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Quality Changes During Storage of Cook-chilled Soybean Sprouts

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Abstract *Sous vide* and packaged cook-chilled soybean sprouts were evaluated for physicochemical quality changes and microbial safety during storage for the purposes of shelf-life extension and industrial application. The physicochemical changes assessed were color, texture, and ascorbic acid concentration. The quality of soybean sprouts became worse with increased periods of storage and better in storage temperature of 3°C more than in 10°C. The concentration of aerobic bacteria decreased from 2.1×10^8 to 6.0×10^2 CFU/g after pasteurization, but increased during storage. These bacteria are the same shape as anaerobic and psychrophilic bacteria, but none of these other organisms were detected after heat treatment. The physicochemical qualities of soybean sprouts and microbial safety were better for products stored at 3°C than at 10°C. In the case of short storage periods, heat treatment at 70°C for 2 min was most effective for quality and microbial safety.

Keywords: minimally processed refrigerated food, *sous vide*, cook-chill, soybean sprout, quality change

Introduction

Over the past two decades there has been increased demand for minimally processed chilled foods and *sous vide* products. In addition, urban and suburban consumers have increased the demand for high quality convenience foods (1).

Minimal processing normally involves washing, trimming, scraping, slicing and/or dicing, shredding and/or chopping, removal of moisture, packaging, and storage of the vegetable at refrigeration temperatures (2). *Sous vide* and cook-chill processes, otherwise known as minimally processed refrigerated (MPR) foods, provide two product types meeting this demand. *Sous vide*, literally meaning 'under vacuum', is a process introduced in France that utilizes vacuum packaging, pasteurization, and controlled temperature storage to provide an unfrozen extended shelf-life. Cook-chill processing is similar to *sous vide* but does not necessarily use a vacuum (1).

The increase in demand by both retail and catering industries for convenient, preservative free, and apparently fresh foods has led to the development of a number of refrigerated and minimally processed products. Most have extended shelf lives due to the application of heat treatment, modified atmosphere (MA), advanced packaging techniques (active packaging and selective barrier films), and/or refrigeration. There is microbiological safety concerns associated with these foods, because chilled foods are more prone to temperature abuse than frozen foods, and the relatively long shelf-life claimed for some products may allow ample time and conditions for a number of psychrotrophic pathogens to grow. Another risk factor associated with these types of product is that many food-borne pathogens can grow and/or produce toxins without causing any visual or sensory defects in the food (1).

Microbiological evaluation and physicochemical quality assessment were carried out to examine the shelf-life extension of cook-chilled and *sous vide* food. The shelf-life of such a product is determined, to an extent, by the processing parameters applied (1). Many studies have been conducted to minimize the processing of vegetables prepared by cook-chill and *sous vide* methods for shelf-life extension and industrial application (2-6).

Soy foods have received much more attention, primarily due to their beneficial effects on human health. A large number of food preparations can be made from soybeans for the human diet. Soybean products extensively used in the Orient include soybean curd, soymilk, fermented food products, germinated products, and soybeans for cooking with rice. Soybean sprouts are a year-round vegetable that is easily cultivated, relatively inexpensive, and known widely as a source of protein, vitamins, and minerals (7). Also, soybean sprouts are a popular food in the Orient, especially in Korea (8).

The objective of this paper was to apply soybean sprouts to *sous vide* and cook-chill processing systems, was and to evaluate the physicochemical qualities and microbial safety of these products during storage.

Materials and Methods

Sample preparation Fresh soybean sprouts was purchased from Sambo foodservice operations in Korea, and the experiments were performed and the products stored in a refrigerated chamber at 3°C until use. Prior to blanching, soybean sprout samples were washed. Blanching was carried out in a heat exchanger and the samples were cooled in ice water. Blanched samples were dehydrated and 500 g portions were vacuum packaged in plastic films (Cryovac C5045; nylon/PE/nylon/PE/nylon/LLDPE, Cryovac Division, Sealed Air Corporation, Duncan, SC, USA) and were heated according to their heat treatment conditions.

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Cooked soybean sprouts and soup were quickly cooled in ice water. Packaged cook-chilled samples were stored at 3 or 10°C.

