhamnoside were found in persimmon[32] and these compounds were presumed to be responsible for antibacterial effect[33]. Arakawa *et al.*[34] stated that the antibacterial activities of persimmon were related with their hydrogen peroxide concentration. Zhang *et al.*[35] also found antibacterial potential in persimmon.

CONCLUSION

Vitamin C content was high in persimmon fruit powder treated soybean sprouts as compared to that of untreated control. It was gradually reduced with storage duration. The reduction rate of vitamin C was high at room temperature (20±1°C) as compared to that of stored at low temperature (4±1°C). Chlorophyll, which also plays a primary roles in chemoprotection and anti-carcinogenic effects, was found to be increased with storage time at room temperature as well as at low temperature. Storage caused decrease in hardness of soybean sprouts. The rate of decrement in hardness was higher at room temperature than in low temperature. Persimmon fruit powder treatment showed lower bacterial growth in soybean sprouts during storage. This study showed that soybean seeds soaking in water containing persimmon fruit powder could better resist in depleting vitamin C content, inhibit bacterial growth, and help maintain the texture of soybean sprouts during storage at room as well as low temperatures.

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