

## Effect of Soy Protein Isolate Coating on Meat Quality of Pork Fresh Cut during Refrigerated Storage

Jinhan Shon · Jin-Ho Kim · Ji-Hyun Eo · Yong-Hwa Choi

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**Abstract** Soy protein isolate (SPI)-based edible coating, with and without carboxymethyl cellulose (CMC), were used to reduce oxidative degradation of cut pork stored at 4°C for 5 days. The SPI coating reduced ( $p < 0.05$ ) thiobarbituric acid-reactive substances (TBARS) and peroxide value (PV), compared with controls. The inhibition of TBARS and PV for SPI-coated porks with and without CMC, compared with the control was 19.1 and 23.9, and 25.7 and 37.7%, respectively. The SPI coating prevented loss of  $L^*$  and  $a^*$  values of porks compared to the control. The ability of the SPI coating to provide a moisture barrier for the porks was reduced ( $p < 0.05$ ). The SPI-coated porks with and without CMC reduced moisture loss by 37.3 and 44.6%, respectively, over the control. However, SPI coating of porks did not inhibit the growth of either total plate counts or *L. monocytogenes*. The result revealed that SPI can effectively be used as a natural antioxidative coating to extend quality and shelf life of pork.

**Keywords** microbial growth · moisture loss · oxidative degradation · shelf life · soy protein coating

### Introduction

Fresh meat and meat products are commonly stored and distributed at refrigerated temperatures. However, many undesirable changes of the products can occur during refrigeration due to lipid oxidation and microbial growth, which may cause quality reduction,

meat spoilage, and economic loss. Lipid oxidation is a major limiting factor for the quality, acceptability, and shelf-life of meat and meat products. It leads to discoloration, off-flavor and off-odor development, and the formation of organic free radicals (Asghar et al., 1988). Various synthetic antioxidants, butylated hydroxytoluene and butylated hydroxyanisole have been used to as food preservatives because of their high efficacy in a variety of food systems (Decker, 1998). In addition, ascorbic acid, phosphates, nitrites, and nitrates have been used to enhance meat color quality (Mercier et al., 1998). However, the use of synthetic antioxidants is limited because consumers are increasingly demanding nonchemical or natural products. Consequently, there is a practical need for the selection of natural antioxidants as effective alternatives in the prevention of food deterioration.

Bacterial contamination deteriorates food quality and reduces shelf life. The growth of microorganisms in meats and meat products may cause both spoilage and foodborne diseases. Lactate, sodium lactate, nitrites, and sodium chloride have also been used in meat industry to improve various quality attributes of meat and to delay growth of meat spoilage organisms as well as foodborne pathogens and lipid oxidation (Cagri et al., 2001; Choi and Chin, 2003).

Research interest in edible coatings or films produced from proteins, polysaccharides, and lipids has intensified in recent years (McHugh et al., 1994). These coatings or films can help maintain and improve the quality of fresh, frozen and processed muscle foods by reducing moisture loss, lipid oxidation and color deterioration, acting as carriers for antimicrobial and antioxidant food additives (Gennadios et al., 1994; Krochta and De Mulder-Johnston, 1997). In recent years, edible films or coatings included soy protein, whey protein, collagen, gelatin, and methyl cellulose-based films (Were et al., 1999; Shon and Haque, 2007; Shon and Chin, 2008; Haque et al., 2009; Shon et al., 2010). Applications of natural products with both antioxidants and antimicrobial additives in meat products may be useful to prolong the shelf life for pork. Therefore, minimizing lipid oxidation and delaying or inhibiting product contamination, growth of spoilage, and pathogenic organisms in the product are major keys for improving

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fresh meat shelf life and increasing consumer safety (McCarthy et al., 2001b).

Soy proteins are widely used as functional and nutritional ingredients in food products and the ability to form films or coatings (Were et al., 1999). Furthermore, antioxidative activity was found for soy protein in liposomal model systems (Peña-Ramos and Xiong, 2002). Also, edible films or coatings based on soy proteins were found to be excellent oxygen barriers (Krochta and De Mulder-Johnston, 1997) and effectively inhibited lipid oxidation and reduced moisture loss in precooked beef patties (Wu et al., 2000) and cut raw Han-Woo beef during storage (Shon et al., 2010). According to Haque et al. (Haque et al., 2009), milk protein films containing carboxymethyl cellulose (CMC) had better antioxidative activities than those based only on protein (sour whey or calcium caseinate) and sorbitol. The objective of this study was to evaluate the effect of soy protein-based edible coating with and without CMC on quality attributes of cut raw pork stored at 4°C for 5 days in aerobic packaging.