Heat treatment Blanching and cook-chilled packaging of soybean sprouts were carried out under various conditions. An appropriate time of blanching was calculated through experiments taking into account sensory evaluation and physicochemical qualities such as texture and ascorbic acid content. Blanching was carried out at 100°C by steaming. *Sous vide* products were heated for a minimum of 10 min at 90°C in order to achieve a 6 log (6D) reduction in the number of psychrotrophic *Clostridium botulinum*. This has since been recommended by the Advisory Committee in the Microbiological Safety of Food (ACMSF, 9) for all vacuum-packaged (VP) and cook-chill packaged foods. A minimum 6D kill of *Listeria monocytogenes* should be achieved by exposure to 70°C for 2 min for a short shelf-life of minimally processed foods (1). Cooked soybean sprout products must be heated for a minimum of 10 min at 90°C and at 70°C for 2 min.

Physicochemical anaylsis The surface color of the soybean sprouts was measured using the Hunter color system ('L', 'a' and 'b' values) with a color difference meter (JC 801; Color Techno System Corporation, Tokyo, Japan). Average values were obtained from 2 random locations on each sample surface. Texture was measured as cutting force (g_f) when a stem of soybean sprout was sliced by blade of 0.26 mm using the Rheometer (Compac-100; Sun Scientific Co., Tokyo, Japan). Soybean sprouts were measured by vertical cutting intensity. Total ascorbic acid was determined by the AOAC method (12). Twenty g of sample was blended in 30 mL of 3% metaphosphoric acid solution, which was homogenized in a homogenizer (AM-8; Nihonseike Kaisha, Tokyo, Japan) and extracted in 50 mL after filtration. Twenty-five mL of extract was then titrated with 0.025% 2,6-dichloroindophenol.

Microbiological analysis Soybean sprouts (8 g) were aseptically transferred into a sterile stomacher bag and 72 mL sterile 0.1% peptone water (Difco Laboratories, Detroit, MI, USA) was added. Samples were then homogenized in a stomacher (Stomacher Blender 400; Seward, London, UK) for 2 min at normal speed and aliquots were plated out directly or as 10-fold dilutions in 0.1% peptone water. Bacterial and fungal colonies were counted and expressed as colony forming units (CFU) per gram. Total viable counts were determined on plate count agar (PCA, Difco) at 35°C for 48 hr. Desoxycholate agar (Difco) was used to measure coliforms by incubation at 37°C for 20±2 hr. Potato dextrose agar (PDA, Difco) adjusted to pH 3.5 with 10% tartaric acid was used for the selective enumeration of yeast and molds by incubation at 25°C for 3-5 days (4). PCA was used to enumerate psychrophilic bacteria at 20°C for 72 hr. Heat resistant bacteria were detected by heat treatment at 80°C for 10 min and enumerated on plates of PCA at 37°C for 48 hr. Anaerobic bacteria were determined by spread plating on PCA and incubating in a BBL anaerobic jar (Difco). All plates were incubated anaerobically at 35°C for 48-72 hr. All experiments were performed twice for 2 separate samples.

Statistical analysis Data from 2 replications × 2 separate treatments were analyzed for physicochemical qualities, change of microbial levels and sensory data by computation with Microsoft Excel 97 (Microsoft Corporation, Redmond, WA, USA) using the standard deviation.

Sensory evaluation The sensory qualities of traditionally cooked soybean sprouts and cook-chilled soybean sprouts were evaluated after 1, 3, and 5 days of storage by an experienced panel of 10 judges. Sensory profile analysis was used to describe flavor, odor, color, texture, juiciness, appearance, fullness after eating, and overall acceptability. The sensory ratings for appearance, color, odor, and flavor were recorded on a score sheet based on numerical scores as follows: extremely fresh – 10, very fresh – 8, moderately fresh – 6, border line/neutral – 5, poor/stale – 4, very poor/very stale – 2, and bad/putrid – 0. Evaluation of texture was carried out using the ratings, firm and elastic – 10, turning soft – 8, elasticity lost – 6, very soft – 4, and extremely soft – 2 (10).

