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## Interaction of Porcine Myofibrillar Proteins and Various Gelatins: Impacts on Gel Properties

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OPEN ACCESS

Received November 28, 2018

Revised January 21, 2019

Accepted February 25, 2019

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**Abstract** The objectives of this study were to determine the interaction between porcine myofibrillar proteins and various gelatins (bovine hide, porcine skin, fish skin, and duck skin gelatins) and their impacts on gel properties of porcine myofibrillar proteins. Porcine myofibrillar protein was isolated from pork loin muscle (*M. longissimus dorsi thoracis et lumborum*). Control was prepared with only myofibrillar protein (60 mg/mL), and gelatin treatments were formulated with myofibrillar protein and each gelatin (9:1) at the same protein concentration. The myofibrillar protein-gelatin mixtures were heated from 10°C to 75°C (2°C/min). Little to no impacts of gelatin addition on pH value and color characteristics of heat-induced myofibrillar protein gels were observed ( $p > 0.05$ ). The addition of gelatin slightly decreased cooking yield of heat-induced myofibrillar protein gels, but the gels showed lower centrifugal weight loss compared to control ( $p < 0.05$ ). The addition of gelatin significantly decreased hardness, cohesiveness, gumminess, and chewiness of heat-induced myofibrillar gels. Further, sodium dodecyl poly-acrylamide gel electrophoresis (SDS-PAGE) showed no interaction between myofibrillar proteins and gelatin under non-thermal conditions. Only a slight change in the endothermic peak (probably myosin) of myofibrillar protein-gelatin mixtures was found. The results of this study show that the addition of gelatin attenuated the water-holding capacity and textural properties of heat-induced myofibrillar protein gel. Thus, it could be suggested that well-known positive impacts of gelatin on quality characteristics of processed meat products may be largely affected by the functional properties of gelatin *per se*, rather than its interaction with myofibrillar proteins.

**Keywords** differential scanning calorimetry, duck skin, gelatin, myofibrillar protein gel, porcine

## Introduction

Gelatin is an inducible protein derived from fibrous protein collagen contained in animal bone, skin, and connective tissues (Karim and Bhat, 2009). In the food industry,

it has been extensively used as a gelling agent and stabilizer in various processed foods, along with the advantages of excellent biocompatibility, high biodegradability, and less antigenicity (Gomez-Guillen et al., 2011; Liu et al., 2015). Edible grade gelatin products are commercially obtained from bovine hide and porcine skin, through acidic- or alkalic-treatments (Karim and Bhat, 2009). However, social issues, regarding the risk of bovine spongiform encephalopathy and foot-and-mouth disease, have aroused the suspicions about the safety of bovine hide and porcine skin consumption (Abedinia et al., 2017). In addition, religious dietary restrictions have been recognized as one of the reasons for searching for an alternative gelatin source (Gomez-Guillen et al., 2011). In this regard, several previous studies have been performed to characterize gelatins derived from fish (skin and fin) and poultry (skin and feet) by-products and to determine their practical availability as an alternative gelatin source (Gomez-Guillen et al., 2011; Karim and Bhat, 2009).

In meat products, gelatin as a non-meat protein is practically used to improve cooking yield and textural properties (Lee and Chin, 2016). It has been known that the positive impacts of gelatin addition into meat products are mostly based on its gel-forming ability and water-binding capacity (Gomez-Guillen et al., 2011). However, inconsistent efficacies of gelatin on meat products have been reported occasionally in previous studies conducted with different gelatins and addition levels (Jridi et al., 2015; Lee and Chin, 2016; Yeo et al., 2014). In fact, the negative impact of gelatin addition on water-holding capacity of emulsified sausage has been observed due to the melting out of gelatin during thermal process (Lee and Chin, 2016). In general, the functional effects of non-meat proteins on processing characteristics of meat products, such as milk proteins (whey and casein proteins) and plant proteins originated from soybean, legume, and oilseed, are determined by their own functional properties (e.g. gel-forming ability, water/oil binding capacities, protein solubility, and emulsifying capacity) and their interaction with myofibrillar proteins (Asgar et al., 2010; Sun and Holley, 2011). However, in most previous studies, the effect of gelatin on meat products has been largely understood based on only functional properties of gelatin (Jridi et al., 2015; Park et al., 2016).

