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ARTICLE

Gelatin Coating on Quality Attributes of Sausage during Refrigerated Storage

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Abstract

Gelatin-based edible coating was used to reduce the oxidative degradation of low-fat sausages (LFSs) stored at 4°C for 8 wk under vacuum packaging. The gelatin coating reduced thiobarbituric acid-reactive substances and peroxide value by 21.5 and 26.5%, respectively, compared with the controls. The moisture barrier effect was significantly better for the gelatin coating compared to the control. The gelatin coating reduced moisture loss in sausages by 32.6% over the control. However, the gelatin coating of sausages did not inhibit the growth of either the total plate counts or *L. monocytogenes*. Data show that gelatin can effectively be used as a natural antioxidative and moisture barrier coating to extend the quality and shelf life of sausages.

Key words: gelatin coating, sausage, lipid oxidation, moisture barrier, shelf life

Introduction

Modern trends in convenience foods have resulted in the increased consumption of precooked, refrigerated, or frozen ready-to-eat (RTE) meats and meat products such as sausages and hams due to not only their nutritional values but also their taste and flavor. However, these products are particularly susceptible to lipid oxidation, which leads to the rapid development of warmed-over flavor during storage (Love, 1988). Lipid oxidation results in off-flavors and odors, loss of polyunsaturated fatty acids, fat-soluble vitamins and pigments and reduced consumer acceptability (Gray *et al*., 1987). Various synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are commonly used to minimize lipid oxidation of food systems (Decker, 1998). However, these synthetic antioxidants have been limited in their applications as food additives by consumers due to health concern, and preferably nonchemical and natural. Synthetic antioxidants have been shown to cause lesion formation in the rat forestomach and internal and external haemorrhaging in some strains of mice and guinea pig at high dose (Shahidi and Wanasundara, 1992). Thus, there is motivation to search for safe and natural antioxidants from various sources originating from sea foods.

Gelatin is a protein resulting from partial hydrolysis of collagen using acid or alkali treatment followed by or accompanied with heating in the presence of water (Liu *et al*., 2008). The principal raw materials used for gelatin manufacture are pork skins and bovine hides, skins and bones (Liu *et al*., 2008). Recently attention has focused on the properties of films or coatings made from fish gelatins (Avena-Bustillos *et al*., 2006). Enzymatic processing is an alternative to acid or alkali hydrolysis for conversion of collagen to gelatin producing a higher purity, better physical properties, and a narrower molecular weight distribution (Hinterwaldner, 1977).

Research interest in edible coatings made from proteins, polysaccharides and lipids has intensified in recent years. These coatings can help maintain and improve the quality of fresh, frozen and processed muscle foods by reducing moisture loss, lipid oxidation and color deterioration, act as carriers for antimicrobial and antioxidant food additives (Gennadios *et al*., 1997).

Gelatin has been used as a preservative coating for meat and other foods (Villegas *et al*., 1999). Gelatin coatings or films have also been used to carry antimicrobials

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and antioxidants. The antioxidative activity of proteins is due to complex interactions between their ability to inactivate reactive oxygen species, scavenging free radicals, chelatate prooxidative transition metals (Elias *et al*., 2008). Previous reports demonstrated that gelatin coatings derived from fish sources effectively inhibited lipid oxidation in cooked ham and bacon (Villegas *et al*., 1999), in turkey steaks (Klose *et al*., 1952), and in cut pork belly (Marggrander and Hofmann, 1997). However, sufficient information about antioxidative and microbiological characteristics of edible gelatin coatings or films from fish sources is not available in literature. The objective of this study was to investigate the effect of gelatin coating would delay the progress of lipid oxidation and preserve color and moisture in cut low-fat sausages (LFSs) during refrigerated storage for 8 wk.

Materials and Methods

Materials

Skate skins used in the present study were obtained from a local skate processing plant (Korea). Glycerol, calcium chloride, carboxymethyl cellulose (CMC), trichloroacetic acid, 2-thiobarbituric acid, methanol, ammonium thiocyanate, and ferrous chloride were all from Sigma Co. (USA). Glycerol and calcium chloride were food grade. Isooctane, 2-propanol, 1-butanol, hydrochloric acid, and barium sulfate were from Fisher Scientific (USA). *L. monocytogenes* strain (ATCC, 43256) was obtained from the American Type Culture Collection (USA) and palcam agar base was obtained from Oxoid Ltd. (England). Plate count agar was obtained from Difco Laboratories (USA). All other reagents were analytical grade.