## Materials and Methods

**Materials.** Soy protein isolate (SPI) was from Archer Daniels Midland (Decatur, IL) and calcium chloride was from Wright Nutrition (Crowley, LA). Glycerol, CMC, trichloroacetic acid, 2-thiobarbituric acid, methanol, ammonium thiocyanate, and ferrous chloride were from Sigma Chemical (St. Louis, MO). Glycerol and calcium chloride were food-grade. Ammonium sulfate, isooctane, 2-propanol, 1-butanol, hydrochloric acid, and barium sulfate were from Fisher Scientific (Fair Lawn, NJ). *L. monocytogenes* strain (ATCC, 43256) was obtained from the American Type Culture Collection (Rockville, MD) and palcam agar base was obtained from Oxoid Ltd. (Cambridge, England). Plate count agar was obtained from Difco Laboratories (Detroit, MI). All other reagents were analytical grade. Fresh porks (loin) were obtained from a wholesale meat market.

**Preparation of soy protein isolate coating solution.** The edible coating solution of SPI was prepared using the modified procedure outlined by Shon and Haque (2007). A 5% (w/v) aqueous solution of SPI was added to glycerol (2.5%, w/v), CaCl<sub>2</sub> (0.125%, w/v) with and without CMC (0.25%, w/v) with constant vortexing, followed by degassing, heat treatment at 90°C for 30 min, homogenization, filtration, cooling and final degassing. The solution was stored at 2°C before use.

For the coating process, fresh pork slices (2.5 cm<sup>3</sup>) were cut from three different pork cuts. The pork slices were randomly assigned for control and coating treatment. The pork slices were dipped into the SPI coating solution for 1 min at a room temperature (22±1°C) and drained for 10 s. The pork slices were then dried under clean air flow for 2 min (22±1°C) and packed immediately in a polystyrene foam food box and refrigerated at 4°C and analyzed for up to 5 days. Control samples were dipped in deionized distilled water (DDW) instead of SPI coating solution.

**Proximate analysis and pH values.** Moisture, fat, and protein contents were determined in triplicate by AOAC (1995). Ten grams of pork cuts were homogenized with 90 mL of DDW for 30 s using a Biomixer (Hamilton Beach, Washington, NC) and pH values were measured in triplicate using a pH meter (Mettler Toledo, MP120, Schwerzenbach, Switzerland).

**Color measurements.** A Minolta 508d spectrophotometer (Hunter Associates Laboratory, Ramsey, NJ), which was standardized using a standard white calibration plate, was used for color evaluation based on CIELAB (1976) color scales, L\* (lightness), a\* (redness) and b\* (yellowness) units. Color measurement was taken at 24 h intervals up to 5 days. Five readings on the upper side of each sample were averaged for color measurement.

**Measurements of antioxidative activity.** The FFA values of the extracted lipids were determined using the procedure described by AOCS (1987) and calculated as follows:

$$\text{FFA (\%)} = \text{mL of titration} \times \text{normality of KOH} \times 28.2 / \text{g of fat.}$$

The inhibition of oxidation in the porks was determined by the modified TBARS procedure outlined by Shon and Chin (2008). Coated and uncoated porks (20 g) were homogenized in DW (50 mL) using a Biomixer (Hamilton Beach, Washington, NC) for 2 min. Pork homogenate (2 mL) was transferred to a centrifuge tube and 2 mL of TBA reagent (15% trichloroacetic acid (w/v) and 0.375% 2-thiobarbituric acid (w/v) in 0.25 M HCl) were added. The solution was then heated in a boiling water bath for 15 min and cooled in an ice bath for 10 min. The resulting mixture was centrifuged at 6,000×g for 5 min (22±1°C), the supernatant was collected and the absorbance was measured at 532 nm using UV-visible spectrophotometer (Shimadzu Co., Kyoto, Japan). The percent inhibition (PI) was recorded as:

$$\text{PI} = 1 - ((\text{TBARS (mg MDA/kg of soy protein coated pork)}) / (\text{TBARS (mg MDA/kg of uncoated control pork)})) \times 100$$

A modified ferric thiocyanate (FTC) method described by Shon and Chin (2008) was used to evaluate the effect of SPI coating on hydroperoxide formation in porks. Coated and uncoated porks (20 g) were homogenized in DDW (50 mL) using a Biomixer. To determine the lipid peroxide value, a 2 mL aliquot was vortexed (10 s, 3 times) with 3 mL of isooctane/ 2-propanol and centrifuged at 2,000×g for 5 min (22±1°C) to obtain the organic solvent phase. An aliquot of this phase (400 µL) was added to 3 mL of methanol/1-butanol, followed by 30 µL of ammonium thiocyanate and 30 µL of the ferrous chloride solution. The absorbance of the solution was measured at 500 nm using an UV-visible spectrophotometer 20 min after addition of the ferrous chloride solution. The PI was calculated using the same formula that was used to calculate TBARS.

