

## Modified Cultivation Methods Improve Shelf-life and Quality of Soybean Sprouts, Effects of Treatment with Oak Charcoal and *Citrus sunki* Seed Extract

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### Abstract

The effects of cultivation methods (cultivation of curled-shaped type, M-1; conventional cultivation, M-2; growing after treatment with a growth regulator, M-3; cultivation by the combination of M-1 and treatment with oak charcoal, M-4) on the quality characteristics of soybean sprouts were studied by the measurement of growth characteristics. This study also investigated the changes in shelf-life stability of the new soybean sprouts (NSB) using M-4, which was cultivated with oak charcoal and treated with antimicrobial *Citrus sunki* seed extract. Among the soybean sprouts grown for six days at the high temperature and humidity environment ( $90 \pm 5\%$  RH,  $25 \pm 1^\circ\text{C}$ ), M-1 revealed no significant difference in terms of quality, such as the harvest yield, the rot rate and the growth characteristics when compared with M-2. M-3 showed no significant difference in growth characteristics, of hardness, and sensory evaluation scores when compared with the soybean sprouts grown by conventional methods. NSB had a low number of total microorganisms and had a better appearance after five days of storage than did the control group (M-2). These findings demonstrate that chemical-free and clean soybean sprouts can be grown by combining oak charcoal and antimicrobial *Citrus sunki* seed extract, thereby meeting the consumer demand for safe, chemical free sprouts.

**Key words:** soybean sprouts, cultivation, oak charcoal, *Citrus sunki* seed extract

### INTRODUCTION

Soybean sprouts have been traditionally for food in Korea since the *Koryeo* dynasty (A.D. 918 ~ 1392). Soybean sprouts can be easily grown throughout the year, thereby providing an inexpensive source of fresh vegetables regardless of season making them an important source of vitamins, inorganic matter and other physiological active substances which are often lacking in the Korean diet. Soybean sprouts are a particularly good source of isoflavones, supplying much more than green vegetables, fruits or seeds (1), and soybean sprout consumption is associated with decreased risk menopausal disorders in women and preventing hormone-related breast cancer (2), osteoporosis (3) and coronary heart disease (4, 5). In light of the demand for a greater variety of food selections among consumers who have a high quality, safety and health orientation, greater preference is given to agricultural chemical-free and clean soybean sprouts.

The production process for soybean sprouts can be largely divided into the selection of raw material soybeans, dipping, germination and sprinkling. They differ considerably in the harvest rate, quality and freshness according to the growing environment. Previous studies of

soybean sprouts have concentrated primarily on changes during germination and growth in the concentrations of nutrients such as vitamins including ascorbic acid, riboflavin and thiamin (6,7); amino acids (8); nitrogen compounds (9), carbohydrates (7), asparagin (10). Research on the growth rates of soybean has focused on increased production through the use of the growth regulators (11), fungicides (12), artificial sunlight (13) and treatment with CO<sub>2</sub> (14). Increasing consumer demand for high quality foods with chemical free certification by the National Agricultural Products Inspection Center has shifted recent research to studies that emphasize the development of technologies for environmentally-friendly production and preservation of freshness by using natural preservatives such as plant extracts (15,16), chitosan (12) and ozonized water (17).

Therefore, this study was designed to determine the growth characteristics of soybean sprouts using a newly-introduced oak charcoal cultivation method without the use of agricultural chemicals such as growth regulators and seed sterilizers. This study also evaluated the antimicrobial activity against soybean-sprout rot microorganisms of an antimicrobial liquid treatment made from *Citrus sunki* seed extract from a species of native citrus

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in Jeju Island. Data from this study should be valuable for the development of production methods for producing high-quality, chemical-free soybean sprouts that are consistent with consumer preference while maintaining a long shelf-life.

