Food Sci. Biotechnol. Vol. 17, No. 1, pp. 166 ~ 171 (2008)

Food Science and Biotechnology

Improvement of Microbiological Safety of *Sous Vide* Processed Soybean Sprouts: Nisin and *Bacillus cereus* Challenge

Hye-Jung Kim, Na-Kyoung Lee[1], Dong-Sun Lee[2], Wansoo Hong[3], Sang-Rak Lee[1], Cheon-Jei Kim[1], and Hyun-Dong Paik[1]*

Gyeongsangnam-do Institute of Public Health and Environmental Research, Changwon, Gyeongnam 641-825, Korea
[1]Division of Animal Life Science, Konkuk University, Seoul 143-701, Korea
[2]Department of Food and Biotechnology, Kyungnam University, Masan, Gyeongnam 631-701, Korea
[3]Department of Foodservice Management and Nutrition, Sangmyung University, Seoul 110-743, Korea

Abstracts Soybean sprouts which are a popular vegetable in Korea, are produced using the techniques of *sous vide*. The purpose of this study was to determine the effect of nisin and storage temperature on the microbiological and physicochemical qualities of *sous vide* processed soybean sprouts during storage in order to improve shelf-life and industrial applications. During storage of the cook-chilled soybean sprouts at 3°C, no development of mesophilic microorganisms was observed. However, at 10°C storage without nisin, the number of mesophilic microorganisms increased markedly, whereas sprouts stored at the same temperature with nisin showed no observed increase. Psychrotrophic microorganisms, anaerobic microorganisms, and *Bacillus cereus* all showed similar trends. The ascorbic acid content, following the sequential heat processing of soybean sprouts through blanching and pasteurization decreased markedly during early storage, and stabilized thereafter. During storage, no major changes in the color or ascorbic acid content of samples at either temperature were observed. With regard to microbial and physicochemical qualities, the presences of nisin and storage temperature are important factors for extending shelf-life of soybean sprout.

Keywords: soybean sprout, *sous vide*, cook-chill, *Bacillus cereus*, nisin

Introduction

The cook-chill and *sous vide* methods for food preparation have received increasing attention from food service and manufacturing industries for their ability to provide high quality chilled ready-to-eat meals having extended shelf-lives (1-3). The *sous vide* process, an improved variant of the cook-chill method, consists of vacuum-packaging raw or partially cooked prepared foods in plastic pouches, pasteurization in hot water and rapid cooling followed by chilled storage (4). The chill-stored products are normally consumed after reheating.

The *sous vide* processing method is subjected to microbial contamination with spore forming and anaerobic bacteria. Pasteurization treatment kills vegetative forms of micro-organisms, but is inadequate to kill bacterial spores. Low oxygen atmospheres created by vacuum packaging have the potential to support the growth of both obligate and facultative anaerobes (5). Among spore-forming bacteria, *Bacillus cereus, Clostridium botulinum,* and *Clostridium perfringens* are regularly implicated in outbreaks of food poisoning (6). Some strains of *B. cereus* and nonproteolytic *C. botulinum* are able to grow in refrigerated, pasteurized foods (7-10). Among spore forming bacteria, members of the *Bacillus* spp. are widely distributed in the environment and in raw and processed food products such as rice, milk and dairy products, spices, vegetables, meat products, farinaceous foods (11), cooked or chilled vegetables (7), and raw soybean sprouts (12). Some strains of *B. cereus* are able to grow at 5 or 7°C (9,10) and could be of concern in refrigerated, pasteurized foods.

The safety of *sous vide* products depends on the type of heat treatment and low temperature storage. However, because of the well-known difficulties in maintaining low storage temperatures along the distribution chain, additional measures such as the use of bacteriocins have been proposed in the manufacture of *sous vide* products to ensure greater levels of safety (13-15). Nisin is a bacteriocin produced by certain strains *Lactococcus lactis* subsp. *lactis*, which exhibits antimicrobial activity toward a large range of Gram-positive vegetative cells and spores (16). It has already been used as an antagonistic additive (17,18) and film coating material (19-21) in the food industry in more than 50 countries (22).

Soybean sprouts are popular vegetable in Korea. It has considered being important food stuff in view of their high nutritive and economic values; little information on their microbial quality is available. So, we investigated the microbiological and physicochemical changes in *sous vide* soybean sprouts stored at various temperatures with or without nisin to improve the shelf-life and industrial application of *sous vide* prepared soybean sprouts.