**Percent moisture loss (PML).** The PML was determined as described by Shon and Chin (2008). The moisture content (MC, wet basis) of the coated and uncoated porks was determined after storage at 4°C for up to 5 days and calculated using the AOAC method (1995). Triplicate samples (about 5 g) were weighed and

then oven dried at 102°C for 18 h. The samples were then cooled in a desiccator to room temperature and reweighed. The MC of all samples was determined before (day 0) and after storage for 5 days at 4°C. The PML was calculated as:

$$\text{PML (\%)} = \frac{(\text{initial MC} - \text{final MC})}{\text{initial MC}} \times 100$$

**Microbial counts.** The porks were inoculated and coated using the modified procedure outlined by (Shon et al., 2010). To prepare the inoculation, an aliquot of *L. monocytogenes* strain (ATCC, 43256) was placed in 9 mL of palcam agar base (PAB) with 1 mL being transferred to a fresh 9 mL PAB and incubated for 24 h at 37°C. Several dilutions were then prepared in sterilized water to obtain a final concentration of 10<sup>6</sup> colony forming units (CFU)/g of pork. Fresh pork samples were cut (10 g) and individually dipped for 1 min into culture broth containing approximately 10<sup>6</sup> CFU/g of *L. monocytogenes*. Porks were removed from the culture broth and allowed to drip free of excess inoculum and dry for 30 min under a laminar hood with blowing air. Pork samples were coated with the SPI film-forming solution by dipping them in it for 1 min and allowing them to drip dry. Control consisted of inoculated pork slices without any SPI coating. After inoculation and coating, the pork samples were packaged immediately using sterile vacuum films (200×300 mm), and stored for up to 5 days in a refrigerator (4°C) for analysis. Control and SPI coated porks were subjected to a temperature of 4°C. The total aerobic bacteria (TPC) and *L. monocytogenes* on the control and SPI-coated sausage samples were counted over the 5 day experimental storage period. Pork samples (10 g) inoculated with about 10<sup>3</sup> CFU/g of *L. monocytogenes* were homogenized in 90 mL of sterilized DDW. The mixtures (0.1 mL) were plated on a plate counts agar for TPC and on palcam agar for *L. monocytogenes*. Colonies were counted after incubation at 37°C for 48 h and results were expressed as log<sub>10</sub> CFU/g.

**Statistical design and analysis.** The experiment was conducted in triplicate and the data were analyzed using a 6 (storage times) × 3 (treatments) factorial design using the general linear model (PROC GLM) procedure of a SAS Statistical Program, version

8.1 (SAS Institute, Cary, NC) for Windows environment (2001). Means were separated using Fisher’s protected least significance test (*p* < 0.05).

**Results and Discussion**

**Proximate analysis and pH values.** The mean values of cut raw pork of moisture, fat, and protein content (%) were 49.1, 37.7, and 10.6%, respectively.

The storage time had a significant effect on pH at the fifth day of storage (*p* < 0.05), but the SPI coating did not affect the pH (*p* > 0.05), regardless of CMC (data not shown). The pH value was 6.14 at the initial storage time and decreased to a final pH of 5.86 for the porks at the fifth day of storage. The pH values of both the control and SPI-coated porks were quite stable until the day 4 of storage and then decreased thereafter (*p* < 0.05). These results mean that porks started to spoil faster after the 4th-day storage due to the availability of oxygen in aerobic packages. The decrease in pH during storage was attributed to activity of lactobacilli (Rubio et al., 2007).

**Color measurements.** The colorimetric L\* value was affected by the SPI coating, regardless of CMC (Table 1). The colorimetric L\* value, which reflects lightness, was 51.1, 50.2, and 50.6 for the control and the SPI-coated porks with and without CMC, respectively, at the initial storage time (Table 1). In the same order, these values significantly decreased (*p* < 0.05) to 25.6, 32.8, and 34.0, respectively, at the fifth day of refrigerated storage (Table 3). Loss of lightness in porks was 49.9 and 34.7, and 32.8% for the control and SPI coating with and without CMC, respectively, during the experimental period. Addition of CMC to the coating solution was reduced loss of lightness. This indicates a synergistic effect of CMC. A decrease in the L\* value during storage represented the formation of darker color in porks due to the browning reaction and the drying of porks in the air flow (Papastamatiou et al., 2007).