## MATERIALS AND METHODS

### Materials

The soybean selected for the cultivation of soybean sprouts (*Glycine max* L. Merrill) was purchased from a local Agricultural Cooperative (*Junjeori* soybean produced on Jeju Island in 2001). The charcoal used for growing soybean sprouts was oak charcoal sold in a local market. The *Citrus sunki* (so called Sanmul) seed from a native species of Jeju Island, was produced in *Seongsanpo*.

### Cultivation methods

**Cultivation of curled-shaped soybean sprouts:** After the *Junjeori* soybeans were carefully chosen from the soybean-sprout plant (Changsuchon Co., north Jeju) on Jeju Island, they were sprayed with bedrock water at  $16 \pm 1^\circ\text{C}$  from a 160 m deep well, by an automatic machine every 4 hr. When the sprouts reached a length of 3 cm, they were transferred to different containers. Curled-shaped soybean sprouts were produced by growing the sprouts for an additional 3 days (M-1). During the cultivation period, the temperature of the cultivation room was kept at an average of  $25^\circ\text{C}$  and the relative humidity was controlled at  $90 \pm 5\%$ .

**General cultivation:** Under the same condition as above M-1, the common upward right-shaped soybean sprouts were grown for five days without changing the cultivation container (M-2).

**Sprouting using treatment with a growth regulator:** M-2 sprouts were conventionally grown using the agricultural chemical treatment processes 2, 3 batches (one kg of soybean per batch) in cultivation baskets. The M-3 sprouts were grown the same as M-2 except that before soybeans were cultivated, they were at first soaked for 2 hr in mixed growth regulator solution which was made from 12 L of 0.3% indol-3-yl acetic acid (Young-il Chem. Co., Seoul) plus 100 g of anhydrous sulfurous acid (M-3).

**Cultivation by treatment with oak charcoal:** Using the method similar to the above M-1, after sound *Junjeori* soybeans were washed with bedrock water at  $16 \pm 1^\circ\text{C}$  from 160 m deep well, one kg of oak charcoal was spread on the bottom of the cultivation baskets. The soybeans were added and water was sprayed by an automatic machine every 4 hr until the sprouts grew to 3 cm. The soybean sprouts were then grown for additional 4 days in new cultivation baskets containing reversely-positioned germinated soybeans (M-4).

### The growth characteristics and the measurement of the yield rate

Using 100 sprouts randomly chosen from each basket at each germination period, measurements were made with a Vernier caliper (Mitutoyo, Japan) to determine the whole length, the body length, the root length and the body thickness. The number of root hairs was also counted. The yield rate of soybean sprouts was counted by the percentage of after-growth weight against initial weight of raw soybeans by randomly choosing 100 sprouts in each basket. The rot rate was marked by the percentage of total soybean-sprout weight after the separation of undeveloped, immature and rotten sprouts in each cultivation group.

### The measurement of hardness

Hardness was measured on the middle part of the sprout trunk, which was the thickest in the embryonic axis, and measured with a Rheometer (Model RT-3005D, Fudo Co., Japan). In order to evaluate the hardness of the soybean sprouts in each experimental group, the results of three time-executed rupture tests were used to determine the mean value with a 5 mm rod type probe with the moving at a rate of 10 mm/min.

### Sensory evaluation

After washing 400 g of ripe sprouts under running water followed by soaking 300 mL of a 3 % salt solution, the soybean sprouts were heated in a pan, boiled for one minute, and blanched in cold water. This sample was given to 20 hotel cooks, who evaluated the color, flavor, and overall taste using the 10-point scale method (18).