Materials and Methods

Strains and culture conditions *Bacillus cereus* employed as an inoculant in *sous vide* soybean sprouts was isolated from raw soybean sprouts at low temperature (10°C), and diarrheal enterotoxin was confirmed to be produced by this strain (23). *B. cereus* cells were cultivated in Tryptic Soy

*Corresponding author: Tel: +82-2-2049-6011; Fax: +82-2-455-1044
E-mail: hdpaik@konkuk.ac.kr
Received October 3, 2007; accepted October 16, 2007

Broth (Difco Laboratories, Detroit, MI, USA) for 3 days at 37°C in a shaking incubator (180 rpm). Stock cultures were stored at −70°C in 20% glycerol. To induce spore formation, cultures were heat-shocked at 70°C for 10 min prior to use, diluted to 10^6 CFU/mL, and inoculated into packaged soybean sprouts.

Cook-chill and *sous vide* procedures used with soybean sprouts Figure 1 shows a flow diagram of cook-chill and *sous vide* procedures used with soybean sprouts. Fresh soybean sprouts were purchased from Sambo foodservice operations in Busan, Korea. Soybean sprouts were transported to the laboratory under low temperature (<7°C), and stored in a refrigerated chamber at 3°C until used for experiments. They were analyzed within 24 hr after sampling. All soybean sprouts were washed prior to blanching. Washed soybean sprouts were blanched in a steam kettle containing approximately 280 L of boiling water at 100°C for 8 min, immediately rinsed in cold water and spun in salad spinner for 1 min to remove excess water. The blanching process was based on established conditions (100°C for 8 min) for optimal quality.

One-hundred kg of blanched soybean sprouts were vacuum-packaged in plastic films (Cryovac C5045; nylon/PE/nylon/PE/nylon/LLDPE; Cryovac Division, Sealed Air Corporation, Duncan, SC, USA) with or without nisin (Sigma Chemical Co., St. Louis, MO, USA) at final concentration of 100 IU/mL, with or without *B. cereus* strains of final concentration of 10^3 CFU/mL. The soybean spout pouches were pasteurized by immersing in water at 97°C for 11.7 min to ensure that the temperature of the soybean sprouts at the geometric center remained above 70°C for 2 min. After pasteurization the pouches were moved to ice water for rapid cooling to 3°C. Cook-chilled packages were then stored at 3 and 10°C, with sample being taken for quality assessment at regular intervals during storage.

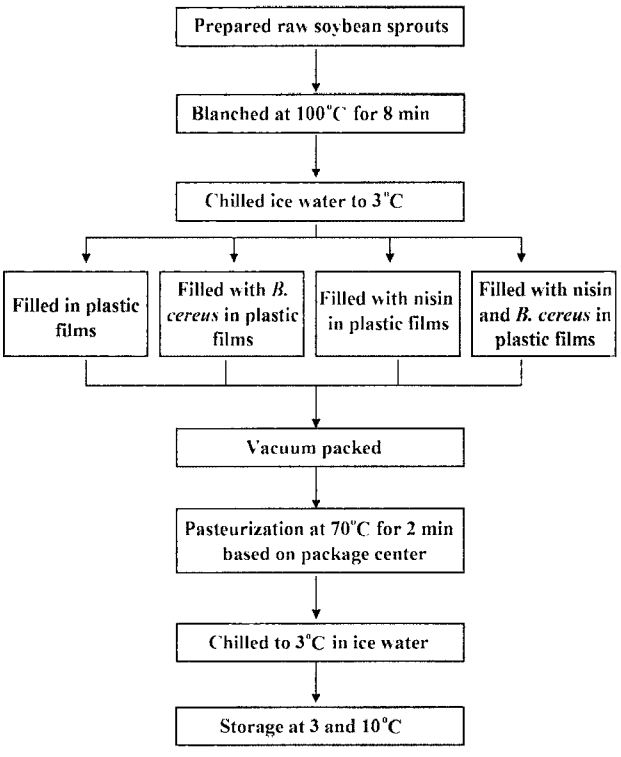

Fig. 1. Flow diagram of cook-chill and *sous vide* procedures with soybean sprouts.

Microbiological analysis For each sampling, 20 g of soybean sprouts was aseptically transferred into a sterile stomacher bag and 20 mL sterile 0.1% peptone water was added. The sample was then homogenized in the stomacher (Lab-Blender, TMC International, Seoul, Korea) for 2 min at normal speed and aliquots were plated out directly or as 10-fold dilutions in 0.1% peptone water. Mesophilic microorganism counts were determined by incubation on Plate Count Agar (PCA; Difco) at 35°C for 48 hr. Psychrotrophic microorganisms were incubated at 10°C for 7 days on PCA. Anaerobic microorganism counts were determined by spread-plating on PCA and incubating anaerobically at 35°C for 48 hr. *B. cereus* counts were determined using Cereus Selective Agar without antibiotics at 30°C for 24 hr. All analyses were performed in duplicate and counts were expressed as colony-forming units per g (CFU/g).