The CIE color scale a\* value, which represents redness, was

**Table 1** The color values of porks with soy protein isolate edible coatings with and without CMC during storage at 4°C for 5 days<sup>1)</sup>

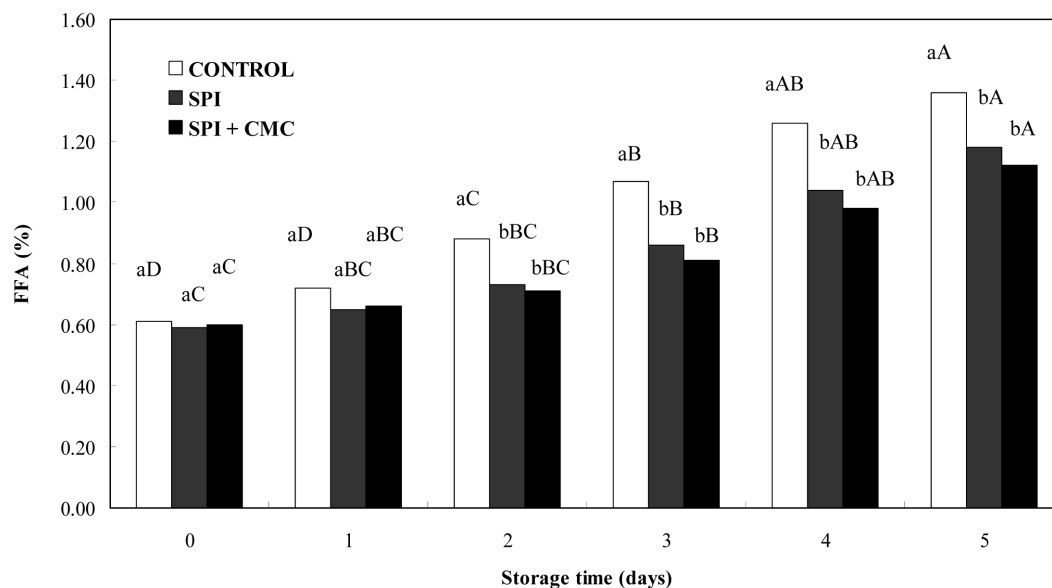
	Treatments	Storage time (days)					
		0	1	2	3	4	5
L*	Control	51.1 <sup>a</sup>	39.9 <sup>bY</sup>	32.5 <sup>cY</sup>	29.3 <sup>dY</sup>	26.7 <sup>eY</sup>	25.6 <sup>eY</sup>
	SPI	50.2 <sup>a</sup>	44.4 <sup>bX</sup>	40.4 <sup>cX</sup>	36.9 <sup>cdX</sup>	34.1 <sup>dX</sup>	32.8 <sup>dX</sup>
	SPI+CMC	50.6 <sup>a</sup>	45.7 <sup>bX</sup>	42.1 <sup>bcX</sup>	39.3 <sup>cX</sup>	36.1 <sup>dX</sup>	34.0 <sup>dX</sup>
a*	Control	9.56 <sup>aX</sup>	8.20 <sup>bY</sup>	5.70 <sup>cZ</sup>	5.41 <sup>dX</sup>	2.04 <sup>eZ</sup>	2.09 <sup>eZ</sup>
	SPI	7.33 <sup>bY</sup>	8.37 <sup>aY</sup>	6.21 <sup>cY</sup>	4.69 <sup>dY</sup>	2.67 <sup>eY</sup>	2.55 <sup>eY</sup>
	SPI+CMC	7.11 <sup>bcY</sup>	8.86 <sup>aX</sup>	7.50 <sup>bX</sup>	5.25 <sup>cX</sup>	3.72 <sup>dX</sup>	3.67 <sup>dX</sup>
b*	Control	3.05 <sup>f</sup>	5.12 <sup>cX</sup>	6.63 <sup>dX</sup>	7.45 <sup>cX</sup>	8.06 <sup>bX</sup>	8.52 <sup>aX</sup>
	SPI	3.19 <sup>f</sup>	4.47 <sup>cY</sup>	5.62 <sup>dY</sup>	6.38 <sup>cY</sup>	6.91 <sup>bY</sup>	7.43 <sup>aY</sup>
	SPI+CMC	3.24 <sup>f</sup>	4.59 <sup>cY</sup>	5.43 <sup>dY</sup>	6.14 <sup>cZ</sup>	6.59 <sup>bZ</sup>	6.98 <sup>aZ</sup>

<sup>1)</sup>Each value represents a mean of five replications.

<sup>a-f</sup>Means within the same row with different letters are significantly different (*p* < 0.05).

<sup>X,Y,Z</sup>Means within the same column with different letters are significantly different (*p* < 0.05).

Control=uncoated pork; SPI=soy protein coated pork; SPI+CMC=SPI with CMC.