### Antimicrobial test of *Citrus sunki* seeds extract

Antimicrobial activity was measured in the citrus seed extract prepared by drying the seeds separated from the flesh part of *Citrus sunki* at  $65^\circ\text{C}$  for one hour, grinding to a size of 80 to 320 mesh, adding 20% glycerine to seed powder, agitating for 24 hr and separating the liquid extract from the residue (16). The bacteria strains used for the antimicrobial test were *E. coli* 026, *S. aureus* 6538, *S. cerevisiae* IBM 4274, *B. Licheniformis* 9945a, *Alcaligenes faecalis*, and *Rhizopus sp.* Nutrient broth (NB, Difco) was used for the culture media. After the culture was shaken for 24 hr, the anti-fungi test was executed by inoculating the strain on a slant medium of potato dextros agar (PDA) and cultivating it for 7 to 14 days at  $30^\circ\text{C}$  (19). Antimicrobial activity was determined by measuring the diameter of a growth inhibition ring by the paper disc method, in which paper disc (5 mm, 3M, Toyo, Japan) with 40  $\mu\text{L}$  sample (*Citrus sunki* seed extract; SSE) was placed on the slant medium and formed a ring free of bacterial colonies which was measured

after 24 hr.

### The measurement of quality characteristics of soybean sprouts treated with *Citrus sunki* seed extract

After dipping 200 g bunches of soybean sprouts in an antimicrobial mixed solution (250 ppm SSE + 1% ascorbic acid) and air drying for 5 min, each bunch was sealed in regular packing paper (polypropylene) and stored in a refrigerator at 5°C. Quality characteristics were surveyed from each sample on the second, fifth, eighth and twelfth days.

In order to evaluate the number of the total organisms, a piece of soybean sprout (50 g) was homogenized in 150 mL sterilized distilled water using a lab blender (Hanil, FM680T, Seoul, Korea). From this liquid, 1 mL was taken and diluted with 0.01% peptone water. 0.1 mL of diluted liquid was then painted onto a medium of tryptone glucose extract agar with 3 g yeast extract and incubated for 48 hr at 35°C. The number of colony forming units were counted (CFU/g). The L-value (lightness) of soybean sprouts was measured according to storage method with a CR-200 (Minolta Chroma, Japan). The sensory qualities of soybean sprouts treated with *Citrus sunki* seed extract were evaluated for flavor and overall taste in accordance with the above method.

### Statistical analysis

SPSS software (SPSS<sup>®</sup> for Windows, Korean Ver 10.0) was used to perform statistical computations (20). All data were expressed as the mean  $\pm$  SD. Significance of differences between control and treatment group were evaluated by T-test. Duncan's multiple range test was used to compare the differences of means among 3 groups. Differences with  $p < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

### The comparison of growth characteristics of soybean sprouts grown by different methods

The high temperature and humidity conditions that are used for sprouting soybeans ( $90 \pm 5\%$  RH,  $25 \pm 1^\circ\text{C}$ ) can cause fast rot in the sprouts. The effects of different growth methods on weight, thickness, body length and total length are shown in Table 1. The growing methods were: curled-shaped soybean sprouts grown in separate baskets (M-1), soybean sprouts grown for five days by the conventional method without changing baskets (M-2) and soybean sprouts grown for seven days using a growth-promoting agent (M-3). Changing baskets to prevent rot caused by heat and poor air circulation during the growth period made little difference in the weight of individual soybean sprouts cultivated by the M-1 and M-2 methods.

**Table 1.** Effect of cultivation methods on growth characteristics of soybean sprouts

	Cultivation methods <sup>1)</sup>		
	M-1	M-2	M-3
Weight (g)/ea	0.48 $\pm$ 0.09 <sup>2)c3)</sup>	0.55 $\pm$ 0.11 <sup>b</sup>	0.73 $\pm$ 0.12 <sup>a3)</sup>
Whole length (cm)	13.82 $\pm$ 1.47 <sup>b</sup>	14.67 $\pm$ 1.90 <sup>a</sup>	15.87 $\pm$ 3.99 <sup>a</sup>
Body length (mm)	7.98 $\pm$ 0.88 <sup>c</sup>	8.23 $\pm$ 0.94 <sup>b</sup>	11.69 $\pm$ 1.27 <sup>a</sup>
Body thickness (mm)	2.47 $\pm$ 0.24 <sup>a</sup>	2.31 $\pm$ 0.19 <sup>a</sup>	2.39 $\pm$ 0.20 <sup>a</sup>

<sup>1)</sup>M-1: curled shape soybean-sprout, M-2: conventional shape soybean-sprout, cultivation, M-3: same as M-1 but treated with cultivated soybean sprout treated by Indol-3-yl acetic acid.