Manufacture of gelatin

The extraction procedures of gelatin from skate skins are described by Shon *et al.* (2011). The skate skins were thoroughly washed with tap water to remove impurities. The cleaned skate skins were soaked with a 0.1 N NaOH solution, followed by washing with deionized distilled water (DDW) to remove alkali. The gelatin extraction was carried out in DDW at 50°C overnight. The extracts were centrifuged at 15,000 g for 1 h at 4°C and the supernatants were immediately collected. The aliquots of the extract were then filtered through Whatman No.1 filter paper and activated carbon (250-350 mesh) and lyophilized to obtain gelatin powder. Gelatin samples were stored in sealed containers at -20°C until needed.

Manufacture of low-fat sausages (LFSs)

The LFSs were manufactured as described by Shon and Chin (2008). Fresh pork hams, from pigs which had been slaughtered a day earlier, were purchased from a local retail meat market, and all visible fats and connective tissues were removed. Trimmed pork hams were ground, vacuum-packaged, and stored at -20°C prior to use. Frozen pork hams were thawed and chopped to reduce the particle size. Salt, sodium nitrite, sodium erythorbate, and half of ice water were then added and chopped for 2 min to extract the salt soluble proteins. Seasonings, flavorants, and the remaining ice water were then added, and the meat batter was chopped until the temperature reached 15 to 16°C. Formulation and non-meat ingredients incorporated into LFSs for this study are listed in Table 1. The meat batter was vacuum-packaged and stuffed into cellulose casing (Suntan, Viscase Crop., USA), smoked, and cooked to an internal temperature of 71.7°C in a smoke chamber (Nu-Vu, ES-13, Food System, USA). After cooking, the sausages were chilled immediately in an ice bath and cut (about 20 cm) and vacuum-packaged in oxygen permeable (45 mL/m^{-2}/24 h) with vacuum film (200 x 300 mm, Fuji Plant Korea Inc., Korea), and stored in a refrigerator (4°C) for 8 wk.

Preparation of gelatin coating solution

The edible coating solution of gelatin was prepared as described by Shon *et al.* (2010). A 5%(w/v) aqueous solution of gelatin was prepared. Sorbitol (2.5%, w/v), $CaCl_2$ (0.125%, w/v), and CMC (0.25%, w/v) were added

Table 1. Formulation of meat and non-meat ingredients incorporated into low-fat sausage

Ingredients	Amounts (%)
Pork (hams)	55.0
Ice water	35.1
Non-meat ingredi*ents*	9.90
- *Fat re*placers[a]	2.50
- Salt	1.25
- Sugar	1.00
- Corn syrup	1.00
- Sodium tripolyphosphate	0.30
- Non-fat dry milk	1.00
- Maltodextrin	1.00
- Hydrolyzed milk protein	0.50
- Frankfurter spices	1.00
- Sodium erythorbate	0.05
- Salt/Sodium nitrite (Cure blend)	0.25
Total (%)	100.0

[a]Fat replacers: konjac flour, carrageenan, and soy protein isolate at the ratio of 1:1:3.

in DDW with constant vortexing, followed by degassing under vacuum. Subsequently, the solution was heated at 90°C for 30 min in a shaking water bath. The solution was homogenized for 2 min in a high-shear probe mixer (Hamilton Beach, USA) and filtered through a layer of cheese cloth to ensure the complete mixing of the lipid components and to remove foam and undissolved materials. The solution was cooled at room temperature (22±1°C) and degassed again with a vacuum pump. The final pH of the solution was adjusted to 6.5 with 0.1 M and/or 1.0 M HCl or NaOH, and the solution was stored at 2°C to allow the viscosity to increase before use.

Fresh LFSs slices (2.5 cm^3) were cut from three different sausages for each treatment for each analysis. The sausage slices were randomly assigned for control and coating treatment. The sausage slices were dipped into the gelatin coating solution for 1 min at room temperature (22±1°C) and drained for 10 s. The sausage slices were then dried under a laminar hood with blowing clean air flow for 2 min (22±1°C). The sausages were vacuum-packaged immediately using vacuum films (200×300 mm) and stored in a refrigerator (4°C) for up to 8 wk for analysis. Control samples were dipped in DDW instead of gelatin coating solution and the process of drying was the same as that for the treatments.