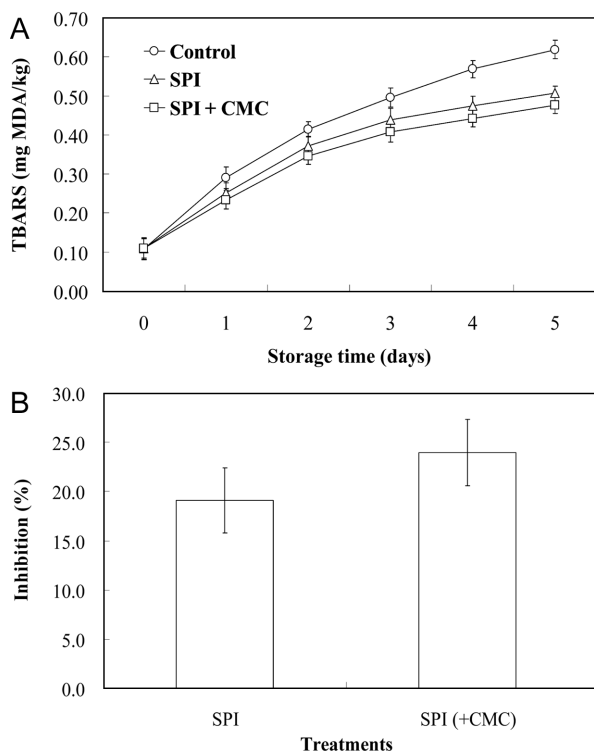
**Fig. 1** Changes in free fatty acid (FFA) (%) in porks with soy protein-based edible coating during storage at 4°C for 5 days. Values represent means of 3 replications. Dissimilar letters above bars indicate significant difference ( $p < 0.05$ ). Control=uncoated pork; SPI=soy protein-coated pork; SPI+CMC=SPI with CMC.

affected by the SPI coating, regardless of CMC (Table 1). The  $a^*$  value of porks decreased from 9.56 to 2.09 (a 78.1% loss) for the control and from 7.33 and 7.11 to 2.55 and 3.67 (a 65.2 and 48.4% loss) for SPI-coated porks with and without CMC, respectively, during refrigerated storage (Table 1). This indicates a synergistic effect of CMC. The  $a^*$  value of the SPI-coated porks tended to increase at day 1 of storage, but decreased significantly ( $p < 0.05$ ) thereafter. Myoglobin pigments are responsible for the red color in raw meat. In general, myoglobin in the ferrous state is oxidized to ferric metmyoglobin, resulting in a brownish-red meat (Hunt et al., 1999). The rate of meat discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation (Yin and Faustman, 1993). The retention of the redness of porks treated with SPI coatings may result from their antioxidative effects and their contribution of pigments.

The color  $b^*$  value (yellowness) was 3.05, 3.19, and 3.24 at the initial storage time and increased to 8.52, 7.43, and 6.98 for the control and SPI-coated porks with and without CMC, respectively, at the fifth day of storage (Table 1). These results indicated that the color of the porks turned a yellowish hue with increased time, indicating oxidation degradation and browning reactions. There was significant difference in the  $b^*$  value were observed between control and SPI-coated porks after a day 1 of storage period ( $p < 0.05$ ) (Table 1). The rapid color change might be mainly due to the lipid oxidation, resulting in the changes of color. However, this mechanism might not be fully understood. Calcium chloride, a divalent salt, is incorporated into the coating solution to improve the texture and color of food products. Previous reports indicated that soy protein coatings with calcium salts controlled darkening of fruits and vegetables (Park et al., 2001) and cut raw Han-Woo beef (Shon et al., 2010).

**Antioxidative activity.** Because FFAs were formed via the hydrolysis of fat tissues, including phospholipids, triglycerides, and diglycerides, they have been considered as an index to assess the degree of lipid oxidation. As expected, FFA values increased ( $p < 0.05$ ) with increased storage time in both the control and SPI coating cuts (Fig. 1). However, FFA values in porks were reduced ( $p < 0.05$ ) by SPI coatings as compared with the control. Especially, porks containing SPI coating with CMC was different from the control ( $p < 0.05$ ). Therefore, the SPI coatings used in this study showed an antioxidative activity as a result of the inhibition of lipolysis.

The antioxidative effect of SPI coating treatments as measured by TBARS, over the 5-day storage period at 4°C, is shown in Fig. 2A. The initial TBARS value was 0.108 in the controls and 0.110 and 0.109 mg MDA/kg in SPI-coated porks with and without CMC, respectively. The control had higher TBARS formation than SPI-coated porks over the 5-day storage period, regardless of CMC. The TBARS formation significantly increased up to 5-day storage time, indicating that lipid oxidation occurred in porks during refrigerated storage under aerobic packaging. The TBARS formation in SPI-coated porks was reduced compared to that in control after day 1 of storage, regardless of CMC (Fig. 2A). Data showed that TBARS formation was 0.62 in the control and 0.51 and 0.48 mg MDA/kg in SPI-coated porks with and without CMC, respectively, at 5-day of storage (Fig. 2A). The percent inhibition (PI) of TBARS formation compared to the control is shown in Fig. 2B. At 5-day of storage, PI-TBARS formation with respect to the control was 19.1 and 23.9% by SPI coating with and without CMC, respectively ( $p < 0.05$ ) (Fig. 2B). Mixing of CMC with the SPI in the coating mixture resulted in a small improvement of the antioxidative activity by 6.92% though the difference was