<sup>2)</sup>Mean  $\pm$  SD.

<sup>3)</sup>Mean value in the same row that are not followed by the same letter are significantly different ( $p < 0.05$ ).

It was thought that growing roots in a reverse position created an obstacle, and the resulting lighter weight in M-3 compared to the unit weight was caused by the use of a growth-promoting agent and the longer cultivation period.

Although M-1 was a little smaller than M-3, both in whole length and body length, which may have resulted from the decrease in weight of each sprout, there was no significant difference in body thickness as shown in Table 1. The harvest yield and rot rate of soybean sprouts according to the cultivation methods are shown in Table 2. The harvest yield was significantly less for M-1 than for M-3 ( $p < 0.05$ ). Judging from this difference, the cultivation period and the growth-promoting agent had an influence on M-3. There was no significant difference in the rot rate in soybeans grown by the different methods. Therefore, we concluded high quality curled-shaped soybean sprouts can be grown in a reverse position by the M-1 method, and that quality depends primarily on spraying and thermal control.

### The characteristics of soybean sprouts cultivated with oak charcoal

The effects of growing sprouts on charcoal on the root length (mm) and the number of roots during the as compared to the controlled group are shown in Fig. 1. The

**Table 2.** Yield and spoilage rate of soybean sprouts by different growing methods

	Cultivation methods <sup>1)</sup>		
	M-1	M-2	M-3
Yield (%)	692 $\pm$ 7.32 <sup>2)a3)</sup>	723 $\pm$ 5.82 <sup>ab</sup>	747 $\pm$ 11.4 <sup>b3)</sup>
Spoilage (%)	0.81 $\pm$ 0.05	0.76 $\pm$ 0.04	0.81 $\pm$ 0.08

<sup>1)</sup>M-1: curled shape soybean-sprout, M-2: conventional shape soybean-sprout, cultivation, M-3: same as M-1 but treated with cultivated soybean sprout treated by Indol-3-yl acetic acid.

<sup>2)</sup>Mean  $\pm$  SD.

<sup>3)</sup>Mean value in the same row that are not followed by the same letter are significantly different ( $p < 0.05$ ).