Physicochemical analysis Physicochemical analyses were carried out at regular intervals throughout the storage period. The surface color of soybean sprouts was measured by the Hunter color system ('L', 'a', and 'b' values) using a color difference meter (model JC 801; Color Techno System Corporation, Tokyo, Japan). Average values were obtained from 6 random locations on each sample surface. Texture was assessed in terms of cutting force required when a soybean sprout stem was sliced by a 0.26 mm blade in a rheometer (model Compac-100; Sun Scientific Co., Tokyo, Japan). A 10 kg load and a blade speed of 60 mm/min were used for the measure-ment. Ascorbic acid content was determined by the AOAC method (24). A 20 g sample was blended in 30 mL of 3% metaphosphoric acid solution, which was homogenized in a homogenizer (model AM-8; Nihonseike Kaisha, Tokyo, Japan) and filtered through Toyo No. 5A filter paper (Toyo Rashi Kaisha, Tokyo, Japan) and then brought up to 50 mL. The extracts were titrated with 0.025% 2,6-dichloroindophenol.

Results and Discussion

Quality of soybean sprouts processed by the *sous vide* method Changes in microbiological and physicochemical qualities were observed at each step of the *sous vide* procedure of preparing soybean sprouts (Table 1). The blanching of soybean sprouts resulted in a lighter yellow color that was further reduced after pasteurization. The texture of blanched soybean sprouts was increased and after pasteurization was reduced. Sequential heat processing of soybean sprouts through blanching and pasteurization greatly reduced the content of ascorbic acid. Prior to cooking, raw soybean sprouts had an ascorbic acid content of approximately 8.37 mg/100 g, 3.45 mg/100 g after blanching, and 2.53 mg/100 g after pasteurization in control samples compared with 2.61 mg/100 g in samples with nisin.

Steam blanching followed by rapid cooling greatly reduced the microbial counts of mesophilic, psychrotrophic, and anaerobic micoorganisms. Prior to cooking, raw soybean sprouts had mesophilic microorganisms counts of

Table 1. Changes in physical, chemical, and microbiological qualities of soybean sprouts at each step through the *sous vide* process

Quality attribute	Raw	Blanched	*Sous vide* processed			
			Without nisin		With nisin	
			Control	*B. cereus*	Control	*B. cereus*
Color 'L'	76.2	72.7	76.2	74.0	75.5	73.9
'a'	-8.8	-10.8	-9.4	-10.6	-9.4	-10.5
'b'	17.9	19.1	15.8	15.0	16.8	16.2
Texture (cutting force, g_f)	707.4	1,156.1	1,000.2	987.1	995.3	998.6
Ascorbic acid content (mg/100 g)	8.37	3.45	2.53	2.34	2.61	2.51
Psychrophilic microorganisms (CFU/g)	2.0×10^8	3.2×10^2	ND[1)]	3.0×10^1	ND	ND
Mesophilic microorganisms (CFU/g)	1.2×10^8	3.2×10^2	ND	1.0×10^1	ND	ND
Anaerobic microorganisms (CFU/g)	3.2×10^7	4.0×10^2	ND	7	ND	ND
Bacillus cereus (CFU/g)	ND	ND	ND	5	ND	ND

[1)]Not detected.

approximately 1.2×10^8 CFU/g, and after blanching 3.2×10^2 CFU/g. After pasteurization, no microorganisms were detected in control samples without *B. cereus*.

Carlin *et al.* (7) reported that *sous vide* processed vegetable puree contained spore forming bacteria even after pasteurization. Raw soybean sprouts harbored large populations of mesophilic, anaerobic, and psychrotrophic microorganisms (>10^7 CFU/g). Most vegetables including soybean sprouts, have a high water content and therefore provide a good environment for the growth of micro-organisms. The numbers of mesophilic, psychrotrophic, and anaerobic microorganisms were below detectable levels in control soybean sprout packages after being cook-chilled. Heat and chill processing destroyed the micro-organisms present in the food or damaged the cells. Damaged cells are not easily detected and enumerated by current microbiological methods. However, these micro-organisms can also pose a food safety and spoilage risk as they can repair themselves if the conditions become favorable. In a previous study, microorganisms such as coliforms, yeasts and molds, fecal *Streptococcus*, and *Pseudomonas* spp. were very high (>10^5 CFU/g) in raw and washed samples, after cook-chill processing were not detected after 10 days at 3 or 10°C (12).