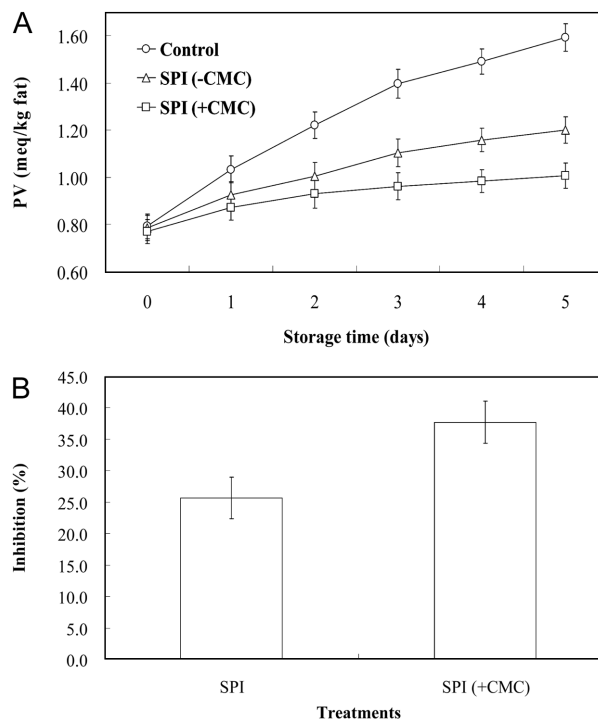


**Fig. 2** A. Changes in thiobarbituric acid-reactive substances (TBARS) (mg MDA/kg) formation in porks with soy protein-based edible coating during storage at 4°C for 5 days. B. Percentage inhibition by soy protein coating on formation of TBARS in porks after 5 days of storage at 4°C. Control=uncoated pork; SPI=soy protein-coated pork; SPI+CMC=SPI with CMC.

not significant. Data show that excellent antioxidative property of SPI by itself. A previous research reported that TBA number at which rancid odor was first perceived by consumers was between 0.5 and 1.0 mg/kg MDA (Gray and Pearson, 1987). This threshold has served as a guide for interpreting TBA test results. The controls in the present study would therefore be perceived as rancid after day 3 of storage, while SPI-coated porks did not exceed until day 4 of refrigerated storage under aerobic packaging (Fig. 2A).

The storage time had a significant effect on the PV and the interaction of treatment and storage time was also significant ( $p < 0.05$ ). The PVs also showed a similar trend to those of TBARS formation. The PVs for both the control and SPI-coated porks increased continuously as storage time increased up to 5-day of refrigerated storage, regardless of CMC (Fig. 3A). However, the SPI-coated porks had a lower PV than the controls. The PI of PV compared to controls is shown in Fig. 3B. The SPI-coated porks, with and without CMC, decreased PV by 25.7 and 37.7%, respectively, over the storage period (Fig. 3B). Furthermore, the addition of CMC increased the antioxidative capacity of this film by 18.5% for the SPI formulations. This indicates a synergistic effect of CMC.

A previous report show that SPI possessed appreciable antioxidative activity in a liposomal model systems (Peña-Ramos



**Fig. 3** A. Changes in peroxide values (PV) (meq/kg) in porks with soy protein-based edible coating during storage at 4°C for 5 days. B. Percentage inhibition by soy protein coating on formation of TBARS in porks after 5 days of storage at 4°C. Control=uncoated pork; SPI=soy protein-coated pork; SPI+CMC=SPI with CMC.

and Xiong, 2002). It is possible that the antioxidative phenolic compounds (about 1.5 mg/g) that are presented in SPI (Seo and Morr, 1984) could have contributed to the direct scavengers of reactive oxygen species (Rojas and Brewer, 2008). The CMC was incorporated into coating solution, and the carboxylic groups, which act as a chelating agent under certain conditions, may have caused the antioxidative activity (Sapers, 1993). Previous reports demonstrated that SPI effectively inhibited lipid oxidation in fresh pork patties (McCarthy et al., 2001a) and in cut raw Han-woo beef during storage (Shon et al., 2010). This may be attributed to the effective O<sub>2</sub> barrier properties of soy protein films or coatings (Brandenburg et al., 1993). During the preparation of the coating material, the mixture was heated at 90°C for 30 min resulting in the formation of intermolecular disulfide and hydrophobic bonds and producing Maillard reaction products (MRPs) that may possess antioxidant activity (Shon and Haque, 2007).