Proximate analysis and pH values

Moisture, fat, and protein contents were determined in triplicate by AOAC (1990). A pH meter (MP120, Mettler Toledo, Switzerland) was used for the pH measurement of the LFSs. Ten grams of sausage slices were homogenized with 90 mL of DDW for 30 s using a Biomixer (Hamilton Beach, USA) and pH values were measured in triplicate.

Color measurements

Color values were measured using a Chroma meter (CR-200, Minolta Corp., USA), which was standardized using as white blank (L* = 91.1, a* = 1.28, b* = -1.54). Samples were placed on a transparent glass plate and five readings on different sites of each sausage sample were averaged for color measurement of on sample. The results were expresses as Hunter L* (lightness), a* (redness), and b* (yellowness) color scale.

Measurements of antioxidative activity

Thiobarbituric acid-reactive substances (TBARS)

Inhibition of oxidation in LFSs was determined as described by Shon *et al.* (2010). Coated and uncoated sausages (20 g) were homogenized with DDW (50 mL) using a Biomixer (Hamilton Beach, USA) for 2 min. Sausages 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 100°C 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 a UV-visible spectrophotometer (Shimadzu Co., Japan). The percent inhibition (PI) was recorded as following:

PI = 1 − [(TBARS (mg MDA/kg of gelatin coated sausage) /(TBARS (mg MDA/kg of uncoated control sausage)] ×100

Peroxide value (PV)

A modified ferric thiocyanate (FTC) method as described by Shon *et al.* (13) was used to evaluate the effect of gelatin coating on hydroperoxide formation in LFSs. Coated and uncoated sausage samples (20 g) were homogenized with DDW (50 mL) using a Biomixer for 2 min. A 2 mL aliquot was vortexed (10 s, 3 times) with 3 mL of isooctane/2-propanol (3:1, v/v) and centrifuged at 2,000 g for 5 min (22°C) to obtain the organic solvent phase. An aliquot of this phase (400 μL) was added to 3 mL of methanol/1-butanol (2:1, v/v), followed by 30 μL of 30% ammonium thiocyanate and 30 μL of the ferrous chloride solution. The absorbance of the solution was measured at 500 nm using a UV-visible spectrophotometer for 20 min after addition of the ferrous chloride solution. The PI was calculated using the same formula that was used for calculating TBARS.

Percent moisture loss (PML)

The PML was determined as described by Shon *et al.* (2010). The moisture content (MC, wet basis) of the samples was determined and calculated using the AOAC method (AOAC) (1990). Triplicate samples (about 5 g) were oven (Dasol Scientific Co. Ltd., Korea) dried at 102°C for 18 h. The samples were then cooled in a desiccator (Dongsung Glass Co. Ltd., Korea) to room temperature and reweighed (Mettler Toledo Group, Switzerland). The MC of all samples was determined before (wk 0) and after storage for 8 wk at 4°C. The PML was calculated as:

PML (%) = [(initial MC final MC)/initial MC] × 100

Inoculation of *Listeria monocytogenes* and coating of sausage samples

The sausages 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 10^6 colony CFU/g of sausage. Fresh sausage samples were cut (25 g) and individually dipped for 1 min into culture broth containing approximately 10^6 CFU/g of *L. monocytogenes.* Sausages 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. Sausage samples were coated with the gelatin film-forming solution by dipping them in it for 1 min and allowing them to drip dry. Control consisted of inoculated sausage slices without any gelatin coating. After inoculation and coating, the sausage samples were packaged immediately using sterile vacuum films (200×300 mm), and stored for up to 8 wk in a re- frigerator (4°C) for analysis. Control and gelatin coated sausages were subjected to a temperature of 4°C. The total aerobic bacteria (TPC) and *L. monocytogenes* on the control and gelatin-coated sausage samples were counted over the 8 wk experimental storage period. Sausage samples (25 g) inoculated with 10^3 CFU/g of *L. monocytogenes* were homogenized in 225 mL of sterilized DDW. The mixtures 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 $\log_{10}$ CFU/g.

Statistical analysis

The experiment was performed in triplicate and data were analyzed using a 2 (treatments) × 6 (storage times, 0, 1, 2, 4, 6, and 8 wk) factorial design using the general linear model (PROC GLM) procedure of a SAS Statistical Program 8.1 (SAS, 2001). Means were separated using Fisher's protected least significance test ($p<0.05$).

