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Effect of Protease Produced from *Bacillus polyfermenticus* SCD on Quality of Jerky

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Abstract The objective of this study was to examine the effects of crude protease from *Bacillus polyfermenticus* SCD and marination time on quality of pork and beef jerky. Neither pork nor beef jerky showed a significant difference in pH among all treatments, and each protease was found to have a greater effect on the color of beef jerky. The hardness was significantly lower in all jerky treated with each protease, however the textural properties of jerky were not significantly different with regard to marination times. Water content was not affected by protease addition or marination times, however the water activity was lower in jerky treated with protease. The rehydration capacity of pork jerky was higher in jerky treated with protease, whereas that of beef jerky was higher in jerky dried after tumbled and held for 24 hr. Sensory characteristics were higher in jerky treated with protease, not affected by holding time after marinated.

Key words: jerky, protease, *Bacillus polyfermenticus* SCD, marination time

Introduction

Texture is an important characteristic of meat products with regard to consumer preference. Meat toughness can be subdivided into actomyosin toughness, which is attributable to changes in myofibrillar proteins, and background toughness, which is attributable to connective tissues (1).

There are several means for tenderizing meat, chemically or physically, which mainly reduce the amount of detectable connective tissue without causing extensive degradation of myofibrillar proteins. Treatment with proteolytic enzymes is one of the popular methods for meat tenderization. Most commercial enzymes currently used at present commercially are derived from plants: e.g., papain and bromelain, and have been widely used as meat tenderizers in America and Europe (2). However, these enzymes often degrade the texture of the meat due to their broad substrate specificity, and result in production of unfavorable taste due to over-tenderization (3).

Microbial proteases from bacteria, yeasts, molds have also been developed for meat tenderization (4,5). In particular, proteases from *Bacillus* sp. have become the most important industrial enzymes based on the world wide enzyme sales (6). *Bacillus polyfermenticus* SCD, which is commonly referred to as a 'Bispan' strain has been effectively used for the treatment of long-term intestinal disorders since live strains in the form of active endospores can successfully reach the target intestine (5). Many studies have addressed the properties of *B. polyfermenticus* SCD, including its industrial utility (7), capacity to inhibit carcinogen-induced DNA damage (8), and anticarcinogenic and antigenotoxic effects (9). However there has been no study on the utility of crude protease from *B. polyfermenticus* SCD as a meat tenderizer.

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Intermediate moisture meat products such as jerky are processed almost everywhere in the world and each product has its own characteristics. Such products include charqui in South America (10), pemmican in North American, mixed ground dried meat with dried fruit or suet known as biltong in South Africa (11), and bundnerfleisch, koppa, and cecina in Europe (12). Because most of the moisture in jerky is removed, it has a stable shelf life, is microbiologically safe (Aw<0.70), easy to prepare, light-weight, has a rich nutrient content, and can be stored without refrigeration (11). But with growing consumer preference for high quality foods with good flavor, texture, and nutrition, one of the drawbacks of jerky is its hard texture (13). It is important to address the tough texture of jerky because it has traditionally been made from sliced whole muscles. This problem may be solved by using crude protease and holding curing meat tumbled with curing solution in which crude protease is added.

Therefore, the purpose of this study was to examine the effects of crude protease from *B. polyfermenticus* SCD and holding time after marinated on the quality of pork and beef jerky.

Materials and Methods

Meat and curing solution preparation Frozen pork ham and beef round (*M. biceps femoris*, *M. semitendinosus*, *M. semimembranosus*) were purchased from a local market at 1 week post mortem. The frozen meat was thawed at low temperature (<4°C) until a core temperature of −1- −2°C was reached, then sliced into 6 to 8 mm thick sections parallel to the muscle fiber. The meat was trimmed of all subcutaneous fat before use in jerky preparation.

The composition (%, w/w) of the curing solution was water (10%), soy sauce (9%), starch syrup (5%), sugar (2%), D-sorbitol (6%), pepper (0.5%), ginger powder (0.1%), garlic power (0.2%), onion power (0.2%), sodium nitrate (0.007%), sodium citrate (0.01%), potassium sorbate

(0.1%), sodium erythorbate (0.036%), and soup stock powder (0.1%).