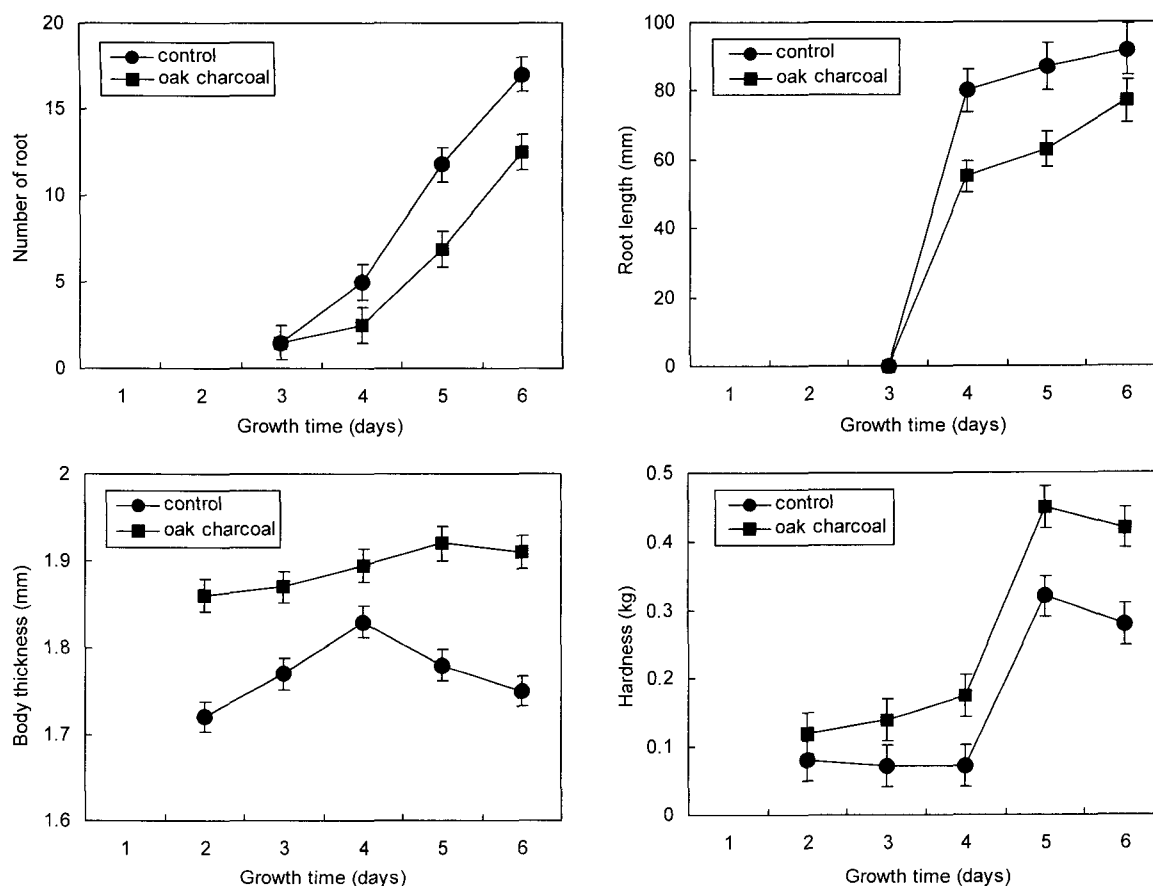


Fig. 1. Growth characteristics of soybean sprouts cultivated with oak charcoal.

roots did not grow until the third day of cultivation, but 80 mm roots in the control group and 56 mm in the group treated with oak charcoal did not appear until the fourth day. This was because undeveloped roots could not be distinguished from its trunk on the third day and such distinction could be made on the fourth day. The root length began to increase gradually until reaching 95 mm in the controlled group on the sixth day. However, the shorter root length of 75 mm in the group treated with oak charcoal was rated higher for texture when cooked.

The number of roots also increased after the fourth day of growth; by the fifth day the numbers of roots were 12 and 7, respectively, in the control group and the group treated with oak charcoal. By the 6th day the numbers of groups had increased to 12 and 18, respectively, which showed a remarkable increase in the group treated with oak charcoal. Park et al. (16) reported that roots appeared on the fourth day of germination, which concurred with the results of this experiment, with both studies demonstrating that the number of roots increases with the growth of soybean sprouts. They also reported that the number of roots of the *Junjeori* soybean was fewer than those of the *Suwon* soybean and the three imported varieties of *Orial*, *Kyeong-deok* and *Yeong-deok*. Our

results demonstrated that treatment with oak charcoal has a deterrent effect on the generation of roots in the early growth stage, but not in later stages.

Body thickness (mm) increased during the early period and began to decrease after 4 to 5 days, as shown in Fig. 1. The body thickness in the group treated with oak charcoal was generally larger than that of the control group. The thickness of the trunk in the control group was the greatest, 1.82 mm, on the fourth day of growth and decreased thereafter while the thickness of the trunk in the group treated with oak charcoal was the greatest, 1.9 mm, on the fifth day of growth.