Results and Discussion

Proximate analysis

The mean values of LFSs of moisture, crude fat, and crude protein content (%) were 76.2±1.11, 0.33±0.02, and 13.7%±0.06, respectively (data not shown).

pH values

Since no interactions ($p>0.05$) were observed between treatment and storage time for pH and Hunter L*, a* and b* values of sausages, data were pooled and are shown in Table 2. The initial pH decreased from 6.11±0.03 to 5.29 ±0.07 for the LFSs during refrigerated storage (Table 2). Thus, the storage time had a significant effect on pH at the 4-wk of storage ($p<0.05$), but gelatin coating did not affect the pH ($p>0.05$). The pH values were quite stable until the 2 wk of storage and then decreased thereafter ($p <0.05$). These results mean that sausages started to spoil after the 2 wk of storage. The reduction in pH was due to formation of lactic acid by lactic acid bacteria that utilized carbohydrate added in the formulation as an energy source (Antara *et al*., 2004).

Color measurements

Hunter color L* value (lightness) was not affected by the gelatin coating. The colorimetric L value decreased from 68.1±0.69 to 65.8±0.64 during refrigerated storage (Table 2) due to the browning reaction (Papastamatiou *et al*., 2007). No differences in lightness were found between the control and gelatin coated sausages under vacuum

Table 2. pH and Hunter color values of sausages with gelatin-based edible coating during storage at 4°C for 8 wk under vacuum package[1)]

	Treatments		Storage time (wk)					
	Control	Gelatin	0	1	2	4	6	8
pH	5.78±0.38$^{N.S}$	5.80±0.36$^{N.S}$	6.11±0.03^{a}	6.13±0.06^{a}	6.14±0.04^{a}	5.65±0.04^{b}	5.32±0.04^{c}	5.29±0.07^{c}
L*	66.8±0.91$^{N.S}$	67.0±0.68$^{N.S}$	68.1±0.69^{a}	67.7±0.27^{b}	66.4±0.45^{c}	67.2±0.34^{b}	66.2±0.40^{c}	65.8±0.64^{d}
a*	12.3±0.40$^{N.S}$	12.6±0.53$^{N.S}$	11.9±0.37^{c}	12.4±0.46^{b}	13.2±0.23^{a}	12.5±0.40^{b}	12.4±0.45^{b}	12.3±0.31^{b}
b*	8.51±0.38$^{N.S}$	8.41±0.34$^{N.S}$	7.97±0.42^{d}	8.17±0.51^{c}	8.42±0.54^{bc}	8.56±0.43^{b}	8.68±0.47^{ab}	8.97±0.34^{a}

[1)]Values represent means of 5 replications±SD.
N.S. = nonsignificant
[a-d]Means within the same row with different superscripts are significantly different ($p < 0.05$).
L*, a*, and b* are Hunter color values as determined by the colorimeter.
Control = uncoated sausage; Gelatin = gelatin coated sausage

package (p>0.05) (Table 2).

The other relevant part of the Hunter a* value (redness), which has been used as an indicator of color stability in meat and meat products, was not affected by the gelatin coating (Table 2). Hunter a* values of the sausage started 11.9±0.37 at the beginning of storage and increased to 13.2±0.23 at the 2-wk of storage, but tended to decrease by the end of the storage period due to the denaturation of nitrosomyoglobin (Table 2). The rate of meat discoloration is related to the rate of myoglobin oxidation induced by lipid oxidation (Yin and Faustman, 1993). Gelatin coating, however, was effective ingredient in improving meat redness in cooked ham and bacon in both aerobic and vacuum packaging (Villegas *et al*., 1999).

The Hunter b* value, which contributes to the appearance of freshness, increased from 7.97±0.42 to 8.97±0.34 during refrigerated storage (Table 2). These results indicated that the sausages turned a yellow color with increased time. The color change might be mainly due to the lipid oxidation. However, this mechanism might not be fully understood. The relationship between lipid oxidation and metmyoglobin formation is that increases in redox potential during lipid oxidation tend to oxidize myoglobin (Hur *et al*., 2004). A previous report found an increase in yellowness due to rancidity during storage of vacuum packaged ham slices (Garcia-Esteban *et al*., 2004). No differences in yellowness were observed between control and gelatin-coated sausages under vacuum package (p > 0.05) (Table 2). Color stability is an important quality attribute contributing to meat shelf-life, texture, and consumer acceptability. Calcium chloride, a divalent salt, is incorporated into the coating solution to improve the texture and color of food products (Garcia-Esteban *et al*., 2001). Whey and soy protein coatings containing calcium chloride were effective in maintaining the reddish color characteristic of cut pork and beef steak (Shon *et al*., 2010; Haque *et al*., 2009), and controlled darkening and oxidative browning of fresh-cut fruit and vegetables (Park *et al*., 2001; Shon and Haque, 2007). Previous reports indicate that edible protein films containing $CaCl_2$, which is as a crosslinking agent, have the potential for improved the mechanical properties (Park *et al*., 2001).