Preparation of tenderizers Tenderizers were prepared with the crude protease from *Streptomyces griseus* (P 5147, EC 3.4.24.31, powder, 4 units/mg solid, Sigma-Aldrich Chemical Co., St. Louis, MO, USA), which is used mainly to tender the fermented sausage (4). *B. polyfermenticus* SCD was purified according to a previous study (14). Each crude protease was added to concentrations of 0.005 or 0.01% (protease weight/raw meat weight) in the curing solution. The jerky samples tested were: control (no added), S-1 (with 0.005% protease from *S. griseus*), S-2 (with 0.01% protease from *S. griseus*), B-1 (with 0.005% protease from *B. polyfermenticus* SCD), and B-2 (with 0.01% protease from *B. polyfermenticus* SCD).

Manufacturing of jerky Sliced beef and pork were mixed with curing solution by hand for 3 min. The meat was then continuously massaged in a tumbler (MHM 20; Vakona, Lienen, Germany) for 30 min. Before drying the cured meat, two manufacturing processes were applied. One process involved drying the cured meat immediately, and the other process was to dry the cured meat after holding at 4°C for 24 hr. Tumbled beef was dried in a dehydrator (Enex-CO-600; Enex, Yongin, Korea) at 72°C for 90 min, 65°C for 60 min, and 55°C for 60 min (11). Tumbled pork was dried at 76.5°C for 90 min, 65°C for 60 min, and 55°C for 60 min (15). After drying, the jerky was cooled at room temperature (25°C) for 30 min, and then put in a polyethylene bag at room temperature and used for analysis.

pH and instrumental color measurements The pH of jerky samples was determined with a pH meter (model 340; Mettler-Toledo GmbH, Schwerzenbach, Switzerland). pH values were measured by blending a 5 g sample with 20 mL distilled water for 60 sec in a homogenizer (Ultra-Turrax T25; Janke & Kunkel, Staufen, Germany).

Instrumental color measurements were taken with a color meter (Chroma meter CR-200; Minolta, Japan, illuminate C, calibrated with white standard plate $L^* = 97.83$, $a^* = -0.43$, $b^* = +1.98$) were by measuring on the surface of samples.

Instrumental texture measurement The textural properties of jerky samples were measured by a cylinder probe (5 mm diameter) attached to a texture analyzer (TA-XT2i; Stable Micro Systems Ltd., Surrey, UK). The test conditions were as follows: stroke, 20 g; test speed, 2 mm/sec; distance, 10.0 mm. Data were collected and analyzed regarding the hardness (N), springiness, cohesiveness, gumminess (N), and chewiness (N) values.

Moisture content and water activity (Aw) Moisture content was determined by weight loss after 24 hr of drying at 105°C in a drying oven (SW-90D; Sang Woo Scientific Co., Bucheon, Korea) using the method of the AOAC (16).

Samples for Aw were minced into pieces approximately 1×1×1 mm in size. The Aw of each sample was determined in duplicate with a hygrometer (BT-RS1; Rotronic Ag., Bassersdorf, Switzerland).

Rehydration capacity The rehydration capacity of jerky samples was analyzed according to the method of Yun *et al.* (17). Samples were cut to a size of 20×20 mm. Cut samples and 50 mL distilled water were combined in a 100 mL beaker and the weight of soaked sample was measured after 15, 30, 45, and 60 min.

Rehydration capacity (%) =
[(sample weight after rehydration − sample weight before rehydration) / (sample weight before rehydration)] × 100

Sensory evaluation Each jerky sample was subjected to sensory evaluation. The samples were served to 10 experienced panel members with previous experience. Panelists were presented with randomly coded samples. The color (1 = extremely undesirable, 10 = extremely desirable), flavor (1 = extremely undesirable, 10 = extremely desirable), tenderness (1 = extremely tough, 10 = extremely tender), juiciness (1 = extremely dry, 10 = extremely juicy), and overall acceptability (1 = extremely undesirable, 10 = extremely desirable) of the samples were evaluated using a 10-point descriptive scale. Panelists were required to cleanse their palate between samples with water (18).

Statistical analysis Analysis of variance was performed on all the variables measured using the general linear model (GLM) procedure of the SAS statistical package (19). The Duncan's multiple range test ($p<0.05$) was used to determine the differences between the means.