Results and Discussion

Heat treatment Changes in texture and ascorbic acid content during the steaming of soybean sprouts at 100°C are shown in Fig. 1. The concentration of ascorbic acid decreased following heat treatment, but decreased slowly after 6 min. The texture (g_f) decreased to 69 and decreased slowly after 8 min of heating. Appropriate blanching conditions were determined by sensory evaluation and other experiments measuring changes in vitamin C and texture. In this manner the appropriate time for blanching conditions was determined to be 8 min, and the core temperature required for cook-chilled sprouts was obtained by heating at 90°C for 10 min and at 70°C for 2 min to eliminate *C. botulinum* and *L. monocytogenes*, respectively. These results are similar to the blanching conditions reported for carrot flowers (2).

Physicochemical anaylsis Table 1 compares raw and blanched soybean sprouts. The change of color was lower after blanching than that reported for green beans by Chourot *et al.* (11). Our examination showed a higher b

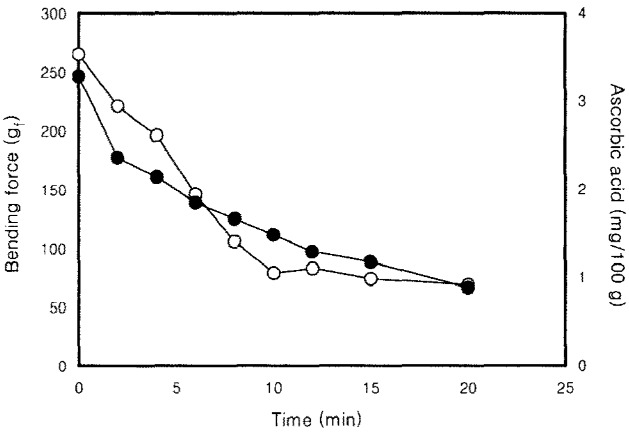

Fig. 1. Changes in texture and ascorbic acid content during steaming of soybean sprouts at 100°C. ○, Texture; ●, ascorbic acid.

Table 1. Changes in physicochemical qualities of soybean sprouts[1)]

	Raw	Blanched at 100°C for 8 min
Color (b value)	5.06±0.56	5.34±0.35
Texture (g_f)[2)]	216±61.95	551±199.10
Ascorbic acid (mg/100 g)	7.19±0.22	2.75±0.06

[1)]Values are means±SD for 5 samples.
[2)]Cutting force.

value for blanched soybean sprouts and the texture value also increased when compared to raw soybean sprouts. The ascorbic acid level of raw soybean sprouts was 7.19 mg/100 g and decreased to 2.75 mg/100 g after blanching. The decrease of ascorbic acid was lower than that reported in a related study (12), and changes in vitamin C levels of steamed broccoli reported by O'Leary *et al.* (13) appeared to vary little during storage.

The surface colors of soybean sprouts as assessed by the Hunter color system, varied according to the heat treatment. As shown in Table 2, all packaged soybean sprouts decreased slowly throughout all storage periods, but decreased suddenly in 4 days. Especially, cook-chilled soybean sprouts at 90°C for 10 min decreased more than at 70°C for 2 min and excelled at 10°C storage than 3°C.

The texture of packaged soybean sprouts was measured using a rheometer and increased during the initial stage but remained relatively constant during storage (Table 3). The texture of packaged soybean sprouts heated at 70°C for 2 min, at storage at 10°C decreased suddenly after 16 days. It is assumed that this was due to the growth of spoilage organisms, however there were no significant changes during the first 16 days which is similar to the results of Knochel *et al.* (14).

Table 4 shows the changes in total ascorbic acid during storage. For all samples, the ascorbic acid levels decreased slowly, and cook-chilled packaged sprouts stored at 3°C were much like those stored at 10°C, and sprouts heated at 70°C for 2 min had better sensory quality than those heated at 90°C for 10 min.