Antioxidative activity

The TBARS values for control and gelatin-coated sausages over the 8 wk of storage periods at 4°C under vacuum package were comparable to the PV patterns (Fig. 1A). Control sausages had higher TBARS formation than the gelatin-coated sausages over the 8 wk of storage periods. The TBARS formation significantly (p<0.05) increased up to 6 wk of storage, indicating that lipid oxidation had occurred in sausages and then decreased thereafter. A decrease in TBARS values after 6 wk of storage could be due to the formed TBARS being lost by further reaction. This decrease could also be attributed to decomposition of malonaldehyde (MDA) by bacteria, such as *Pseudomonas* and Enterobacteriaceae, which obtained the ability to selectively attack and utilize carbonyl compounds, including MDA (Moerck and Ball, 1974). The TBARS formation for gelatin-coated sausages were reduced compared to the control (Fig. 1B). There was significant inhibition of TBARS formation by 21.5% compared to the control for gelatin coating after 8 wk of refrigerated storage under vacuumed packaging (p<0.05) (Fig. 1B). Previous reports indicated that gelatin coating effectively inhibited lipid oxidation in cooked ham and bacon (Villegas *et al*., 1999), in turkey steaks (Klose *et al*., 1952), and

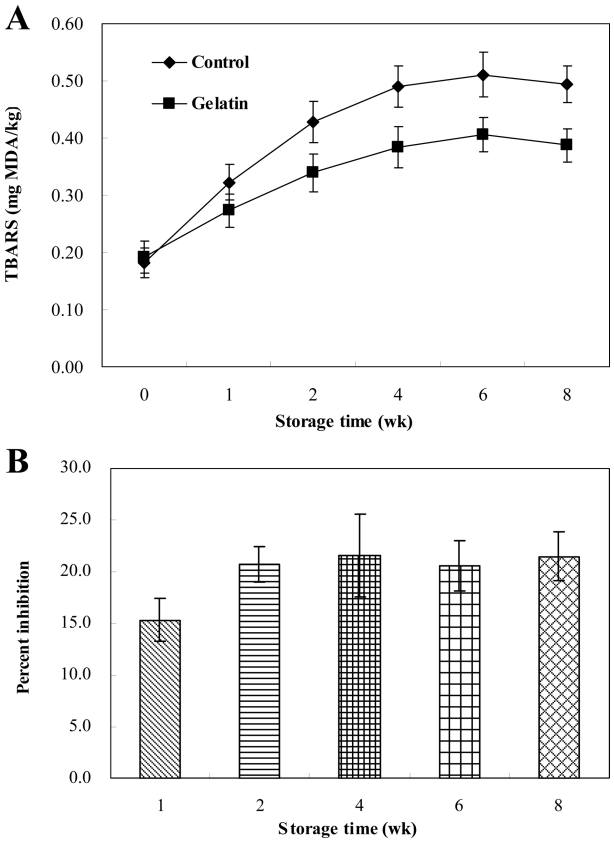

Fig. 1. (A) Changes in thiobarbituric acid-reactive substances (TBARS) (mg MDA/kg) formation in sausages with gelatin-based edible coating during storage at 4°C for 8 wk under vacuum package. (B) Percentage inhibition by gelatin coating on formation of TBARS in sausages after 8 wk of storage at 4°C under vacuum package. Control, uncoated sausage; gelatin, gelatin-coated sausage

in cut pork belly (Marggrander and Hofmann, 1997), and improved retention of flavor, taste and aroma of cut pork belly during storage (Marggrander and Hofmann, 1997). This may be attributed to the good oxygen barrier properties of gelatin films or coatings (Krochta and De Mulder-Johnston, 1997). Our data suggest that these oxygen barrier properties can help reduce lipid oxidation and color deterioration during refrigerated storage of meat products.