Result and Discussion

pH and instrumental color Figure 1 shows the pH of pork and beef jerky prepared with various crude protease levels and holding times after marinated. Regardless of the treatment, the pH of pork jerky was 5.80-5.82 and the pH of beef jerky was 5.84-5.86. In addition, the kind of crude protease and holding time did not have a significant effect on the pH of pork or beef jerky ($p>0.05$). Jose *et al.* (20) reported that the average pH of beef jerky ranged widely from 4.72 to 6.73. In addition, Han (21) reported that pH of pork jerky was 5.71-5.75.

Table 1 shows the color values (CIE-L*, a*, b*) of pork and beef jerky prepared with various crude protease levels and holding times after marinated. The CIE-L* value (lightness) of pork jerky was significantly lower in the group treated with 0.01% crude protease than in the control ($p<0.05$), however jerky held for 24 hr after marinated showed no significant difference in CIE-L* value. The CIE-a* (redness) and b* (yellowness) values were not significantly different regardless of the kind of crude protease and holding time ($p>0.05$). While Song (22) reported that the color of beef jerky treated with humectants was different from that of untreated jerky, Han (21) reported that the addition of humectants had an only slight effect on the color of pork jerky.

Instrumental texture Table 2 shows the texture values of pork and beef jerky prepared with various crude protease levels and holding times after marinated. The hardness of pork jerky was significantly lower in the crude protease treated samples than in the control, and the

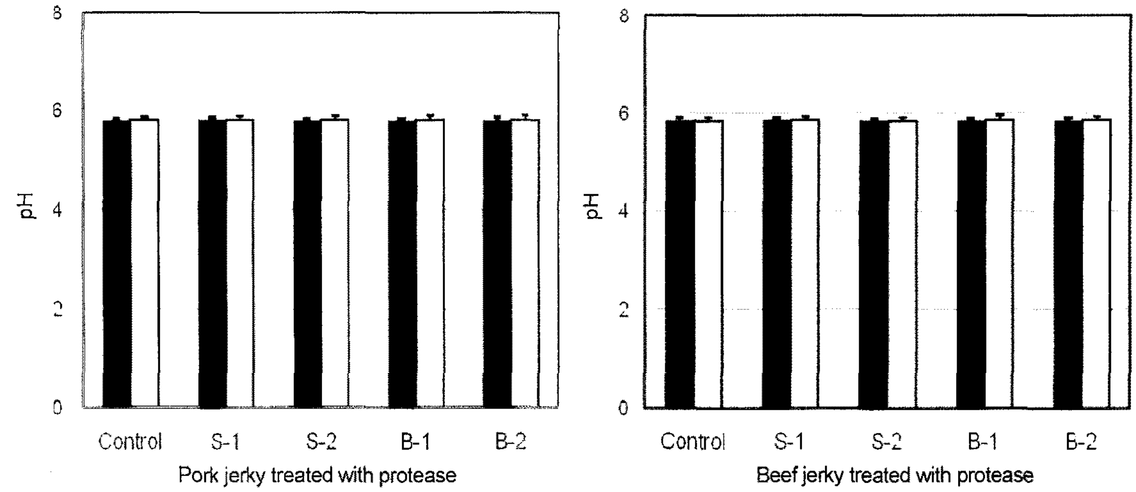

Fig. 1. Comparison of the pH of pork and beef jerky prepared with various protease levels and holding times after marinated. Bars represent standard deviations. ■, Holding time (0 hr); □, holding time (24 hr). Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

springiness of jerky marinated for 24 hr was significantly higher when treated with 0.01% crude protease from *B. polyfermenticus* SCD than in the control or jerky treated with 0.01% crude protease from *S. griseus* ($p<0.05$). In addition, the cohesiveness of jerky dried just after tumbling was higher in the jerky treated with 0.005% crude protease from *S. griseus* than in those with 0.01% crude protease from *S. griseus*, however the cohesiveness of jerky held for 24 hr after marinated showed the opposite tendency ($p<0.05$). The gumminess and chewiness of jerky dried just after tumbling were lower in jerky treated with 0.01% crude protease than the control or those treated with 0.005% crude protease ($p<0.05$). The gumminess and chewiness of jerky dried after holding for 24 hr were higher in samples treated with 0.01% crude protease from *S. griseus* than samples treated with 0.005% crude protease from *S. griseus* or 0.01% crude protease from *B. polyfermenticus* SCD ($p<0.05$). The hardness of beef jerky showed no significant difference ($p>0.05$), but the springiness was significantly lower in crude protease treated samples ($p<0.05$). The cohesiveness was higher in jerky treated with crude protease from *S. griseus* than in jerky treated with crude protease from *B. polyfermenticus* SCD ($p<0.05$). The gumminess and chewiness were significantly lower in jerky treated with 0.01% crude protease from *B. polyfermenticus* SCD than the other jerky samples. Holding time after marinated did not have a significant effect on the hardness of pork and beef jerky. The result of present study differed from the results of Gerelt *et al.* (23).