Heat-induced gelation of myofibrillar proteins plays an important role in forming desirable water-holding capacity and texture of meat products (Sun and Holley, 2011). However, few studies previously investigated an interaction between myofibrillar protein and gelatin and corresponding impacts on its gel properties. According to Yang et al. (2007), the addition of porcine skin gelatin into porcine myofibrillar protein gels could decrease denaturation temperature of myosin. As a result, this would contribute to increased elasticity and viscosity of heat-induced porcine myofibrillar protein gels. In contrast, Brewer et al. (2005) have indicated that porcine skin gelatin may have negative impacts on the gelation of porcine myofibrillar proteins, since those two proteins have different mechanisms for their gelation without interaction. Thus, it would be necessary to clearly elucidate the interaction between myofibrillar proteins and gelatin under equivalent conditions to evaluate the availability of various gelatins as a protein additive.

Therefore, the objectives of this study were to determine the interaction between porcine myofibrillar proteins and various gelatins (bovine hide, porcine skin, fish skin, and duck skin) and to evaluate the physicochemical and textural properties of heat-induced porcine myofibrillar protein-gelatin gels.

## Materials and Methods

### Raw materials

Six boneless pork loins (*M. longissimus dorsi thoracis et lumborum*) for isolating myofibrillar proteins were purchased from a local market at 24 h postmortem. Commercial gelatin powders, such as bovine hide gelatin (gel strength: 280 bloom,

particular size: 5–15 mesh, Hangzhou Qunli Gelatin Chemical Co. Ltd.), porcine skin gelatin (gel strength: 280 bloom, particular size: 5–15 mesh, Hangzhou Qunli Gelatin Chemical Co. Ltd.), and fish skin gelatin (gel strength: 250 bloom, particular size: 5–15 mesh, Hangzhou Qunli Gelatin Chemical Co. Ltd.), were purchased. Duck skin gelatin powder (gel strength: 210 bloom), which was extracted through acid treatment (pH 4), super-heated steam extraction (150°C for 10 min), and lyophilization, was kindly provided by the Korea Food Research Institute.

### **Myofibrillar protein isolation**

Porcine myofibrillar protein was isolated from pork loins according to the procedure of Wu et al. (2009) with minor modification. Briefly, trimmed and chopped pork loin was homogenized with 4 volumes of isolating buffer {0.1 M NaCl, 10 mM sodium phosphate (pH 7.0), 2 mM MgCl<sub>2</sub>, 1 mM ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA)} for 3 min. The homogenate was centrifuged at 3,000×g for 15 min (4°C). The supernatant was discarded, and the residual pellets were resuspended in the isolating buffer (4 volumes). This process was repeated three times. The final pellets were homogenized again with 4 volumes of 0.1 M NaCl solution, and a few drops of 1 N HCl were added to adjust the pH of the myofibrillar protein suspension to pH 6.2 and centrifuged again at the same conditions above. The supernatant was discarded, and the protein concentration of myofibrillar proteins was determined by the biuret reaction method (Gornall et al., 1949). The isolated porcine myofibrillar protein was stored in a 4°C refrigerator and used within 48 h after isolation. In total, three independent batches were performed.

### **Preparation of heat-induced porcine myofibrillar protein-gelatin gels**

The porcine myofibrillar protein was suspended with dilution buffer {50 mM sodium phosphate (pH 6.2), 0.6 M NaCl} in 50 mL conical tubes (Wu et al., 2009), in which the protein concentration of all treatments was equally fixed as 60 mg/mL. For gelatin treatments, the ratio of porcine myofibrillar protein and each gelatin was 9:1 at the same protein concentration, which is one of the optimal mixing ratios for improving gel strength (Lan, 1994). The porcine myofibrillar protein-gelatin mixtures were heated from 10 to 75°C in an 80°C water bath, in which the heating rate was 2°C/min. After cooling in a 4°C refrigerator for 12 h, the heat-induced porcine myofibrillar protein-gelatin gels were blotted with paper towels, weighed, and used to evaluate gel properties.

### **Analyses of gel properties**

#### **pH measurement**

The pH value of heat-induced porcine myofibrillar protein-gelatin gels was measured in triplicate using an electric pH meter equipped with an insert-type pH probe (HI 99163, Hanna Instruments Inc., Woonsocket, RI).

#### **Instrumental color evaluation**

The instrumental color of heat-induced porcine myofibrillar protein-gelatin gels (cross-sections) was measured using a colorimeter (CR-400, Minolta, Osaka, Japan) equipped with an 8 mm observer (diameter). The setting for the illuminant was D<sub>65</sub> source. Calibration of the instrument was conducted with a calibration tile (CIE L\*: +93.01, CIE a\*: -0.25, CIE b\*: +3.50), according to the manufacturer's manual. The CIE L\*, a\*, and b\* values were taken from five random locations, and chroma (color intensity) was calculated as follows: chroma,  $\sqrt{a^{*2} + b^{*2}}$  (American Meat Science Association, 2012).