The PVs for the control and gelatin-coated sausages increased continuously as storage time increased up to the 6-wk of refrigerated storage and then decreased thereafter (Fig. 2A). It is possible that the rate of hydroperoxide decomposition was higher than the rate of its formation (Georgantelis *et al*., 2007). As the refrigerated storage time increased, the PV also increased, however, the gelatin-coated sausages had a lower PV than the controls. Treatment with gelatin coating decreased PV formation

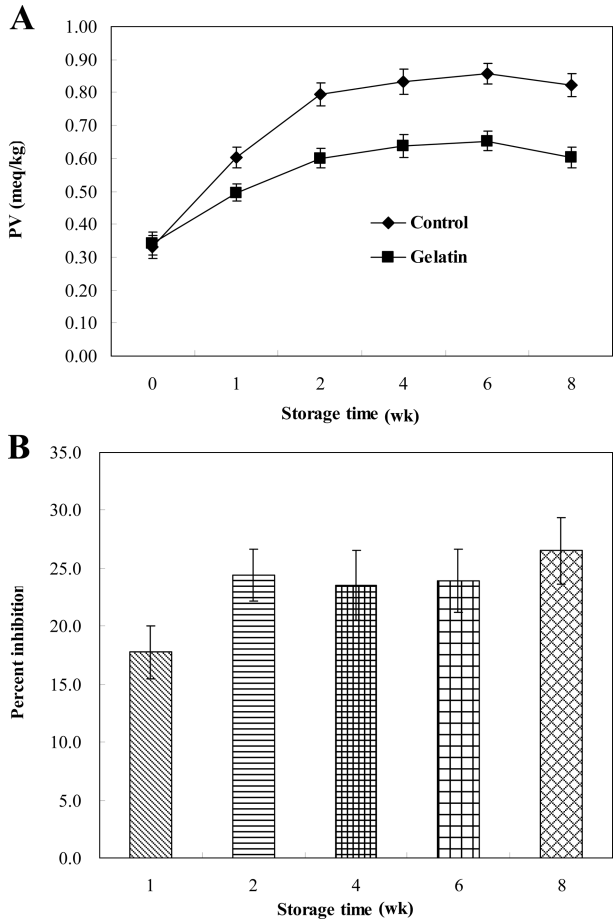

Fig. 2. (A) Changes in peroxide values (PV) (meq/kg) in sausages with gelatin-based edible coating during storage at 4°C for 8 wk under vacuum package. (B) Percentage inhibition by gelatin coating on PV in sausages after 8 wk of storage at 4°C under vacuum package. Control, uncoated sausage; gelatin, gelatin-coated sausage

by more than 26.5% over the storage period ($p<0.05$) (Fig. 2B). 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). 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). The increase in antioxidative activity was likely due to an increase, exposure of free radical scavenging amino acid residues, since heating increased sulfhydryl exposure and peroxyl radical scavenging capacity, but decreased iron chelation capacity (Elias and McClements, 2007; Elias *et al*., 2006). Skin gelatin hydrolysates from hoki fish (Mendis *et al*., 2005) and Alaska Pollock (Kim *et al*., 2001) skin have demonstrated noticeable free-radical scavenging activity and linoleic acid peroxidation inhibition.

Percent moisture loss (PML)

The storage time was found to have a significant effect on moisture content (MC), while the interaction of treatment and storage time was also significant ($p<0.05$) (Table 3). The mean initial MC was 76.2±0.56 and 75.8±0.54% for the control and gelatin-coated sausages, respectively, during refrigerated storage (Table 3). Initial MC decreased to a final MC of 67.3±0.41 and 69.9±0.37% for the control and gelatin-coated sausages, respectively. This reflected a 11.7 and 7.85% loss of moisture for the control and gelatin-coated sausages, respectively, during the experimental period.

The PML was greatly affected by the change of moisture content in sausages during refrigerated storage. The storage time was found to have a significant effect on PML, while the interaction of treatment and storage time was also significant ($p<0.05$) (Table 3). The PML for both control and gelatin-coated sausages increased with increased storage period. There was a significant effect in PML of gelatin-coated sausages compared to the control sausages after 2 wk of storage period ($p < 0.05$) (Table 3). The PML was 3.94±0.47 and 3.17±0.52% after the first wk of storage and then continued to increase to 11.7±0.81 and 7.85±0.72% for the control and gelatin-coated sausages at the end of storage. The control sausages were less protected with PML values of 11.7±0.81 compared to 7.85±0.72% for the gelatin-coated sausages. Thus, gelatin-coated sausages had less moisture loss than the control. A significant reduction of PML by 32.6% over the