Microbiological analysis Storage at 10°C was used to represent mild temperature abuse, since surveys of retail cases in supermarket and domestic refrigerators indicate that 20% exceeded temperatures of 10°C (25,26). The inoculum level of *B. cereus* used in this study was substantially higher than contamination levels which have been found in either fresh or processed vegetables products (7,27). Therefore, the inoculum level and the storage conditions at 10°C used in this study represent the worst case scenario for this kind of *sous vide* product. Nisin, when used, was added to 100 IU/g, which can effectively control growth and spoilage in high acid canned vegetable products (16).

The effect on microbial growth of added nisin during the storage of packaged soybean sprouts is shown in Fig. 2. At 10°C storage, the numbers of mesophilic micro-organisms in control samples was markedly increased after 4 days, and reached more than 10^5 CFU/g by 28 days, the recommended limit in guidelines for *sous vide* processing. But the growth of mesophilic microorganisms in samples containing nisin was inhibited at 3 and 10°C According to Carlin *et al.* (7), the mesophilic microorganisms that survive the pasteurization process grow even at refrigerated temperatures and cause spoilage under temperature abuse conditions. At 30°C storage, the numbers of mesophilic microorganisms in the control samples was 7×10^7 CFU/g after 2 days, while samples containing nisin exceeded 10^5 CFU/g after 28 days, and there was no observable spoilage such as visible swelling (data not shown). The effect of nisin in *sous vide* processed soybean sprouts delayed microbiological growth and spoilage such as swelling. The important microorganisms at refrigeration temperatures are psychrotrophic microorganisms because these pathogens are capable of growth at these temperatures (28) and hence, pose a health risk for *sous vide* products (29). The number of psychrotrophic microorganisms in control samples at 10°C increased after 6 days, and after 34 days exceeded Department of Health and Social Security (DHSS) guidelines specifying that a bacterial load of 10^5 CFU/g of food is the allowable limit in a cooked chilled food product. In samples containing nisin, there were no significant changes during storage. The number of anaerobic microorganisms increased markedly at 10°C storage in soybean sprouts packages without nisin, but samples with nisin and the control at 3°C storage showed a marginal increase in microbial counts.

During storage of the *sous vide* processed soybeans sprouts inoculated with *B. cereus,* no increase in micro-biological counts was observed at 3°C with or without nisin (Fig. 3). The numbers of mesophilic microorganisms in *B. cereus* inoculated samples without nisin at 10°C storage increased after 2 days, and after 10 days exceeded the DHSS guidelines. In the presence of nisin, microbial growth was not detected until 2 days and increased thereafter, but remained below 10^3 CFU/g through 40 days of storage. At 3°C storage, the numbers of mesophilic microorganisms in the absence of nisin were 27±6 CFU/g, and in the presence of nisin were 4±4 CFU/g for 40 days of storage. Psychrotrophic microorganisms and anaerobic micro-organisms showed similar trends.