**Moisture barrier property.** The storage time had a significant effect on moisture content (MC), while the interaction of treatment and storage time was also significant ( $p < 0.05$ ) (Table 2). The mean initial MC ranged from 73.9 for the control to 74.3 and 74.5% for the SPI-coated porks with and without CMC, respectively (Table 2). The initial MC decreased to 54.4, 61.8, and 64.5% for the control and SPI-coated porks with and without CMC, respectively, at the 5-day of refrigerated storage (Table 2). This reflected a 26.3% for the control compared with 16.8 and

**Table 2** Moisture contents and percent moisture loss of porks with soy protein isolate edible coatings with and without CMC during storage at 4°C for 5 days<sup>1)</sup>

		Storage time (days)					
		0	1	2	3	4	5
MC (%)	Control	73.9 <sup>a</sup>	69.7 <sup>b</sup>	64.3 <sup>cY</sup>	60.2 <sup>dY</sup>	56.9 <sup>eY</sup>	54.4 <sup>fY</sup>
	SPI	74.3 <sup>a</sup>	70.4 <sup>b</sup>	68.1 <sup>b<sup>c</sup>X</sup>	66.9 <sup>c<sup>X</sup></sup>	63.8 <sup>d<sup>X</sup></sup>	61.8 <sup>e<sup>X</sup></sup>
	SPI+CMC	74.5 <sup>a</sup>	71.2 <sup>b</sup>	69.9 <sup>b<sup>c</sup>X</sup>	67.8 <sup>c<sup>X</sup></sup>	65.7 <sup>d<sup>X</sup></sup>	63.5 <sup>e<sup>X</sup></sup>
PML (%)	Control	0	5.68 <sup>c</sup>	12.9 <sup>d<sup>X</sup></sup>	18.5 <sup>c<sup>X</sup></sup>	23.0 <sup>b<sup>X</sup></sup>	26.3 <sup>a<sup>X</sup></sup>
	SPI	0	5.27 <sup>d</sup>	8.35 <sup>c<sup>Y</sup></sup>	9.96 <sup>b<sup>Y</sup></sup>	14.1 <sup>a<sup>b</sup>Y</sup>	16.8 <sup>a<sup>Y</sup></sup>
	SPI+CMC	0	4.52 <sup>d</sup>	6.22 <sup>c<sup>Z</sup></sup>	8.97 <sup>b<sup>c</sup>Y</sup>	11.8 <sup>b<sup>Z</sup></sup>	14.8 <sup>a<sup>Y</sup></sup>

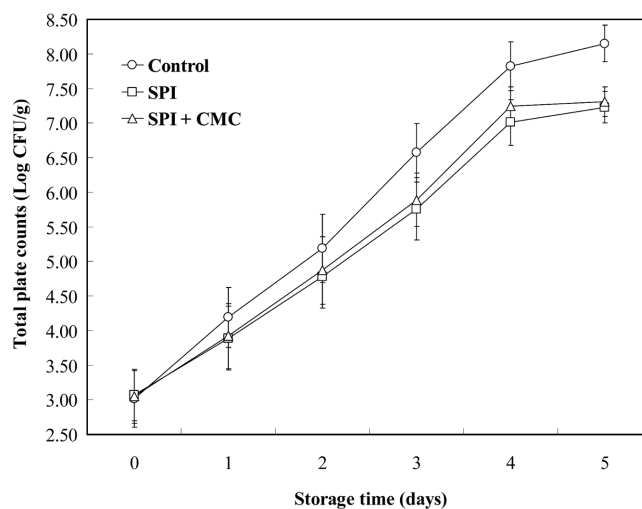
<sup>1)</sup>Each value represents a mean of three replications.

<sup>a-f</sup>Means within the same row with different letters are significantly different ( $p < 0.05$ ).

<sup>X,Y,Z</sup>Means within the same column with different letters are significantly different ( $p < 0.05$ ). Control=uncoated pork; SPI=soy protein coated pork; SPI+CMC=SPI with CMC; MC=moisture content (%); PML=percent moisture loss (%).

14.8% loss of moisture for the SPI-coated porks with and without CMC, respectively, during the experimental period.