Table 3. Moisture contents and percent moisture loss of sausages with gelatin-based edible coating during storage at 4°C for 8 wk under vacuum package

		Storage time (wk)					
		0	1	2	4	6	8
MC (%)	Control	76.2±0.56^{a}	73.2±0.54^{b}	70.9±0.76^{cY}	68.8±0.47^{dY}	67.6±0.42^{eY}	67.3±0.41^{eY}
	Gelatin	75.8±0.43^{a}	73.4±0.23^{b}	72.2±0.57^{cX}	70.8±0.42^{dX}	70.3±0.36^{dX}	69.9±0.37^{dX}
PML (%)	Control	-	3.94±0.47^{d}	6.96±0.57^{cX}	9.71±0.66^{bX}	11.3±0.73^{aX}	11.7±0.81^{aX}
	Gelatin	-	3.17±0.52^{d}	4.75±0.62^{cY}	6.54±0.74^{bY}	7.30±0.69^{abY}	7.85±0.72^{aY}

[1)]Values represent means of 3 replications±SD.
[a-d]Means within the same row with different superscripts are significantly different ($p < 0.05$).
[X,Y]Means within the same column with different superscripts are significantly different ($p < 0.05$).
Control, uncoated sausage; gelatin, gelatin coated sausage; MC, moisture content (%); PML, percent moisture loss (%)

control was achieved by gelatin coating at the end of storage. This may be attributed to the good moisture-barrier properties of gelatin coating. Previous reports demonstrated that whey protein coating reduced the moisture loss in cut LFSs (Shon and Chin, 2008) and cut beef steak (Haque *et al*., 2009). During the preparation of the coating solution, the mixture is heated at 90°C for 30 min, producing disulfide bonds and greater surface hydrophobicity (Shon and Haque, 2007). It is conceivable that the gelatin coating was effective as a moisture-barrier because of its hydrophobic nature (Shon and Haque, 2007). Data indicate that calcium chloride incorporated into the coating solution improved the moisture-barrier properties of gelatin films. Protein-based edible coatings containing calcium salts, which are as a cross-linking agent, have the potential to increased hydrophobicity (Park *et al*., 2001) and decrease water vapor permeability in cut Korean beef (Shon *et al*., 2010) and fresh-cut vegetables and fruit (Shon and Haque, 2007). In addition, 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 emulsifying ability of the proteins correlated with surface hydrophobicity and contributed to improve the moisture-barrier (Shon and Haque, 2007).

Microbial counts

The effect of gelatin edible coating on microbial growth in sausages was determined over the 8-wk of storage periods at 4°C under vacuum package (Fig. 3). As shown in Fig. 3, the total aerobic bacteria (TPC) were approximately 3.23±0.31 and 3.27±0.24 log CFU/g for the control and gelatin-coated sausages, respectively, at the beginning of the storage period. A significant linear increase in the TPC was observed as storage time increased for both control and gelatin-coated sausages during refrigerated storage (Fig. 3). The TPC progressively reach to 7.38±0.27 and 7.08±0.24 log CFU/g for control and for

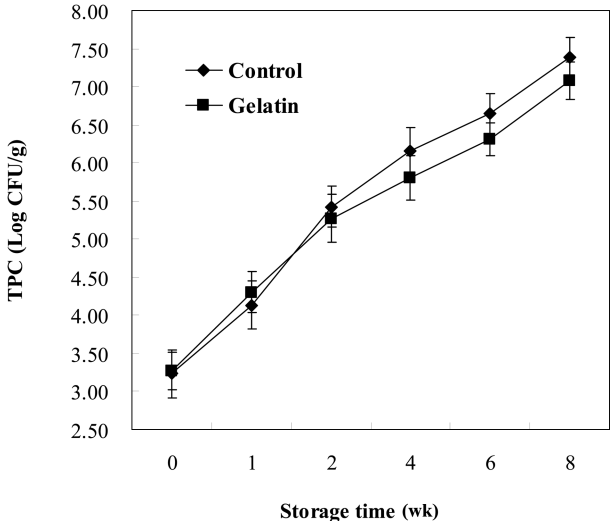

Fig. 3. Microbial changes (total aerobic bacteria) (Log CFU/g) of sausages coated with gelatin during storage at 4°C for 8 wk under vacuum package. Control, uncoated sausage; gelatin, gelatin coated sausage