Nisin remains stable indefinitely at refrigeration or

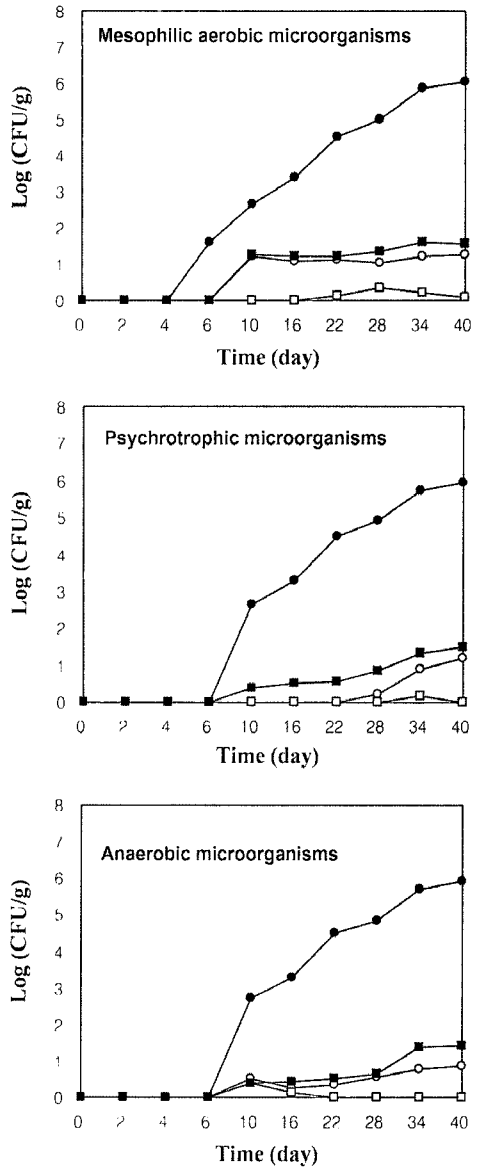

Fig. 2. Microbiological changes of *sous vide* processed soybean sprouts with or without nisin during storage. (○), Control samples; (□), samples with nisin. Open and closed symbols represented storage at 3 and 10°C, respectively.

freezing temperature, therefore when added to food its stability starts to decrease over time (16). In cheese, a 15% reduction in nisin activity occurred following 15 min at 85-95°C, and at 30°C a 55 to 70% reduction in nisin activity after 6 months can be expected. This lead to the loss of activity during storage for Swiss cheese, canned mushrooms, chocolate milk, and cooked ham. *B. cereus* was the dominant aerobic bacteria in cooked pasteurized and chilled vegetable soybean sprouts, because of the potential survival of their spores following pasteurization after packing. *Bacillus* spp. is also detected in other cooked chilled foods (7,26,27). Many *Bacillus* spp. are able to grow in anaerobic conditions, thus oxygen depletion created by packing under vacuum would not prevent their growth (7). Control of *B. cereus* also depends on storage temperature. Choma *et al.* (27) did not detect *B. cereus* in commercial cook-chilled foods containing vegetables at 4°C. However, surveys of retail cases in supermarket and domestic refrigerators indicated that 20% exceeded temperatures of 10°C (25,26). Especially in warm climates, inadequate refrigeration facilities during transportation and storage of properly *sous vide* processed soybean sprouts can reduce the shelf-life considerably. Because of the well-known difficulties in maintaining a low storage temperature along the distribution chain, and to ensure a greater level of safety, it has been proposed that additional safeguards such as the use of bacteriocins are necessary in the manufacture of *sous vide* products (13-15). The majority of bacteriocins retain their activity after mild heating. Nisin is particularly effective at controlling the growth of spore formers such as *Bacillus* and *Clostridium* genera as well as species of *Listeria monocytogenes*, *Staphylococcus*, and many lactic acid bacteria species (30). Only nisin has been approved for use by the FDA in several commodities.

The number of *B. cereus* of inoculated samples increased during 10°C storage, and after 28 days was above 10^4 CFU/g, the maximum limit recommended in France for some processed vegetables (27). Spoilage in soybean sprouts at 3°C storage was not detected for 40 days. No major changes in *B. cereus* growth at 3°C storage were observed during storage. As 4°C is the recommended storage temperature for cook-chilled products in France (27), *B. cereus* should not be a hazard whenever refrigeration is properly maintained throughout the shelf-life of the product.

The increase in numbers of microorganisms during storage depends on the storage temperature and the type of *sous vide* vegetable. It may also depend on differences in the initial microbial contamination, and *sous vide* products made from vegetables receiving a milder heat treatment may harbor strains more apt to grow in vegetables and cause spoilage under refrigeration.

Physicochemical analysis Texture was assessed as the cutting force required when a soybean sprout stem was sliced by a 0.26 mm blade using a rheometer. The texture of raw materials was 707.4 g_f and that of blanched soybean sprouts increased to 1,156.1 g_f but decreased to 1,000.2 and 987.1 g_f after cook-chill processing (Fig. 4). The texture of control samples and inoculated samples at 10°C storage did not show significant changes until after 28 days and decreased markedly after 34 days. The texture of control samples at 10°C storage decreased markedly after 34 days, but samples with nisin at 10°C, much like the control and nisin-containing samples at 3°C storage, did not show significant changes during storage. Knochel *et al.* (31) did not observe significant changes in texture during 25 days of storage of *sous vide* cooked green beans. Xie (32) studied the effect of *sous vide* cook-chill (SVCC) and traditional cook-chill processes on the textural changes of dry peas.