Consumers usually prefer soybean sprouts with a thicker body. Soybean sprouts with the thinner body thickness scored lower on texture; and the fastest selling soybean sprouts in the marketplace have a larger body thickness. However, trunk thickness is usually increased by adding a growth-promoting agent such as 0.3% indol-3-yl acetic acid. Growing sprouts by only adding water results in a thinner body in the embryonic axis. Therefore, it is not easy to produce soybean sprouts with a thickness of 2.5 mm or more using pure clean with current technology. However, the increased thickness from 0.08 to 0.12 mm in the group treated with oak charcoal, compared to the

control group; suggests that inorganic compounds may be able to enhance the cell division and growth of the trunk.

The hardness of the sprout trunks during the growth period is shown in Fig. 1. Until the fourth day of growth, the trunk hardness in the control group was almost the same as that of the trunk in the group treated with oak charcoal, but the hardness began to increase from the fifth day of growth. However, the hardness of the control sprouts increased until the fifth day to 0.31, after which it decreased to 0.27 kg; whereas the hardness of the sprouts treated with oak charcoal increased gradually from the second day of growth to the highest level of 0.4 kg on the fifth day. As mentioned earlier, the soybean sprouts that scored highest on the sensory evaluation were harvested on the fifth day, and the group treated with oak charcoal scored highest on the hardness of trunk test.

A frequently used method for judging the quality of soybean sprouts in a soybean-sprout factory is chewing and feeling the uncooked sprouts. The group treated with oak charcoal had a more savory flavor and a more crunchy texture than the control group. In order to assess the flavor of soybean sprouts when cooked, they were seasoned with salt. The sensory evaluation which checked for color, flavor and overall taste showed no difference in color, but overall taste and flavor of the oak charcoal group was better than that of the control group (Table 3). These results clearly demonstrate that soybean sprouts

**Table 3.** Sensory evaluation scores for overall taste, flavor and color of control soybean sprouts and soybean sprouts grown with oak charcoal

	Cultivation methods <sup>1)</sup>	
	M-1	M-4
Flavor	6.76 ± 2.09 <sup>2)</sup>	7.63 ± 1.02*
Color	7.1 ± 1.47	7.15 ± 1.90
Overall taste	6.85 ± 2.22	7.55 ± 2.94*

<sup>1)</sup>M-1: general type soybean-sprout, M-4: soybean sprout cultivated with oak charcoal.

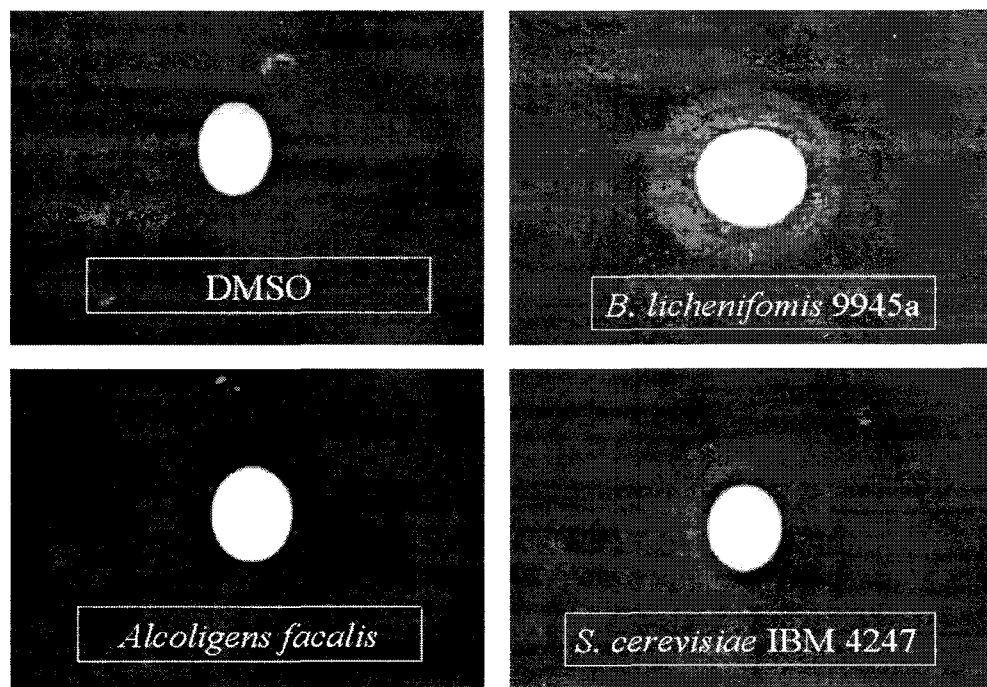
<sup>2)</sup>Mean ± SD.