Table 1. Comparison of the instrumental color (CIE-L*, a*, b*) of pork and beef jerky processed with various protease levels and holding times after marinated[1)]

Traits	Jerky	Holding time after marination (hr)	Control	S-1	S-2	B-1	B-2
CIE-L*	Pork	0	44.30±1.06^{A}	43.74±1.03^{AB}	42.93±1.25^{B}	43.99±1.05^{AB}	43.25±1.77^{B}
		24	44.07±0.91	43.38±1.32	42.82±1.27	43.40±1.46	43.00±1.16
	Beef	0	42.32±1.16	41.90±1.26	41.72±1.97	41.38±0.95	41.79±0.33
		24	42.02±1.44^{A}	41.61±1.38^{AB}	41.53±2.07^{AB}	40.22±1.18^{B}	41.29±0.72^{AB}
CIE-a*	Pork	0	9.38±1.20	9.44±0.78	9.40±0.77	9.27±0.66	9.31±1.14
		24	9.50±0.98	9.38±0.69	9.43±0.74	9.08±0.59	9.23±1.08
	Beef	0	5.28±0.72^{A}	4.29±1.00^{B}	4.03±0.76^{B}	3.92±0.74^{B}	3.54±0.55^{B}
		24	5.21±0.71^{A}	3.97±0.74^{B}	4.13±0.85^{B}	3.96±0.77^{B}	3.80±0.63^{B}
CIE-b*	Pork	0	3.12±0.32	3.22±0.22	3.13±0.48	3.03±0.43	3.31±0.77
		24	3.16±0.31	3.19±0.79	3.18±0.51	3.05±0.39	3.34±0.21
	Beef	0	1.12±0.28	1.01±0.20	1.07±0.31	1.19±0.35	1.03±0.18
		24	1.11±0.33	0.98±0.30	1.05±0.21	1.18±0.28	1.05±0.19

[1)]All data are means±SD. [A,B] Means with different superscripts within the same row are significantly different ($p<0.05$). Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

Table 2. Comparison of the instrumental texture of pork and beef jerky processed with various protease levels and holding times after marinated[1)]