The surface colors of the soybean sprouts with or without nisin were measured by the Hunter color system after storage at various temperatures. During storage, the 'b' value of soybean sprouts, which can be interpreted as the degree of yellow color in the Hunter color scale, decreased while the 'L' and 'a' values did not show any noticeable changes from their respective initial values of 76.2 and −9.4. The changes of color increased after

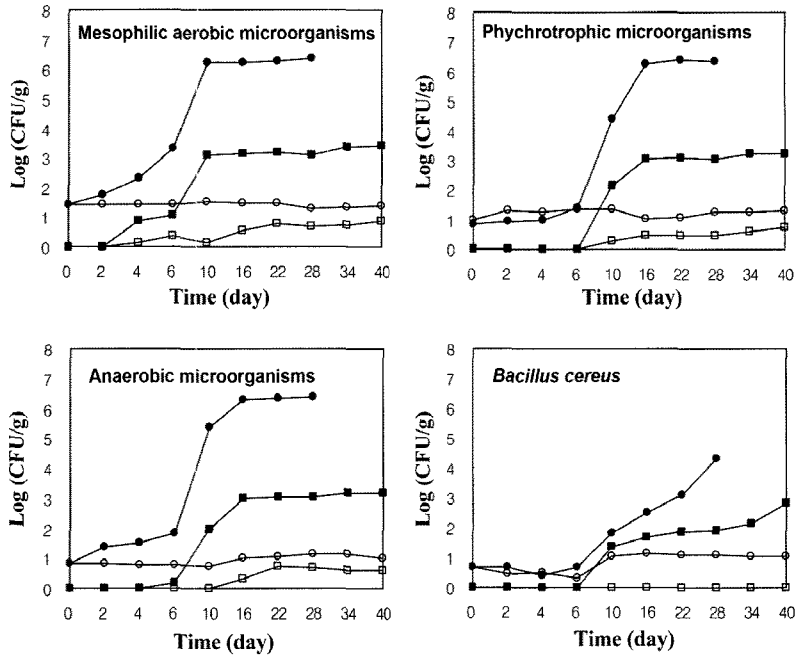

Fig. 3. Microbiological changes of *sous vide* processed soybean sprouts with *Bacillus cereus* in the absence or presence of nisin during storage. (○), Control samples; (□), samples with nisin. Open and closed symbols represent storage at 3 and 10°C, respectively.

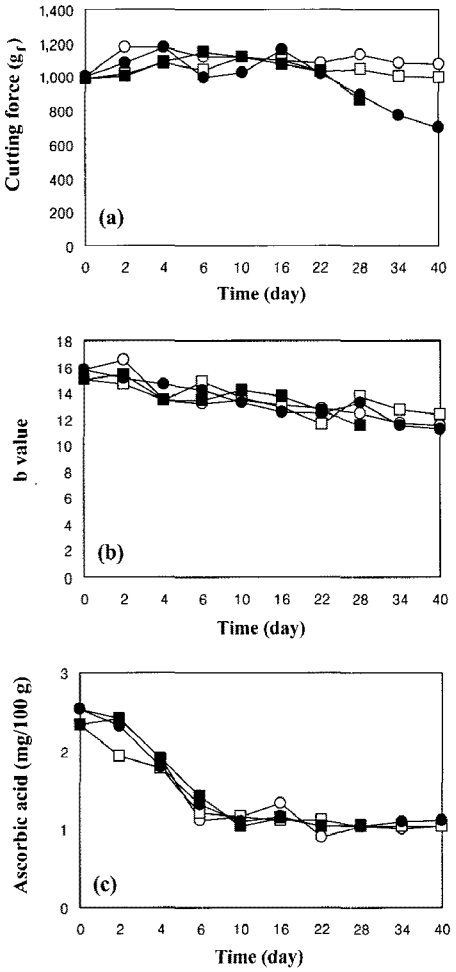

Fig. 4. Changes of physicochemical properties in the absence or presence of nisin in *sous vide* processed soybean sprouts during storage. (○), Control samples; (□), samples with nisin. Open and closed symbols represent storage at 3 and 10°C, respectively.

blanching and decreased after cook-chilled processing. The changes observed were a lighter yellow color due to blanching, which decreased further after processing. No major changes were observed in the color of *sous vide* cooked soybean sprouts with regard to the presence of nisin or storage temperature (3 and 10°C). This result is in contrast to a similar study by Chourot *et al.* (33) involving raw and blanched green beans.

The ascorbic acid content of the control and nisin-containing samples decreased slowly after a sharp decrease during initial storage. However, no major changes in ascorbic acid content were observed after 10 days of storage. Prior to cooking, raw soybean sprouts had an ascorbic acid content of approximately 8.37 mg/100 g with a decrease of 59% after blanching, 70% after pasteurization, and 87% after 40 days of storage (Table 1). This progressive deterioration results in an insubstantial amount of ascorbic acid in the food by the time it is consumed (34). However, in this study, no major changes in the ascorbic acid content of samples with or without nisin at either temperature were observed during storage. Based on these results, the loss of ascorbic acid was lower in comparison to other studies involving soybean sprouts (35), and the change in vitamin C of steamed broccoli (36) which also showed a little variation during storage.