\*Significant at  $p < 0.05$ .

with sensory excellence can be produced using the oak charcoal cultivation method without the use of a growth-promoting agent or agricultural chemicals.

#### The antimicrobial characteristic of *Citrus sunki* seed extract

The measurement of the antimicrobial activity of *Citrus sunki* seed extract (SSE) by the paper disc method indicated little effectiveness as illustrated in Fig. 2. More extensive and additional research is needed for maximizing the antimicrobial activity of the extract by using different extraction methods, the diversification of the exploration methods for the antimicrobial activity, and for screening various citrus components for potent antimicrobial activity. The exploration of minimal inhibition concentrations of the certified strain should be made in order to check for the existence of living organisms by



**Fig. 2.** Growth inhibition of extract from *Citrus sunki* seed (SSE) against *B. licheniformis* 9945, *A. facalis* and *S. cerevisiae* IBM 4247.

cultivating a septic-certified strain on a medium containing a specific amount of SSE.

### The shelf-stability and sensory quality of soybean sprouts cultivated with *Citrus sunki* seed extract

Because soybean sprouts are grown at a high temperature and humidity environment, microorganisms grow quickly; the number of the total organisms shortly after their growth was  $10^7 \sim 10^8$  CFU/g. As a result, the fast multiplication of microorganisms during the distribution period can cause lower quality and therefore a higher dissatisfaction rate among consumers. In order to solve this problem, the treatment of soybean sprouts with 250 ppm SSE + 1% ascorbic acid was shown to reduce the total number of organisms (Fig. 3). The number of the total organisms shortly after the treatment of soybean sprouts with SSE + ascorbic acid was significantly less than the control group; however, the inhibition effect decreased after the second day of storage and microorganism counts were similar to that of the controlled group by the fifth day and thereafter increased. Therefore, the inhibition effect of the antimicrobial solution appeared to last for five days. This may be due to the fact that microorganisms in the initial stage of growth in soybean sprouts can not limit the growth of other organisms through competition. Therefore, an antimicrobial agent is the only factor in limiting growth. In spite of the partial decrease in the number, the microorganism multiplication proceeds at a fast pace under optimal conditions. When soybean sprouts were treated with only seed extract (control group), the L-value of the control group after 2 days of storage was lower than that of SSE-mixture group which was treated with 250 ppm SSE plus 1% ascorbic acid and packed with cast plastic films (Fig. 4). This indicates that the browning reaction of soybean sprout treated

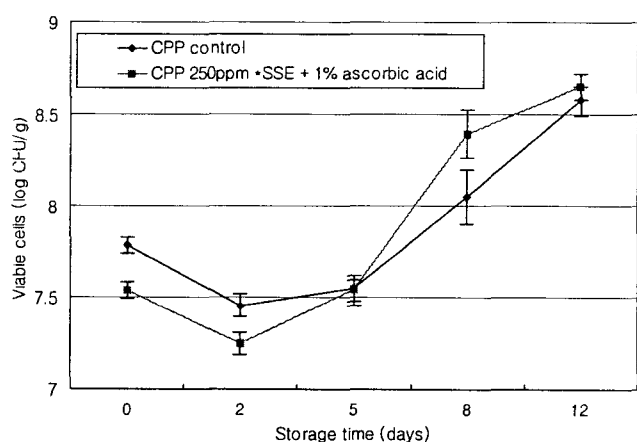


Fig. 3. Changes in total aerobic bacteria counts of soybean sprouts treated with 250 ppm SSE (*Citrus sunki* seed methanol extract) + 1% ascorbic acid and packed with CPP (cast plastic films) after storage at 5°C.