### **Cooking yield**

The cooking yield of heat-induced porcine myofibrillar protein-gelatin gels was calculated as a percentage difference between initial and cooked weights.

### **Centrifugal weight loss**

The water-holding capacity of heat-induced porcine myofibrillar protein-gelatin gels was determined in triplicate by the centrifugal method described by Xia et al. (2010) with minor modification. Briefly, 3 g of intact gel were placed in a 50 mL conical tube, and the tube was centrifuged at 3,000×g for 15 min (4°C). The water-holding capacity was calculated as follows: centrifugal weight loss (%) = [(the weight before centrifuge (g) – the weight after centrifuge (g))/the weight before centrifuge (g)]×100. A high value of centrifugal weight loss would indicate poor water-holding capacity.

### **Texture profile analysis (TPA)**

Texture profile analysis of heat-induced porcine myofibrillar protein-gelatin gels was conducted using a texture analyzer (CT3, Brookfield Engineering Laboratories, INC. Middleboro, MA). The gels were equilibrated to room temperature at 25°C for 3 h, and four samples (cylinder shape of 2.5 cm height and 2 cm diameter) were taken from the central portion of each gel. A twice compression cycle test (70% compression of the original sample height) was performed with a cylinder probe (diameter in 4 cm). Sample deformation curves were obtained with a 50 kg maximum load cell and the analysis condition were as follows: pre-test speed 1.0 mm/s, post-test speed 5.0 mm/s, test speed 2.0 mm/s. Value for hardness (kg), springiness (ratio), cohesiveness, gumminess (kg), and chewiness (kg) were determined according to the calculating method of Bourne (1978).

### **Sodium dodecyl sulfate poly-acryl amide gel electrophoresis (SDS-PAGE)**

SDS-PAGE of porcine myofibrillar protein-gelatin mixtures was performed by the Laemmli method (1970), using 12% running gels and 5% stacking gels. Gelatin samples and protein mixtures were diluted with 25 mM sodium phosphate buffer (pH 7.2) containing 1% sodium dodecyl sulfate (SDS) and 3.5 M urea, in which the protein concentration of samples was 50 mg/mL. The sample mixtures were heated at 85°C for 1 h and centrifuged at 8,500×g for 5 min to discard insoluble residues. The samples were mixed with 5× Laemmli sample buffer (312.5 mM Tris-HCl (pH 6.8), 50% glycerol, 5% SDS, 5% β-mercaptoethanol, 0.05% bromophenol blue, EBA-1052, Elpisbiotech, Daejeon, Korea) at the ratio of 4:1 and heated at 100°C for 5 min. Fifteen microliters of each sample were loaded at 100 V for 2 h. The loaded gel was stained with Coomassie Brilliant Blue R250 (B7920, Sigma, USA) and destained in methanol:distilled water:acetic acid (50:40:10). The separated protein bands were identified by comparison with a standard protein marker (pre-stained DokDo-MARK, EBM-1032, Elpisbiotech, Daejeon, Korea).

### **Differential scanning calorimetry (DSC)**

The thermal denaturation temperature of porcine myofibrillar protein-gelatin mixtures was measured in triplicate using a DSC machine (Q200, TA Instrument, New Castle, NJ). Approximately 15 mg of protein mixtures were accurately weighed into an aluminum pan, and an empty pan was used as a reference. Samples were heated from 5 to 95°C at a constant rate of 10°C/min (Doerscher et al., 2004). The temperature of endothermic peaks was analyzed using a Universal Analysis 2000 program (TA Instruments, New Castle, NJ) according to the manufacturer's guidelines.

## Statistical analysis

The experimental design was a completely randomized block design with three independent batches. Analysis of variance was performed on the measured variables, such as pH, color, cooking yield, centrifugal weight loss, TPA, and DSC results, using one-way ANOVA procedure of the SPSS program (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to determine significant differences between means ( $p < 0.05$ ).

## Results and Discussion

### pH and water-holding capacity

The pH value and water-holding capacity of heat-induced porcine myofibrillar protein-gelatin gels are shown in Table 1. The pH value of porcine myofibrillar protein-gelatin gels ranged from 5.71 to 6.01, which was unaffected by gelatin addition ( $p > 0.05$ ). In general, it has been reported that the addition of gelatin has little impact on the pH value of meat products (Lee and Chin, 2016; Yeo et al., 2014), since acid- or alkali-extracted gelatin is necessarily neutralized to pH 5.0–7.0. While the duck feet gelatin (pH 3.04) used in this study was not neutralized, no significant effect on the pH value of heat-induced porcine myofibrillar protein gel was observed.