Shelf-life was investigated in relation to storage temperature and microbial growth, especially that of *B. cereus* (37). Changes in physicochemical qualities were greater at 10°C than 3°C, however the difference between storage temperatures was less pronounced than changes in microbial quality. The mild and gradual changes in physicochemical qualities make it hard to define probable shelf-life based on these criteria. It is difficult to determine the critical limit of various qualities for the product. If changes in physicochemical qulalities are used for this purpose, a shorter shelf-life will be suggested than if

microbial criteria are considered. Thorough sensory studies may be helpful for this purpose, along with their correlation with the results of microbial tests.

Acknowledgments

This work was supported by Korea Science and Engineering Foundation (project # R01-2005-000-10235-0) and the Brain Korea 21 project, Korea.

References

1. Church IJ, Parsons AL. Review: *Sous vide* cook-chill technology. Int. J. Food Sci. Tech. 28: 563-574 (1993)
2. Creed PG, Reeve W. Principles and applications of *sous vide* processed foods. pp. 57-88. In: *Sous Vide* and Cook-Chill Processing for the Food Industry. Ghazala S (ed). Aspen Publishers, Gaithersburg, MA, USA (1998)
3. Gould GW. Methods for preservation and extension of shelf life. Int. J. Food Microbiol. 33: 51-64 (1996)
4. Creed PG. Sensory and nutritional aspects of *sous vide* processed foods. pp. 57-88. In: *Sous Vide* and Cook-Chill Processing for the Food Industry. Ghazala S (ed). Aspen Publishers, Gaithersburg, MA, USA (1998)
5. Caterine JM, Elizabeth AS. Microbiological safety aspects of cook-chill foods. pp. 311-336. In: *Sous Vide* and Cook-Chill Processing for the Food Industry. Ghazala S (ed). Aspen Publishers, Gaithersburg, MA, USA (1998)
6. Kramer JM, Gilbert RJ. *Bacillus cereus* and other *Bacillus* species. pp. 21-70. In: Foodborne Bacterial Pathogens. Doyle MP (ed). Marcel Dekker, New York, NY, USA (1989)
7. Carlin F, Guinebretiere MH, Choma C, Pasqualini R, Braconnier A, Nguyen-The C. Spore-forming bacteria in commercial cooked, pasteurized, and chilled vegetable purees. Food Microbiol. 17: 153-165 (2000)
8. Valero M, Leontidis S, Fernandez PS, Martinez A, Salmeron MC. Growth of *Bacillus cereus* in natural and acidified carrot substrates over the temperature range 5-30°C. Food Microbiol. 17: 605-612 (2000)
9. van Netten P, van de Moosdjik A, van Hoensel P, Mossel DAA, Perales I. Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. J. Appl. Bacteriol. 69: 73-79 (1990)
10. Dufrenne J, Soentoro P, Tatini S, Day T, Notermans S. Characteristics of *Bacillus cereus* related to safe food production. Int. J. Food Microbiol. 23: 99-109 (1994)
11. Jang JH, Lee NA, Woo GJ, Park JH. Prevalence of *Bacillus cereus* group in rice and distribution of enterotoxin genes. Food Sci. Biotechnol. 15: 232-237 (2006)
12. Kim HJ, Kim GN, Lee DS, Paik HD. Distribution of indicator organisms and incidence of pathogenic bacteria on soybean sprouts in cook-chill system. Food Sci. Biotechnol. 11: 412-416 (2002)
13. Ben Embarek PK, From Jeppesen V, Huss HH. Antibacterial potential of *Enterococcus faecium* strains isolated from *sous-vide* cooked fish fillets. Food Microbiol. 11: 525-536 (1994)
14. Thomas LV, Raghavan GSV, Alikhani Z. Nutritive quality of whole soybeans heat-treated in a continuous flow processor. J. Agr. Eng. Res. 61: 49-56 (1995)
15. Vessoni P, Moraes DA, Fajardo DN. The effect of nisin on kinetics from activated *Bacillus cereus* spores in cooked rice and in milk. J. Food Protect. 65: 419-422 (2002)
16. Bibek R. Nisin of *Lactococcus lactis* ssp. *lactis* as a food bio-preservative. pp. 207-264. In: Food Biopreservatives of Microbial Origin. Bibek R (ed). CRC Press, Boca Raton, FL, USA (1992)