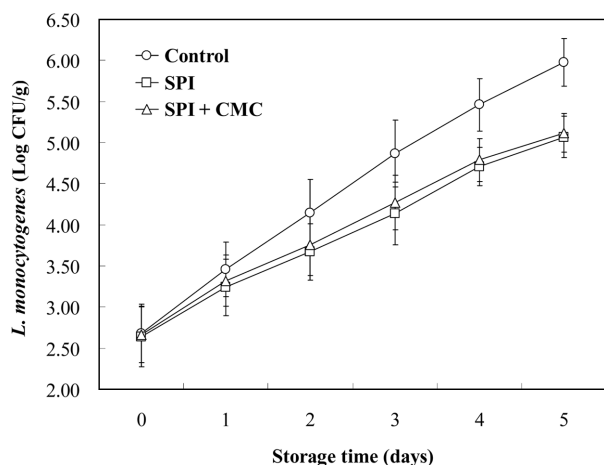
The PML was greatly affected by the change of MC in porks during storage. The storage time had a significant effect on PML, while the interaction of treatment and storage time was also significant ( $p < 0.05$ ) (Table 2). The PML for both the control and SPI-coated porks significantly increased during storage period. There was significant effect on PML in SPI-coated porks compared to control after a 2-day of storage period ( $p < 0.05$ ) (Table 2). The PML was 5.68, 5.27, and 4.52% at day 1 of storage and then continued to increase to 26.3, 16.8, and 14.8% for the control and SPI-coated porks with and without CMC, respectively, at the end of storage. Moisture loss was due to evaporation of moisture during refrigerated storage. The control samples were less protected, with a PML value of 26.3 compared to 16.8 and 14.8% for SPI-coated porks with and without CMC, respectively (Table 2). Thus, SPI-coated porks had less moisture loss than the control. Significant reduction of PML by 37.3 and 44.6% over control at the end of storage was achieved by SPI coating (Table 2). This may be attributed to the good moisture-barrier properties of soy protein coating. The PML varied between treatment and in the control with storage (Table 2). Addition of CMC to the coating solution was reduced loss of moisture. This indicates a synergistic effect of CMC. Mixing of CMC with the proteins in the coating mixture resulted in significant improvement of the PML value of SPI by 12.4%. There was a small improvement in PML of the SPI treatment though the difference was not significant. Previous reports demonstrated that soy protein coating reduced the moisture loss in precooked beef patties (Wu et al., 2000) and cut raw Han-Woo beef (Shon et al., 2010). During the preparation of the coating solution, the mixture was heated at 90°C for 30 min, producing disulfide bonds and greater surface hydrophobicity (Shon and Haque, 2007). This process is known to improve the moisture-barrier capability of protein-based edible films (Park et al., 2001). Calcium chloride incorporated into the coating solution improved the moisture-barrier properties of SPI films. The SPI films formulated with calcium salts have potential as moisture-barriers for food systems and as coating agents for food application



**Fig. 4** Microbial changes (total plate counts) (log CFU/g) of porks coated with soy protein during storage at 4°C for 5 days. Control=uncoated pork; SPI=soy protein-coated pork; SPI+CMC=SPI with CMC.

or intermediate-moisture foods due to the hydrophobicity of protein (Park et al., 2001). Furthermore, the coating mixture was homogenized for 2 min in a high-shear probe mixer resulting in the formation of oil-in-water emulsion. Therefore, good emulsification properties of the proteins correlated with surface hydrophobicity and contributed to improve the moisture-barrier (Shon and Haque, 2007).

**Microbial counts.** The effect of SPI edible coating on total plate bacteria (TPC) in porks was determined over the 5-day storage period at 4°C (Fig. 4). The increase in storage time produced significant proliferations in TPC for both the control and SPI-coated porks during refrigerated storage (Fig. 4). The initial TPC of porks ranged from approximately 3.01 in the control to 3.07 and 3.05 log CFU/g in SPI-coated porks with and without CMC, respectively. However, TPC of both the control (7.82) and SPI-coated porks with (7.01) and without (7.24) CMC exceed the



**Fig. 5** Microbial changes (*L. monocytogenes*) (log CFU/g) of porks coated with soy protein during storage at 4°C for 5 days. Control=uncoated pork; SPI=soy protein-coated pork; SPI+CMC=SPI with CMC.

maximal recommended limit of log 10<sup>7</sup>/g CFU/g for TPC in raw meat by the day 4 of storage at 4°C (Verma and Sahoo, 2000), indicating a shelf life of about 4 days (Fig. 4). However, no significant difference was observed between the control and SPI-coated porks. Data show that the SPI coating without antimicrobial agents did not effectively inhibit the growth of TPC.

The effect of SPI edible coating on the *L. monocytogenes* in porks over 5-day storage at 4°C is shown in Fig. 5. The storage time had a significant ( $p < 0.05$ ) effect on the growth of *L. monocytogenes*, while the SPI coating had no inhibitory effect ( $p < 0.05$ ) on the growth of this microorganism. The microbial counts for *L. monocytogenes*, which represents psychrotrophic nature, also showed a similar trend to those of TPC. The *L. monocytogenes* populations increased from 2.68, 2.64, and 2.66 log CFU/g to 5.98, 5.07, and 5.12 log CFU/g for control and SPI-coated porks with and without CMC, respectively, at the end of the storage time (Fig. 5). A previous report demonstrated that some plant proteins have antimicrobial properties. The SPI hydrolysates incorporated into frankfurters improved the shelf life of products during storage at 25°C under aerobic packaging (Vallejo-Cordoba et al., 1987). However, in the present study, SPI coating did not reduce the TPC count and *L. monocytogenes* growth compared with the control when the porks were stored at 4°C in aerobic packaging.

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