Traits	Jerky	Holding time after marination (hr)	Control	S-1	S-2	B-1	B-2
Hardness (N)	Pork	0	60.70 ± 5.02^{A}	55.96 ± 2.96^{B}	53.30 ± 4.22^{B}	56.79 ± 5.41^{B}	55.04 ± 6.05^{B}
		24	59.27 ± 3.62^{A}	53.83 ± 2.74^{B}	50.59 ± 3.07^{B}	53.52 ± 4.44^{B}	51.41 ± 4.24^{B}
	Beef	0	49.37 ± 4.67	47.69 ± 4.08	46.51 ± 4.18	47.04 ± 4.72	46.24 ± 4.15
		24	47.31 ± 5.47	45.77 ± 4.52	43.92 ± 4.86	46.41 ± 4.43	44.00 ± 3.85
Springiness	Pork	0	0.91 ± 0.04	0.91 ± 0.07	0.92 ± 0.03	0.92 ± 0.05	0.92 ± 0.04^{b}
		24	0.88 ± 0.07^{C}	0.93 ± 0.03^{AB}	0.91 ± 0.05^{B}	0.92 ± 0.05^{AB}	0.95 ± 0.02^{Aa}
	Beef	0	0.93 ± 0.02^{A}	0.89 ± 0.03^{B}	0.89 ± 0.04^{B}	0.91 ± 0.04^{AB}	0.91 ± 0.03^{AB}
		24	0.93 ± 0.02^{A}	0.89 ± 0.03^{B}	0.89 ± 0.03^{B}	0.90 ± 0.03^{B}	0.90 ± 0.04^{B}
Cohesiveness	Pork	0	0.13 ± 0.01^{AB}	0.14 ± 0.02^{Aa}	0.12 ± 0.01^{Bb}	0.13 ± 0.01^{AB}	0.13 ± 0.02^{AB}
		24	0.13 ± 0.02^{B}	0.12 ± 0.01^{Cb}	0.16 ± 0.01^{Aa}	0.14 ± 0.01^{AB}	0.13 ± 0.02^{B}
	Beef	0	0.20 ± 0.02^{B}	0.23 ± 0.02^{A}	0.23 ± 0.02^{A}	0.21 ± 0.02^{B}	0.20 ± 0.02^{B}
		24	0.20 ± 0.02^{AB}	0.23 ± 0.02^{A}	0.23 ± 0.02^{A}	0.21 ± 0.03^{AB}	0.19 ± 0.02^{B}
Gumminess (N)	Pork	0	8.07 ± 0.91^{A}	7.72 ± 0.88^{Aa}	6.33 ± 0.85^{Bb}	7.64 ± 0.84^{A}	6.92 ± 0.71^{B}
		24	7.75 ± 0.81^{AB}	6.64 ± 0.87^{Bb}	8.02 ± 0.92^{Aa}	7.33 ± 0.88^{AB}	6.63 ± 0.87^{B}
	Beef	0	9.91 ± 0.98^{B}	10.78 ± 1.01^{A}	10.61 ± 0.74^{AB}	9.99 ± 0.91^{B}	9.02 ± 0.96^{Ca}
		24	9.27 ± 0.86^{B}	10.53 ± 0.98^{A}	10.07 ± 0.84^{A}	9.62 ± 0.76^{AB}	8.35 ± 0.75^{Cb}
Chewiness (N)	Pork	0	7.34 ± 0.72^{A}	6.98 ± 0.88^{Aa}	5.82 ± 0.77^{Bb}	6.98 ± 0.75^{A}	6.36 ± 0.66^{B}
		24	6.83 ± 0.94^{AB}	6.19 ± 0.81^{Bb}	7.31 ± 0.74^{Aa}	6.76 ± 0.78^{AB}	6.31 ± 0.90^{B}
	Beef	0	9.21 ± 0.93^{A}	9.59 ± 0.79^{A}	9.45 ± 0.87^{A}	9.05 ± 0.91^{A}	8.17 ± 0.89^{Ba}
		24	8.63 ± 0.81^{AB}	9.35 ± 0.76^{A}	8.98 ± 0.87^{AB}	8.69 ± 0.72^{A}	7.53 ± 0.63^{Bb}

[1)]All data are means±SD. ^{A,B}Means with different superscripts within the same row are significantly different ($p<0.05$). Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

Moisture content and water activity Figure 2 shows the moisture content and water activity of pork and beef jerky prepared with various crude protease levels and holding times after marinated. In manufacturing intermediate moisture foods, it is important to control the water content because water activity is closely related to water content (24). The water content was 30-32% in pork jerky and 31-33% in beef jerky, and the type of crude protease and holding times after marinated had no significant effect on the water content of jerky. According to the reports of Jung