The water-holding capacity of porcine myofibrillar protein-gelatin gels was determined through the measurement of cooking yield and centrifugal weight loss (Table 1). The addition of gelatin led to a decrease in cooking yield of heat-induced porcine myofibrillar protein gels ( $p < 0.05$ ). In particular, duck skin gelatin treatment (68.97%) exhibited a significantly lower cooking yield compared to control (87.27%). Further, the addition of gelatin slightly decreased centrifugal weight loss of heat-induced porcine myofibrillar protein gels ( $p < 0.05$ ), indicating improved water-holding capacity. In particular, bovine hide gelatin and porcine skin gelatin treatments (7.10% and 7.79%, respectively) showed significantly lower centrifugal weight loss than control (11.08%). These results could indicate no positive interaction between gelatin and porcine myofibrillar proteins during heating process, but gelatin addition may be effective in retaining moisture within myofibrillar protein heat-induced gels. Moreover, the decreased cooking yield of duck skin treatment was likely due to relatively low pH toward isoelectric point of myofibrillar protein, although the pH difference between treatments was not significant.

Previous studies have reported inconsistent results regarding the impact of gelatin on water-holding capacity of meat products, especially on cooking yield or loss. According to Yeo et al. (2014), the addition of duck feet gelatin (2–3 g/100 g), as a fat replacer, could decrease the cooking loss of emulsion sausages. In contrast, Lee and Chin (2016) reported that the

**Table 1.** pH and water-holding capacity of heat-induced porcine myofibrillar protein-gelatin gels

Traits	Control (no added gelatin)	Gelatin treatments				SEM	Significance of p value
		Bovine hide gelatin	Porcine skin gelatin	Fish skin gelatin	Duck skin gelatin		
pH value	6.02	6.01	6.03	6.05	5.71	0.058	NS
Water-holding capacity							
Cooking yield (%)	87.27 <sup>a</sup>	82.03 <sup>a</sup>	82.02 <sup>a</sup>	79.09 <sup>a</sup>	68.97 <sup>b</sup>	1.961	0.014
Centrifugal weight loss (%)	11.08 <sup>a</sup>	7.10 <sup>c</sup>	7.79 <sup>bc</sup>	10.14 <sup>ab</sup>	10.02 <sup>ab</sup>	3.400	0.014

Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration.

<sup>a-c</sup> Means sharing different letters within a row are significantly different ( $p < 0.05$ ).

NS, non-significance ( $p \geq 0.05$ ).

cooking loss of model sausages increased by increasing the added level of porcine skin gelatin (0.5–1.5 g/100 g) and explained that the observed phenomenon might be due to “melting out” of gelatin during the thermal process. In addition, they suggested that the positive impact of gelatin on water-holding capacity could be influenced by fat content in meat products, since gelatin may interact with lipid molecules, as an emulsifying agent (Lee and Chin, 2016). In a heat-induced myofibrillar protein gel (nearly fat-free system), thus, gelatin may have no positive effect for minimizing water release during the thermal process. However, gelatin gel after heating could contribute to improved water-holding capacity, thereby entrapping moisture within its gel matrix structure.

### Color characteristics

The color characteristics of heat-induced porcine myofibrillar protein-gelatin gels are shown in Table 2. CIE L\* (lightness) and b\* (yellowness) of the gels were affected by gelatin addition ( $p < 0.05$ ); however, there were no significant differences in CIE a\* (redness) and chroma (color intensity). All gelatin treatments exhibited more light and yellow color than control, in which duck skin gelatin treatment showed the highest lightness and yellowness among all treatments ( $p < 0.05$ ). Similarly, Yeo et al. (2014) noted that the addition of duck feet gelatin to emulsion sausages increased yellowness but did not affect redness. The color of gelatin is mainly influenced by raw materials and extraction conditions, such as pH, temperature, and time (Aykin-Dincer et al., 2017). Commercial bovine hide gelatin and porcine skin gelatin are generally colorless, whereas the gelatins obtained from poultry by-products (e.g. skin and feet) show slightly whitish-yellow color (Park et al., 2013). According to Ockerman and Hansen (1988), however, the color of gelatin may have no influence on its functional properties.