gelatin-coated sausages, respectively, at the end of the refrigerated storage period (Fig. 3). However, no differences in TPC were observed between control and gelatin-coated sausages. These results indicated that the gelatin coating on LFSs was not effective by itself in inhibiting the growth of TPC. A previous report indicated that off-odor and slime starts when the total viable counts on meat tissue exceed log 10^{7}/g (Verma and Sahoo, 2000). Therefore, sausages may develop off flavors on spoilage with increased storage, and the gelatin coating did not inhibit the microbial growth. Approximately 8-wk was required to reach total bacterial counts of 7 log CFU/g in both the control and gelatin-coated sausages.

The effect of gelatin edible coating on the *L. monocytogenes* in LFSs over the 8-wk of storage period at 4°C under vacuum package is shown in Fig. 4. The storage time had a significant effect on *L. monocytogenes*, while the gelatin coating did not affect the microbial growth

(Fig. 4). The initial inoculated level of *L. monocytogenes* in the sausages was around 10^3 CFU/g. The *L. monocytogenes* populations also increased from 2.92±0.23 and 3.04±0.22 log CFU/g at the initial storage to 6.59±0.28 and 6.15±0.16 log CFU/g at the end of the storage time for control and gelatin-coated sausages, respectively (Fig. 4). The slight differences in the initial populations of *L. monocytogenes* might be due to differences in the attachment of bacteria to sausage surfaces. The gelatin coating alone had no inhibitory effect on the growth of *L. monocytogenes*. Data indicates that both TPC and *L. monocytogenes* counts were not reduced by gelatin coating alone compared to the control during refrigerated storage under vacuum packaging. A previous report indicated that gelatin and a metaphosphate polymer (Keil *et al*., 1960) as coatings on processed meats such as sausages, Canadian bacon and boned hams were reduced mould growth and lipid oxidation.

Edible antimicrobial coatings and films are one of the approaches to control contamination of pathogens on the surface of meats and meat products. In other case, whey protein films containing sorbic acid or *p*-aminobenzoic acid inhibited the growth of *L. monocytogenes* on bologna and summer sausages (Cagri *et al*., 2002). The SPI hydrolysates incorporated into frankfurters also extended the shelf life of products stored aerobically at 25°C (Vallejo-Cordoba *et al*., 1987). Thus, incorporation of antimicrobial compounds and hydrolysates into gelatin coatings or films may provide synergistic or additional safety and shelf life for RTE meats and meat products.

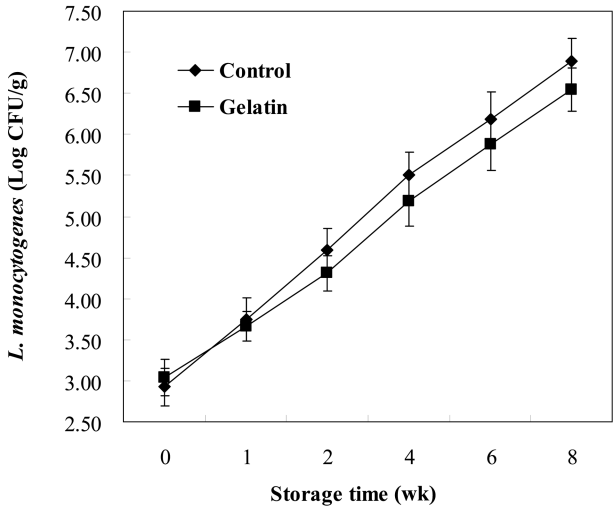

Fig. 4. Microbial changes (*Listeria monocytogenes*) (Log CFU /g) of sausages coated with gelatin during storage at 4°C for 8 wk under vacuum package. Control, uncoated sausage; gelatin, gelatin coated sausage

In conclusion, gelatin edible coating delayed oxidation of sausages as reflected by TBARS and PV. The inhibition of TBARS and PV, as compared to the control, was 21.5 and 26.5% for gelatin coated sausages. The PML of the sausages was significantly reduced by 32.6% with gelatin coating compared to the controls. However, gelatin coating on sausages was not effective to inhibit the growth of total aerobic bacteria and *L. monocytogenes.* The results of this study suggested that gelatin coating used in this study was capable of acting as a natural antioxidative and moisture-barrier coating to retard the lipid oxidation of sausages packed vacuumed during refrigerated storage.

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