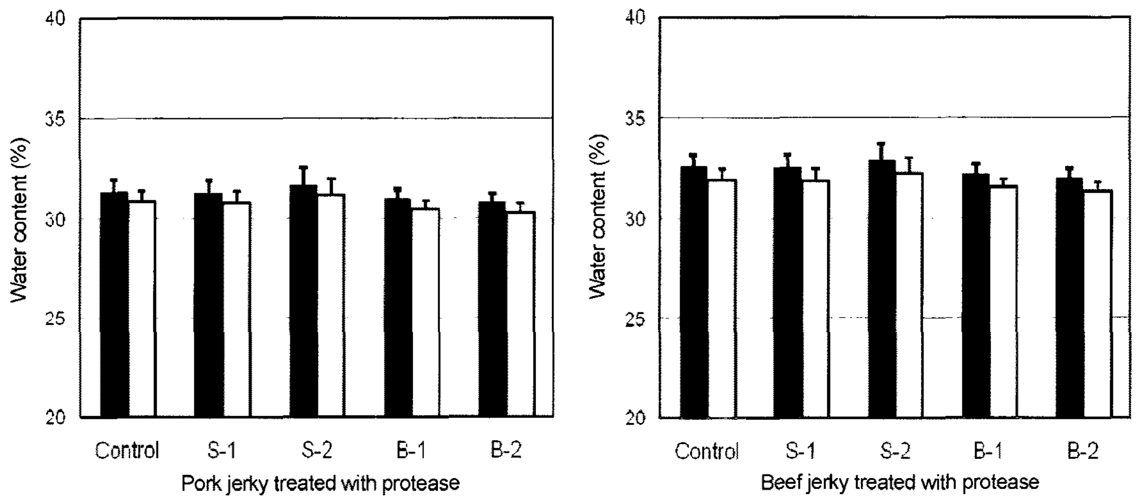

Fig. 2. Comparison of the moisture content of pork and beef jerky prepared with various protease levels and holding times after marinated. Bars represent standard deviations. ■, Holding time (0 hr); □, holding time (24 hr). Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

Table 3. Comparison of the water activity (Aw) of pork and beef jerky processed with various protease levels and holding times after marinated[1)]

Jerky	Holding time after marination (hr)	Control	S-1	S-2	B-1	B-2
Pork	0	0.71±0.01	0.70±0.01^{a}	0.70±0.02^{a}	0.70±0.01	0.70±0.01
	24	0.70±0.01^{A}	0.67±0.01^{Bb}	0.67±0.02^{Bb}	0.68±0.01^{AB}	0.68±0.01^{B}
Beef	0	0.71±0.01	0.70±0.02	0.70±0.02	0.71±0.01	0.70±0.02
	24	0.71±0.01^{A}	0.69±0.01^{B}	0.70±0.02^{AB}	0.70±0.01^{AB}	0.70±0.01^{AB}

[1)]All data are means±SD. ^{A,B} Means with different superscripts within the same row are significantly different (p<0.05). Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

et al. (25) and Yang and Lee (26), the water content of jerky in the market is 20-25%.

The Aw was 0.67-0.71 in pork jerky and 0.69-0.71 in beef jerky (Table 3). Jerky dried just after marination showed no significant difference among the samples treated crude proteases for both pork and beef (p>0.05), however crude protease treated jerky dried after holding for 24 hr showed significantly lower Aw than the control (p<0.05). Pork jerky treated with crude protease from *S. griseus* and held for 24 hr had the lowest Aw (p<0.05). Yang and Lee (26) reported that the average Aw of jerky in the domestic market was 0.743, and Bone (27) reported that the range of Aw of ordinary intermediate moisture foods was 0.65-0.90, which is consistent with the results of this study.

Rehydration capacity Figure 3 shows the rehydration capacity of pork and beef jerky prepared with various