Table 2. Color (b value) changes of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	5.49±0.51	5.10±0.46	5.49±0.51	5.10±0.46
2	5.31±0.56	5.27±0.36	-	-
4	5.08±0.59	4.84±0.17	5.38±0.29	5.14±0.09
6	4.47±0.47	4.31±0.35	-	-
8	3.91±0.21	3.32±0.32	4.12±0.05	3.88±0.23
12	4.17±0.13	3.68±0.20	4.19±0.39	4.12±0.20
16	3.79±0.20	2.95±0.20	4.19±0.11	3.94±0.29
20	3.91±0.18	2.92±0.49	3.94±0.34	3.68±0.21
24	3.64±0.21	2.68±0.33	3.81±0.22	3.58±0.21
30	-	-	3.95±0.07	3.81±0.03
36	-	-	4.06±0.14	3.28±0.34

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; -, not performed.

Table 3. Changes in texture of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	795±140.39	636±219.51	795±140.39	636±219.51
2	815±249.28	844±154.40	-	-
4	821±132.76	846±134.63	784±143.75	746±162.71
6	807±149.98	843±176.82	-	-
8	780±188.60	811±186.43	770±146.04	735±167.31
12	765±105.11	811±86.66	792±105.13	745±87.79
16	756±128.70	834±105.68	788±160.94	727±127.91
20	388±110.14	848±87.03	778±170.98	760±70.85
24	98±12.29	761±145.20	797±143.04	785±88.19
30	-	-	777±111.78	745±113.76
36	-	-	711±164.68	685±143.77

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; -, not performed.

Table 4. Changes in ascorbic acid levels of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	1.26±0.09	1.17±0.05	1.26±0.09	1.17±0.05
2	1.03±0.08	1.05±0.05	-	-
4	0.85±0.07	0.81±0.05	0.96±0.06	0.93±0.05
6	0.82±0.05	0.79±0.00	-	-
8	0.79±0.00	0.70±0.05	0.93±0.03	0.84±0.08
12	0.71±0.04	0.68±0.00	0.72±0.03	0.68±0.00
16	0.68±0.00	0.68±0.00	0.73±0.06	0.73±0.03
20	0.62±0.07	0.56±0.00	0.70±0.03	0.68±0.00
24	0.64±0.06	0.53±0.04	0.74±0.00	0.70±0.03
30	-	-	0.64±0.03	0.53±0.04
36	-	-	0.58±0.03	0.52±0.06

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; -, not performed.

Table 5. Changes in aerobic bacterial counts of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	2.78±0.00	ND	2.78±0.00	ND
2	2.74±0.17	ND	-	-
4	3.23±0.23	ND	2.30±0.34	ND
6	5.54±0.03	ND	-	-
8	5.79±0.06	ND	2.30±0.34	ND
12	6.57±0.04	ND	2.81±0.14	ND
16	7.85±0.14	ND	7.67±0.31	ND
20	3.40±0.12	ND	6.67±0.02	ND
24	3.57±0.33	ND	6.90±0.07	ND
30	-	-	6.57±0.05	ND
36	-	-	5.28±0.23	ND

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; ND, not detected; -, not performed.

Therefore, samples heated at 70°C for 2 min had the best physicochemical qualities through 16 days of storage.

Microbiological analysis Table 5 shows the changes in aerobic bacterial counts of packaged soybean sprouts during storage. At 10°C storage, samples heated at 70°C for 2 min had suddenly increased aerobic bacterial counts after 4 days of storage, however sprouts heated at 90°C for 10 min did not show such changes. Microbial changes in packaged samples stored at 3°C that were heated at 70°C for 2 min increased suddenly after 12 days of storage, but not with samples heated at 90°C for 10 min. The results of Edgar and Aidoo (2) regarding carrot, spring onion, and baby sweet corn showed that total viable microbial counts decreased to 50% after blanching. In our experiments, the initial microbial counts in packaged soybean sprouts was 8.32 log CFU/g which was reduced to 2.78 log CFU/g after blanching. This result was due to the fact that mesophilic counts were lower in vacuum cooled samples than in water-immersion cooled samples (15).

Changes in anaerobic bacterial counts are shown in Table 6. The initial counts were 7.18 log CFU/g, however none were detected after heat treatment. In samples heated at 70°C for 2 min, storage at 10°C resulted in increased microbial counts at day 0, and samples heated at 90°C for 10 min showed an increased microbial count after 2 days of storage. This difference was due to 3 critical control points in the *sous vide* process; (i) the cooking time and temperature, (ii) the cooling rate, and (iii) the storage time and temperature (1).