### Textural properties

The textural properties of heat-induced porcine myofibrillar protein-gelatin gels are shown in Table 3. The addition of gelatin significantly decreased hardness, cohesiveness, gumminess, and chewiness of heat-induced porcine myofibrillar gels. However, there was no significant difference in springiness of the gels. Similarly, Brewer et al. (2005) noted that the addition of gelatin could cause negative impacts on myofibrillar protein gelation, resulting in a decrease in gel strength. They suggested that those two proteins, myofibrillar protein and gelatin, may have compatible mechanisms on gelation without interaction, which could in turn weaken the gel strength (Brewer et al., 2005). In this study, the gelatin type had little to no impacts on the textural properties of heat-induced porcine myofibrillar protein gels. Among gelatin treatments, the only significant difference in hardness was found between fish and duck skin gelatin treatments, while duck skin gelatin had a

**Table 2.** Color characteristics of heat-induced porcine myofibrillar protein-gelatin gels

Traits	Control (no added gelatin)	Gelatin treatments				SEM	Significance of p value
		Bovine hide gelatin	Porcine skin gelatin	Fish skin gelatin	Duck skin gelatin		
CIE L* (lightness)	82.94 <sup>c</sup>	83.25 <sup>bc</sup>	83.14 <sup>bc</sup>	84.40 <sup>b</sup>	86.08 <sup>a</sup>	0.354	0.002
CIE a* (redness)	-3.18	-2.64	-2.71	-2.60	-2.65	0.095	NS
CIE b* (yellowness)	-2.99 <sup>b</sup>	-2.06 <sup>b</sup>	-2.13 <sup>b</sup>	-1.64 <sup>b</sup>	1.31 <sup>a</sup>	0.473	0.012
Chroma (color intensity)	4.47	3.56	3.61	3.16	2.96	0.186	NS

Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration.

<sup>a-c</sup> Means sharing different letters within a row are significantly different ( $p < 0.05$ ).

NS, non-significance ( $p \geq 0.05$ ).

**Table 3.** Textural properties of heat-induced porcine myofibrillar protein-gelatin gels

Traits	Control (no added gelatin)	Gelatin treatments				SEM	Significance of p value
		Bovine hide gelatin	Porcine skin gelatin	Fish skin gelatin	Duck skin gelatin		
Hardness (kg)	10.01 <sup>a</sup>	3.53 <sup>bc</sup>	2.94 <sup>bc</sup>	2.30 <sup>c</sup>	3.92 <sup>b</sup>	0.766	<0.001
Springiness (ratio)	0.80	0.75	0.73	0.62	0.68	0.032	NS
Cohesiveness	0.47 <sup>a</sup>	0.25 <sup>b</sup>	0.25 <sup>b</sup>	0.24 <sup>b</sup>	0.26 <sup>b</sup>	0.025	0.000
Gumminess (kg)	6.32 <sup>a</sup>	0.90 <sup>b</sup>	0.72 <sup>b</sup>	0.56 <sup>b</sup>	1.06 <sup>b</sup>	0.693	0.007
Chewiness (kg)	0.65 <sup>a</sup>	0.12 <sup>b</sup>	0.09 <sup>b</sup>	0.06 <sup>b</sup>	0.13 <sup>b</sup>	0.061	0.000

Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration.

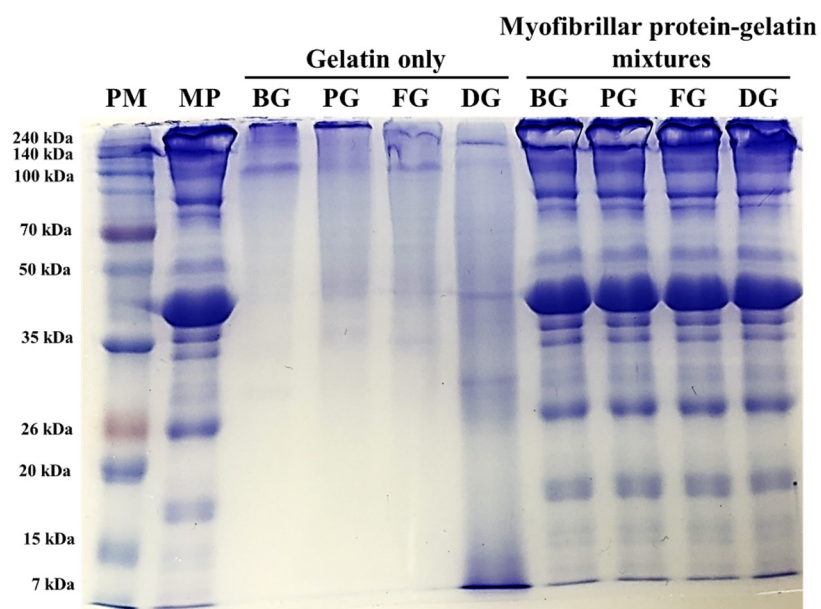
<sup>a-c</sup> Means sharing different letters within a row are significantly different ( $p < 0.05$ ).