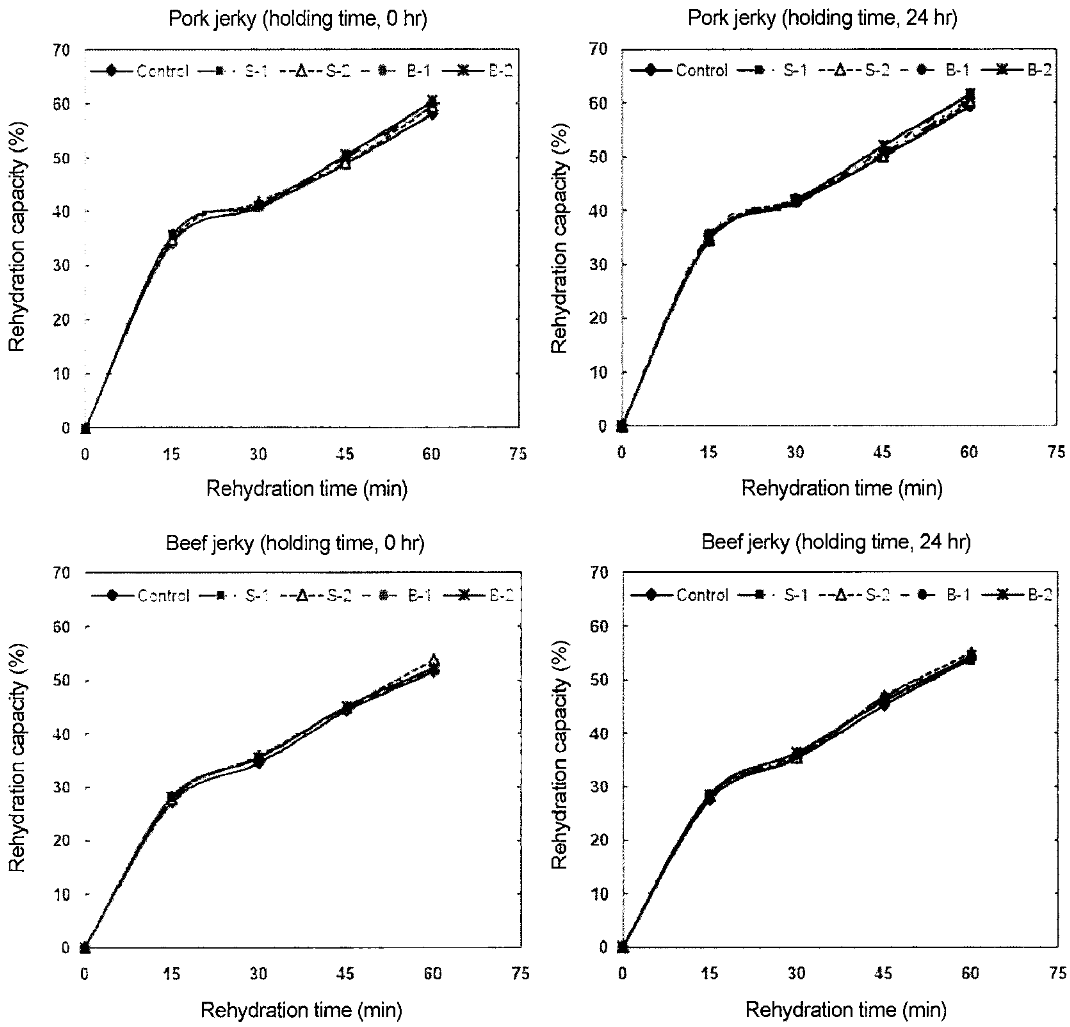

Fig. 3. Comparison of the rehydration capacity of pork and beef jerky prepared with various protease levels and holding times after marinated. Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

crude protease levels and holding times after marinated. The rehydration capacity of pork jerky and beef jerky showed a tendency to rise with increased rehydration time, and after 60 min of rehydration, pork jerky and beef jerky showed rehydration capacities of 58-62 and 55%, respectively. When pork jerky was rehydrated for 45 min, the jerky treated with 0.01% crude protease from *B. polyfermenticus* SCD showed a significantly higher rehydration rate than the control or jerky treated with 0.01% crude protease from *S. griseus*. When rehydrated for 60 min, pork jerky showed a significantly higher rehydration rate than the control ($p<0.05$). On the other hand, beef jerky rehydrated for 60 min and treated with 0.01% crude protease from *S. griseus* showed a significantly higher rehydration rate, and jerky held for 24 hr showed a higher rehydration rate than jerky made just after marination. Seol (28) reported that the rehydration capacity of Korean traditional beef jerky was approximately 40% and decreased during storage.

Sensory evaluation The sensory characteristics of jerky are affected by various factors including marination, drying temperature and time (29), and the condition of the raw meat (30). Figure 4 shows the sensory evaluations of pork and beef jerky prepared with various crude protease levels and holding times after marinated. The color and flavor were not significantly different between pork and beef jerky ($p>0.05$). However, the tenderness of jerky treated with crude protease was greater than the control, as was beef jerky treated with 0.01% crude protease ($p<0.05$). These results are consistent with those reported in instrumental texture analysis. The juiciness and overall acceptability of jerky dried just after marination were higher in pork jerky treated with crude protease than the control, and were higher for jerky held for 24 hr and treated with 0.01% crude protease ($p<0.05$). However, for beef jerky made just after marinated, jerky treated with 0.01% crude protease was rated significantly higher for juiciness and overall acceptability than the control ($p<0.05$). When holding for 24 hr after marinated, no significant difference was observed. Holding time after marinated did not have a significant effect on the sensory characteristics of pork jerky. Yang (31) reported that pork jerky had quite a high overall acceptability, and in particular, domestic beef jerky was more acceptable than imported jerky.