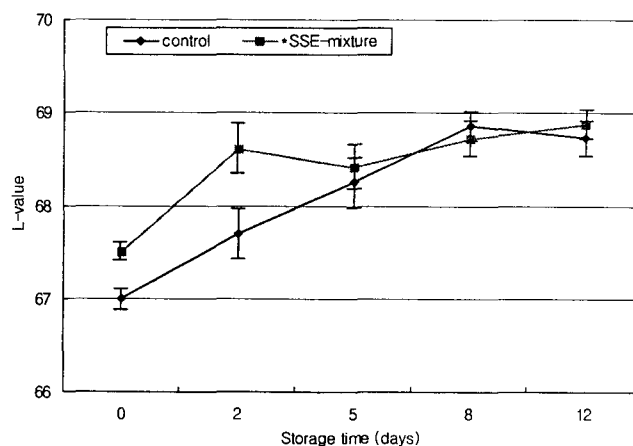


Fig. 4. Changes in L value (lightness) of soybean sprouts treated with 250 ppm SSE (*Citrus sunki* seed methanol extract) + 1% ascorbic acid and CPP (packed with cast plastic films) during storage at 5°C.

Table 4. Sensory evaluation scores for flavor and overall taste of soybean sprouts treated with 250 ppm SSE and 1% ascorbic acid

	Samples	
	Control	SCS <sup>1)</sup>
Flavor	6.95 ± 3.25 <sup>2)</sup>	6.71 ± 2.61
Overall taste	7.02 ± 4.22	6.97 ± 3.89

<sup>1)</sup>SCS: soybean sprout treated with 250 ppm SSE and 1% ascorbic acid.

<sup>2)</sup>Mean ± SD.

with *Citrus sunki* seed methanol extract is able to partly be prevented by adding a reducing agent such as ascorbic acid.

The results of sensory evaluation for flavor and overall taste showed no significant difference between the control group (cultivated by conventional method, without any other treatment) and the SSE-mixture group as shown in Table 4.

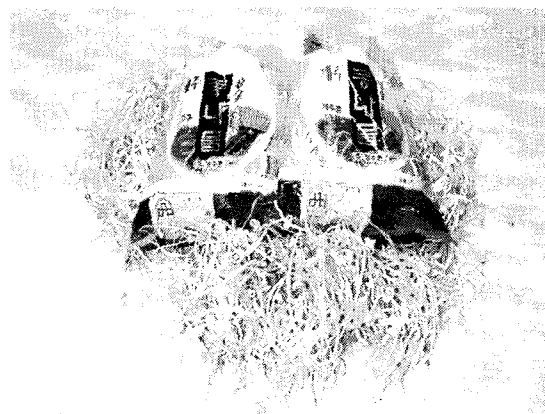
In conclusion, we demonstrated that the combined treatment of *Citrus sunki* seed extract and ascorbic acid had a partial deterrent effect on the growth of septic microorganisms in soybean sprouts. If soybean sprouts are grown by the combination of using a separation basket with the cultivation basket, treatment with oak charcoal, and packed after being dipped in a mixed solution of *Citrus sunki* seed extract and ascorbic acid (Fig. 5), they can be distributed for approximately five days and retain their desirable sensory characteristics.

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(I)



(II)

Fig. 5. Soybean sprouts (I) cultivated with oak charcoal, and market soybean sprout (II) treated with *Citrus sunki* seed extract plus ascorbic acid.

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