NS, non-significance ( $p \geq 0.05$ ).

lower bloom value than fish skin gelatin. This result was likely due to the relatively high amount of released water in duck skin gelatin treatment, as shown by a decrease in cooking yield.

### SDS-PAGE

Protein pattern of porcine myofibrillar protein-gelatin mixtures was analyzed to determine the non-thermal interaction of those two proteins using SDS-PAGE in the presence of  $\beta$ -mercaptoethanol (Fig. 1). All gelatin samples (gelatin only) showed two evident bands at 100–140 kDa, which could be expected as  $\alpha$ 1- and  $\alpha$ 2-chains (~100 kDa), major subunits of gelatin (Kim et al., 2014). Bovine hide gelatin, porcine skin gelatin, and fish skin gelatin additionally presented dimers and trimers of  $\alpha$ -chains, namely  $\beta$ -chain (~200 kDa) and  $\gamma$ -chain (> 240 kDa). However, such dimers and trimers were not observed in duck



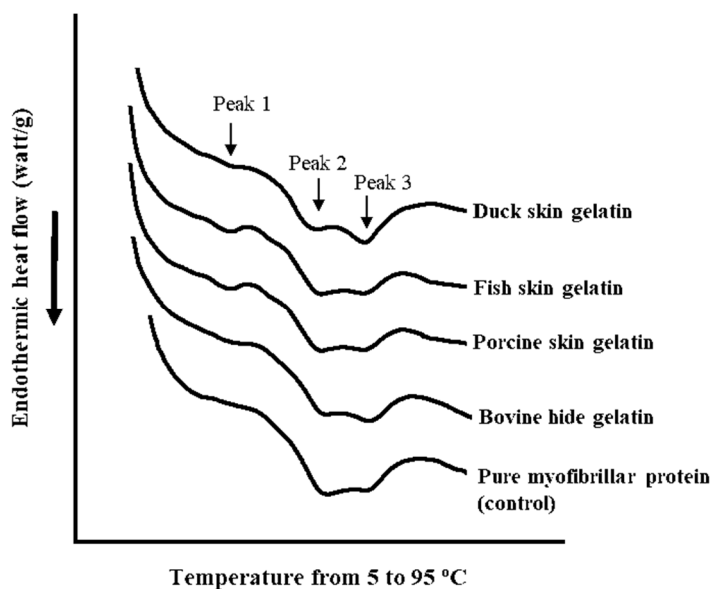
**Fig. 1.** SDS-PAGE (12% acrylamide gel) photograph of porcine myofibrillar protein-gelatin mixtures. Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration. PM, standard protein marker; MP, pure myofibrillar protein; BG, bovine hide gelatin; PG, porcine skin gelatin; FG, fish skin gelatin; DG, duck skin gelatin.



skin gelatin, although many protein bands at diverse molecular weights below 100 kDa (mainly ~40 kDa and ~30 kDa) were found. Those proteins at lower molecular weights compared to  $\alpha$ -chains were probably derived from  $\alpha$ -chains of duck skin gelatin, due to the relatively higher extracting temperature of super-heated steam compared to the commercial heating temperature for gelatin extraction. Similarly, Gomez-Guillen et al. (2002) indicated that the high temperature for gelatin extraction could result in a fragmentation of  $\alpha$ -chains. When compared to the protein pattern of porcine myofibrillar proteins, porcine myofibrillar protein-gelatin mixtures exhibited no differences in protein pattern and intensity. This result may indicate no interaction between porcine myofibrillar proteins and gelatin under non-thermal conditions. Furthermore, no changes in band intensity at the molecular weights of gelatin components (e.g.  $\alpha$ -chains at 100–140 kDa and  $\beta$ -chain at 200 kDa) were found. According to Kim et al. (2014), the addition of 3%–6% duck feet gelatin into duck muscle increased the band intensity at the molecular weights of  $\alpha$ - and  $\beta$ -chains. The different result of the current study was likely due to the relatively low ratio of gelatin to porcine myofibrillar protein.