The important microorganisms in foods stored at refrigeration temperatures are psychrophilic bacteria such as *Aeromonas hydrophila* and *L. monocytogenes* (16). Table 7 shows changes in the psychrophilic bacterial counts in packaged soybean sprouts during storage. The initial counts were 8.15 log CFU/g. Packaged soybean sprouts heated at 70°C for 2 min had an increased psychrophilic bacterial count after 10°C storage for 2 days, and after 6 days of 3°C storage. Samples prepared under the different conditions showed no significant variation in microbial counts. The results of Francis and O'Beirne (17) showed that the survival and growth of *L. monocytogenes*

Table 6. Changes in anaerobic bacterial counts of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	ND	ND	ND	ND
2	2.78±0.02	ND	-	-
4	3.32±0.33	ND	2.57±0.12	ND
6	5.20±0.25	ND	-	-
8	5.99±0.04	ND	4.54±0.37	ND
12	6.56±0.19	ND	4.72±0.06	ND
16	3.43±0.16	ND	3.55±0.23	ND
20	3.42±0.11	ND	4.16±0.12	ND
24	2.24±0.09	ND	3.18±0.08	ND
30	-	-	2.97±0.22	ND
36	-	-	2.88±0.04	ND

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; ND, not detected; -, not performed.

Table 7. Changes in psychrophilic bacterial counts of cook-chilled soybean sprouts during storage[1)]

Storage period (day)	Stored at 10°C		Stored at 3°C	
	Heat treatment conditions			
	70°C/2 min	90°C/10 min	70°C/2 min	90°C/10 min
0	ND	ND	ND	ND
2	ND	ND	-	-
4	2.89±0.10	ND	ND	ND
6	5.59±0.08	ND	-	-
8	5.26±0.18	ND	2.83±0.12	ND
12	6.78±0.10	ND	2.95±0.12	ND
16	7.87±0.01	ND	6.23±0.05	ND
20	3.00±0.06	ND	6.23±0.08	ND
24	3.68±0.08	ND	6.84±0.40	ND
30	-	-	6.55±0.19	ND
36	-	-	4.76±0.45	ND

[1)]Values are means±SD for 2 readings on each of 2 soybean sprout samples; ND, not detected; -, not performed.

did not change significantly during storage at 8°C with modified atmosphere packaged vegetables, however our experiments carried out for all psychrophilic bacteria. Psychrophilic bacteria had a slower growth rate in the initial stages of storage. Heating and chilling processes destroyed the microorganisms present in the food or damaged the cells and slowed down their growth rates (18).

Yeast and molds, heat resistant bacteria and coliforms were not detected after pasteurization, however the initial microbial counts were 4.81, 4.43, and 6.13 log CFU/g, respectively. Because these organism were inactivated by heat treatment, it is assumed they are not a concern for food safety. Edgar and Aidoo (2) found that yeast counts were 3.8, 3.5, and 3.4 log CFU/g in carrots, baby sweet corn, and spring onions, respectively. These yeast cell counts were higher than those obtained in this study. Very low level of microorganisms in food samples is an indication that the products have not been contaminated by direct contact with humans (4).

Heat-treated packaged samples had decreased microbial levels when heated at 90°C for 10 min relative to samples heated at 70°C for 2 min, and showed greater inhibition of further microbial growth. However, the sensory evaluation of packaged cook-chilled sprouts was better for samples heated at 70°C for 2 min relative to samples heated at 90°C for 10 min. In addition, samples heated at 70°C for 2 min and stored for a period of 10 days had safe microbial levels. Considering all experimental evaluations, cook-chilled soybean sprouts are best when heated at 70°C for 2 min.