17. Lo'pez-Mendoza M, Ruiz P, Mata CM. Combined effects of nisin, lactic acid, and modified atmosphere packaging on the survival of *Listeria monocytogenes* in raw ground pork. Int. J. Food Sci. Tech. 42: 562-566 (2007)
18. Ko SH, Kim HS, Jo SC, Cho SH, Park WS, Lee SC. Evaluation of pH-sensitive eudragit E100 microcapsules containing nisin for controlling the ripening of *kimchi*. Food Sci. Biotechnol. 14: 358-362 (2005)
19. Mauriello G, De Luca E, La Storia A, Villani F, Ercolini D. Antimicrobial activity of a nisin-activated plastic film for food packaging. Lett. Appl. Microbiol. 41: 464-469 (2005)
20. Lee CH, Park HJ, Lee DS. Influence of antimicrobial packaging on kinetics of spoilage microbial growth in milk and orange juice. J. Food Eng. 65: 527-531 (2004)
21. Lee CH, An DS, Lee SC, Park HJ, Lee DS. A coating for use as antimicrobial and antioxidative packaging material incorporate nisin and α-tocoferol. J. Food Eng. 62: 323-329 (2003)
22. Ohmomo S, Murata S, Katayama N, Nitisinprasart S, Kobayashi M, Nakajima T, Yajima M, Nakanishi K. Purification and some characteristics of enterocin ON-157, a bacteriocin produced by *Enterococcus faecium* NIAI 157. J. Appl. Microbiol. 88: 81-89 (2000)
23. Kim HJ, Lee DS, Paik HD. Characterization of *Bacillus cereus* isolates from raw soybean sprouts. J. Food Protect. 67: 1031-1035 (2004)
24. AOAC. Official Methods of Analysis Intl. 16th ed. Association of Official Analytical Communities, Arlington, VA, USA (1995)
25. Harris RD. Kraft build safety into next generation refrigerated foods. Food Proc. 50: 111-114 (1989)
26. Simpson MV, Smith JP, Simpson BK, Ramaswamy H, Doods KL. Storage studies on a *sous vide* spaghetti and meat sauce product. Food Microbiol. 11: 5-14 (1994)
27. Choma C, Guinebretiere MH, Carlin F, Schmitt P, Velge P, Granum PE, Nguyen-The C. Prevalence, characterization, and growth of *Bacillus cereus* in commercial cooked chilled foods containing vegetables. J. Appl. Microbiol. 88: 617-625 (2000)
28. Bolton DJ, McMahon CM, Doherty AM, Sheridan JJ, McDowell DA, Blair IS, Harrington D. Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef under laboratory conditions and in *sous-vide* prepared minced and solid beef cooked in a commercial retort. J. Appl. Microbiol. 88: 626-632 (2000)
29. Schofield GM. Emerging food-borne pathogens and their significance in chilled foods. J. Appl. Bacteriol. 72: 267-273 (1992)
30. Mansour M, Milliere JB. An inhibitory synergistic effects of a nisin-monolaurin combination on *Bacillus* sp. vegetative cells in milk. Food Microbiol. 18: 87-94 (2001)
31. Knochel S, Vangsgaard R, Johansen LS. Quality changes during storage of *sous vide* cooked green beans (*Phaseolus vulgaris*). Z. Lebensm. Unters. F. A 205: 370-374 (1997)
32. Xie G. Comparison of textural changes of dry peas in *sous vide* cook-chill and traditional cook-chill systems. J. Food Eng. 43: 141-146 (2000)
33. Chourot JM, Lauwers J, Massoji N, Lucas T. Behaviour of green beans during the immersion chilling and freezing. Int. J. Food Sci. Tech. 36: 179-187 (2001)
34. McErlain L, Marson H, Ainsworth P, Burnett SA. Ascorbic acid loss in vegetables: Adequacy of hospital cook-chill system. Int. J. Food Sci. Nutr. 52: 205-211 (2001)
35. Kim HY. A study in total vitamin C content changes in process of food products flow and holding time of cooked soybean sprouts and fresh vegetable salads in food service operations. Korean Diet. Culture 13: 9-17 (1998)
36. O'Leary E, Gormley TR, Butler F, Shilton S. The effect of freeze-chilling on the quality of ready-meal components. Lebens.-Wiss. Technol. 33: 217-224 (2000)
37. Lee DS, Hwang KJ, Seo I, Park JP, Paik HD. Estimation of shelf life distribution of seasoned soybean sprouts using the probability of *Bacillus cereus* contamination and growth. Food Sci. Biotechnol. 15: 773-777 (2006)