### DSC

The DSC thermograms and endothermic peak temperature of porcine myofibrillar protein-gelatin mixtures are shown in Fig. 2 and Table 4, respectively. Pure porcine myofibrillar proteins (control) showed two evident endothermic peaks at 57.22°C (peak 2) and 68.64°C (peak 3), which could be expected as the thermal denaturation temperature of myosin and actin (Liu et al., 2000). The addition of gelatin resulted in the appearance of a new endothermic peak at 30°C–40°C (Fig. 2). The highest temperature of endothermic peak 1 was observed in the fish skin gelatin treatment, although there was no significant difference (Table 4). The appearance of endothermic peak 1 was likely due to gelatin melting. Thus, the different temperature of endothermic peak 1 could be associated with thermal properties of each gelatin, since the melting temperature of gelatin is species-specific (Gomez-Guillen et al., 2011). Moreover, the addition of gelatin altered the temperature of endothermic peak 2 ( $p < 0.05$ ; Table 4), whereas a similar temperature of endothermic peak 3 was observed among all treatments regardless of



**Fig. 2.** DSC thermogram of porcine myofibrillar protein-gelatin mixtures. Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration.



**Table 4. Endothermic peak temperatures of porcine myofibrillar protein-gelatin mixtures**

Endothermic peaks	Control (no added gelatin)	Gelatin treatments				SEM	Significance of p value
		Bovine hide gelatin	Porcine skin gelatin	Fish skin gelatin	Duck skin gelatin		
Peak 1	- <sup>1)</sup>	37.10	35.65	38.02	35.96	0.371	NS
Peak 2	57.22 <sup>a</sup>	57.16 <sup>a</sup>	56.89 <sup>a</sup>	56.83 <sup>a</sup>	54.81 <sup>b</sup>	0.259	<0.001
Peak 3	68.64	69.08	68.27	68.41	68.23	0.221	NS

Control was prepared with only porcine myofibrillar protein (60 mg/mL), and porcine myofibrillar protein-gelatin mixtures were formulated with porcine myofibrillar protein and each gelatin (9:1 ratio) at the same protein concentration.

<sup>1)</sup> not detected.

<sup>a-c</sup> Means sharing different letters within a row are significantly different ( $p < 0.05$ ).

NS, non-significance ( $p \geq 0.05$ ).

gelatin addition ( $p > 0.05$ ). The porcine myofibrillar protein-gelatin mixtures showed significantly lower temperatures at endothermic peak 2 compared to pure porcine myofibrillar protein (control). Previously, Yang et al. (2007) reported that the addition of gelatin decreased the denaturation temperature of pure myosin from 52°C to 47.5°C and suggested that the decreased denaturation temperature of myosin might be due to the interaction between myosin and gelatin. The previous suggestion was consistent with our observation. In particular, the noticeable decrease in peak 2 temperature might be related to the presence of many fragmented peptides, since the fragmentation of gelatin subunits could improve the solubility and/or interaction with other biological molecules (Gomez-Guillen et al., 2002).

## Conclusion

The results of this study show that the addition of gelatin at low concentration (6 mg/mL) could attenuate water-holding capacity and textural properties of heat-induced porcine myofibrillar protein gel. In particular, the inadequate cooking yield and hardness were found in duck skin gelatin and fish skin gelatin treatments, respectively. While the result for thermal properties determined by DSC could provide limited information on weak thermally induced interactions between myosin and gelatin, it would be insufficient to override the negative impacts. Thus, it could be suggested that well-known positive impacts of gelatin on quality characteristics of meat products may be mainly related to the functional properties of gelatin *per se*, rather than its interaction with myofibrillar proteins. In addition, results of the current study indicate that mammalian gelatins extracted from bovine hide and porcine skin could be effective in improving water-holding capacity of heat-induced porcine myofibrillar gel.

## Conflicts of Interest

The authors declare no potential conflict of interest.

## Acknowledgements

This research was supported through the High Value-added Food Technology Development Program (2018-118011) by the Ministry of Agriculture, Food, and Rural Affairs (Korea).

## Author Contributions

Conceptualization: Choi YS, Kim HW. Data curation: Ham YK. Formal analysis: Noh SW, Kim TK. Methodology: Noh SW, Kim TK. Software: Ham YK, Song DH. Validation: Ham YK, Song DH. Investigation: Ham YK, Song DH. Writing - original draft: Noh SW. Writing - review & editing: Noh SW, Ham YK, Song DH, Choi YS, Kim HW, Kim HW.

## Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

## References

- Abedinia A, Ariffin F, Huda N, Nafchi AM. 2017. Extraction and characterization of gelatin from the feet of Pekin duck (*Anas platyrhynchos domestica*) as affected by acid, alkaline, and enzyme pretreatment. *Int J Biol Macromol* 98:586-594.
- American Meat Science Association [AMSA]. 2012. Meat color measurement guidelines. American Meat Science Association, Champaign, IL, USA.
- Asgar MA, Fazilah A, Huda N, Bhat R, Karim AA. 2010. Nonmeat protein alternatives as meat extenders and meat analogs. *Compr Rev Food Sci Food Saf* 9:513-529.
- Aykin-Dincer E, Koc A, Erbas M. 2017. Extraction and physicochemical characterization of broiler (*Gallus gallus domesticus*) skin gelatin compared to commercial bovine gelatin. *Poult Sci* 96:4124-4131.
- Bourne MC. 1978. Texture profile analysis. *Food Technol* 32:62-66.
- Brewer MS, Peterson WJ, Carr TC, Mccusker R, Novakofski J. 2005. Thermal gelation properties of myofibrillar protein and gelatin combinations. *J Muscle Food Banner* 16:126-140.
- Doerscher DR, Briggs JL, Lonergan SM. 2004. Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. *Meat Sci* 66:181-188.
- Gomez-Guillen MC, Gimenez B, Lopez-Caballero ME, Montero MP. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocoll* 25:1813-1827.
- Gomez-Guillen MC, Turnay J, Fernandez-Diaz MD, Ulmo N, Lizarbe MA, Montero P. 2002. Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocoll* 16:25-34.
- Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177:751-766.
- Jridi M, Abdelhedi O, Souissi N, Kammoun M, Nasri M, Ayadi MA. 2015. Improvement of the physicochemical, textural and sensory properties of meat sausage by edible cuttlefish gelatin addition. *Food Biosci* 12:67-72.
- Karim AA, Bhat R. 2009. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocoll* 23:563-576.
- Kim HW, Park JH, Yeo EJ, Hwang KE, Song DH, Kim YJ, Ham YK, Jeong TJ, Choi YS, Kim CJ. 2014. Effect of duck feet gelatin concentration on physicochemical, textural, and sensory properties of duck meat jellies. *Korean J Food Sci Anim Resour* 34:387-394.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.

- Lan Y. 1994. Comparison of thermal gelation properties of pork, beef, fish, chicken and turkey. Ph.D. Dissertation, University of Illinois, Urbana, IL, USA.
- Lee CH, Chin KB. 2016. Effects of pork gelatin levels on the physicochemical and textural properties of model sausages at different fat levels. *LWT-Food Sci Technol* 74:325-330.
- Liu D, Nikoo M, Boran G, Zhou P, Regenstein JM. 2015. Collagen and gelatin. *Annu Rev Food Sci Technol* 6:527-557.
- Liu G, Xiong YL, Butterfield, DA. 2000. Chemical, physical, and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. *J Food Sci* 65:811-818.
- Ockerman HW, Hansen CL. 1988. Glue and gelatin animal by-product processing. Ellis Horwood Ser Food Sci Technol, New York, NY, USA. pp 132-157.
- Park JH, Choe JH, Kim HW, Hwang KE, Song DH, Yeo EJ, Kim HY, Choi YS, Lee SH, Kim CJ. 2013. Effects of various extraction methods on quality characteristics of duck feet gelatin. *Korean J Food Sci Anim Resour* 33:162-169.
- Park SY, Kim GW, Kim HY. 2016. Study on physicochemical properties of emulsion-type sausage added with pork skin gelatin. *J Korean Soc Food Sci Nutr* 45:209-214.
- Sun XD, Holley RA. 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. *Compr Rev Food Sci Food Saf* 10:33-51.
- Wu M, Xiong YL, Chen J, Tang X, Zhou G. 2009. Rheological and microstructural properties of porcine myofibrillar protein-lipid emulsion composite gels. *J Food Sci* 74:E207-E217.
- Xia X, Kong B, Xiong Y, Ren Y. 2010. Decreased gelling and emulsifying properties of myofibrillar protein from repeatedly frozen-thawed porcine *longissimus* muscle are due to protein denaturation and susceptibility to aggregation. *Meat Sci* 85:481-486.
- Yang YL, Zhou GH, Xu XL, Wang Y. 2007. Rheological properties of myosin-gelatin mixtures. *J Food Sci* 72:C270-C275.
- Yeo EJ, Kim HW, Hwang KE, Song DH, Kim YJ, Ham YK, He FY, Park JH, Kim CJ. 2014. Effect of duck feet gelatin on physicochemical, textural, and sensory properties of low-fat frankfurters. *Korean J Food Sci Anim Resour* 34:415-422.