In conclusion, the results of this study show that treatment with crude protease from *B. polyfermenticus* SCD improved the tough texture of jerky and this effect was particularly high for pork jerky. Also, holding for 24 hr after marinated with crude protease did not have a significant effect on the quality of jerky. Thus, further studies on holding time after marinated with crude protease are needed.

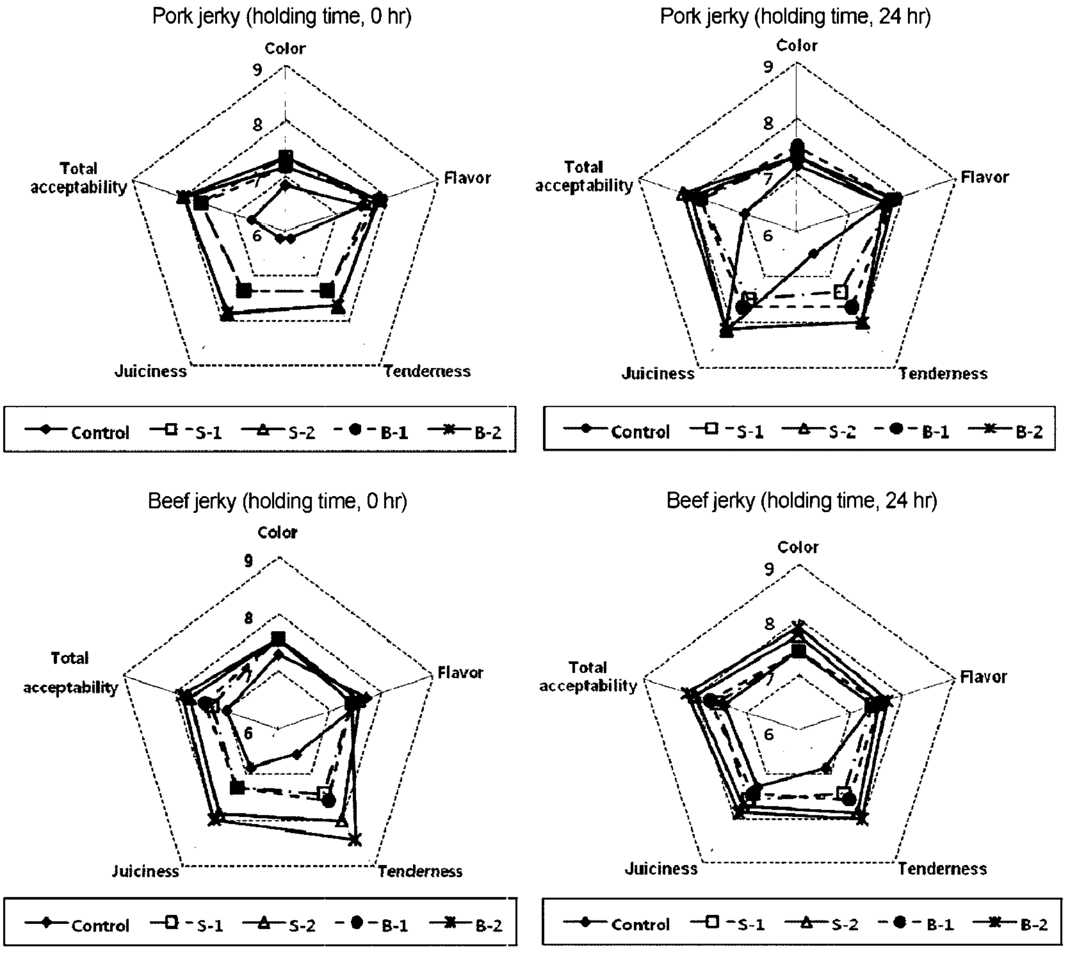

Fig. 4. Comparison of the sensory evaluations of pork and beef jerky processed with various protease levels and holding times after marinated. Control, not treated; S-1, treated with 0.005% protease from *S. griseus*; S-2, treated with 0.01% protease from *S. griseus*; B-1, treated with 0.005% protease from *B. polyfermenticus* SCD; B-2, treated with 0.01% protease from *B. polyfermenticus* SCD.

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