Sensory evaluation Table 8 shows the average sensory scores of cook-chilled soybean sprouts during storage. All sensory scores were over 5 points, and scores for odor were higher after 1 day of storage than after 3 and 5 days, however no significant difference was observed among these values. The appearance, color, flavor, odor, texture, juiciness, fullness after eating, and overall acceptability of traditionally cooked and cook-chilled soybean sprouts are presented in Table 9, 10, and 11, respectively. In Table 9, the sensory scores for flavor, texture, color, juiciness, and

Table 8. Average sensory scores for cook-chilled soybean sprouts during storage

	Storage period (day)			
	1	3	5	F-value
Flavor	5.60±1.84	5.62±2.19	5.94±2.14	0.234
Odor	6.17±1.90	5.57±2.00	6.08±1.57	0.413
Texture	5.96±2.33	6.32±2.54	6.28±1.72	0.245
Color	5.51±2.56	6.08±2.59	5.79±1.71	0.436
Juiciness	6.13±0.24	7.05±2.07	6.68±2.02	1.461
Appearance	5.98±2.48	6.24±2.74	5.95±1.43	0.125
Fullness after eating	5.54±2.23	5.89±2.34	5.70±2.23	0.186
Overall acceptability	5.56±1.96	5.94±2.17	6.02±2.08	0.237
Overall mean	5.81±1.63	6.11±1.81	6.05±1.30	0.457

Table 9. Sensory evaluation of cook-chilled and traditionally cooked soybean sprouts after 1 day of storage

	Traditional cooking	Cook-chill processing	*t*-value
Flavor	5.72±2.13	5.60±1.86	0.270
Odor	6.01±1.89	6.17±1.92	0.264
Texture	5.97±2.98	5.96±2.32	1.593
Color	6.39±1.93	5.51±2.54	1.605
Juiciness	6.27±1.73	6.13±2.04	0.337
Appearance	6.81±1.96	5.98±2.48	1.510
Fullness after eating	5.15±2.52	5.54±2.23	0.548
Overall acceptability	5.39±1.79	5.56±1.96	0.204
Overall mean	5.83±1.41	5.81±1.63	0.786

Table 10. Sensory evaluation of cook-chilled and traditionally cooked soybean sprouts after 3 days of storage

	Traditional cooking	Cook-chill processing	*t*-value
Flavor	5.72±2.13	5.62±2.19	0.153
Odor	6.01±1.89	5.75±2.00	0.472
Texture	5.97±2.98	6.32±2.54	0.513
Color	6.39±1.93	6.08±2.59	0.490
Juiciness	6.27±1.73	7.10±2.07	1.453
Appearance	6.81±1.96	6.24±2.74	0.846
Fullness after eating	5.15±2.52	5.89±2.32	1.088
Overall acceptability	5.39±1.79	5.94±2.17	0.984
Overall mean	5.83±1.41	6.11±1.81	0.784

appearance were higher for traditionally cooked soybean sprouts, however the difference was not significant. The sensory evaluation of cook-chilled soybean sprouts and traditionally cooked sprouts after 3 days of storage are shown in Table 10. Traditionally cooked soybean sprouts had a higher sensory score for flavor, odor, color, and appearance than cook-chilled soybean sprouts. Table 11 shows the sensory evaluation after 5 days of storage, revealing that cook-chilled soybean sprouts were rated higher for flavor, odor, texture, juiciness, fullness after eating, and overall acceptability than traditionally cooked sprouts. No significant difference was observed for storage times beyond 5 days.

Cook-chilled soybean sprouts tolerated 5 days of storage since the sensory qualities did not change during this period of storage. Further studies should be focused on predictive microbial modeling to ensure microbial safety and quality.

Table 11. Sensory evaluation of cook-chilled and traditionally cooked soybean sprouts after 5 days of storage

	Traditional cooking	Cook-chill processing	*t*-value
Flavor	5.72±2.13	5.94±2.14	0.363
Odor	6.01±1.89	6.08±1.57	0.146
Texture	5.97±2.98	6.28±1.77	0.823
Color	6.39±1.93	5.79±1.71	1.171
Juiciness	6.27±1.73	6.68±2.00	0.779
Appearance	6.81±1.96	5.95±1.43	1.764
Fullness after eating	5.15±2.52	5.70±2.23	0.823
Overall acceptability	5.39±1.79	6.02±2.08	1.143
Overall mean	5.83±1.41	6.05±1.03	0.927

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