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Gel Properties of Surimi-like Materials from Cardiac and Skeletal Muscle of Pigs

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ABSTRACT : To investigate the gel properties of surimi-like materials (SLM) made from pig heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs, the three muscles were diced, chopped and washed with 25 mM sodium phosphate buffer (pH 7.0) to extract myofibrillar protein. SLM from SM had significantly ($p<0.05$) higher moisture content and lower crude protein content compared with PH and PM samples. The cooked SLM from PH was darker than that from PM and SM. Gel from PH had significantly ($p<0.05$) lower L* and hue values, and higher b* and chroma values compared to gels from PM and SM. The cooked SLM from PH had poor water-holding capacity (WHC) resulting in higher cooking loss. SDS-PAGE showed that the bands of myosin and tropomyosin/troponin had reduced staining intensity in the PH sample, and some unidentified bands that were not in PM and SM samples were observed in PH samples. (**Key Words :** Gel Property, Myofibrillar Protein, Pig Heart, Surimi-like Materials)

INTRODUCTION

Interest in utilizing edible meat by-products as value-added products has increased in recent years. The use of beef heart muscle as an ingredient in restructured meat products has been limited by low protein functionality and less desirable flavor compared with skeletal muscle (Kenney et al., 1992; Srinivasan and Xiong, 1996). It was suggested that heart muscle had a problem because of higher fat, heme pigment and collagen content in processing surimi-like material (SLM) (Park et al., 1996). McKeith et al. (1988) demonstrated that SLM prepared from beef hearts had improved textural properties when compared with fish surimi. Kenney et al. (1992) also showed that incorporation of washed beef cardiac muscle into restructured beef enhanced sensory and instrumental texture traits.

Considerable work investigating the role of various proteins in thermal gelation of muscle foods has indicated that myosin and actomyosin are superior to sarcoplasmic proteins. Cardiac muscle, which is composed mainly of red fibers having two isoforms of myosin, is considered to be relatively poor in functional properties, possibly as a consequence of the low solubility of its myofibrillar proteins and its high connective tissue content. However, there is little information on gel properties of SLM derived from porcine cardiac and skeletal muscle. An understanding of the characteristics of porcine muscles will assist in determining recommendations for how to handle the SLM from pork muscles for manufacture of surimi products. The objectives of this study were to investigate the characteristics of cardiac and skeletal muscles and to evaluate the gel properties of SLM from porcine muscles.

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MATERIALS AND METHODS

Sampling and gel preparation

Fresh heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs were obtained at the Meat Plant of Gyeongsang National University in Korea. A total of 5 pigs were slaughtered on 3 alternate days (2 pigs in day 1, 2 pigs in day 2 and 1 pig in day 3) and then external fat tissues immediately removed and the lean muscle was diced into approximately 20 mm cubes, and ground through a 4.7 mm diameter orifice with a mincer to manufacture SLM. The SLM was manufactured 3 times for each muscle type (treatment) from the 3 alternate days.

The SLM manufacturing procedure was a modification

Table 1. Proximate composition of surimi-like materials from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs

Treatment	Moisture %	Crude protein %	Crude fat %
PH	73.66±1.75[b]	28.71±1.79[a]	<0.02
PM	72.75±1.65[b]	29.52±1.58[a]	<0.01
SM	77.05±0.80[a]	25.03±0.84[b]	<0.01

[a, b] Means±SD with different superscripts within a column differ significantly ($p<0.05$).

of the method of Kang et al. (2004) and Srinivasan et al. (1996). The minced muscles were chopped in a homogenizer (Model AM-7, Nihouseiki Kaisha LTD, Japan) with ten volumes (v/w) of cold 25 mM phosphate buffer (NaH_2PO_4/Na_2HPO_4), pH 7.0. The resulting slurry was filtered through a 1 mm-mesh metal screen to remove connective tissues. The filtrate was centrifuged (15 min/2,220×g, 4°C) and the supernatant containing fat and water-soluble proteins was discarded.

The sediment was mixed with cold 25 mM phosphate buffer, the volume of which was equal to that of the supernatant discarded in the previous step, homogenized again and filtered through a 500 μm mesh metal sieve and centrifuged (15 min/2,220×g, 4°C) again. A final wash was done in five volumes (v/w based on original weight of mince) of iced water. The washing procedure was repeated a third time. The resulting residue was centrifuged at 2,220 ×g for 15 min at 4°C and the supernatant discarded. Finally, sediment (SLM) concentration was adjusted to 5% protein by adding a solution containing 3% NaCl, 0.5% tripolyphosphate (TPP) and 4% sorbitol. The adjusted SLM was prepared by heating 15 ml of SLM in capped 1.5 cm diameter tubes for 20 min in a water bath at a constant 75°C. After cooking, a total of 15 tubes for each treatment were cooled (~15 min) at room temperature (about 20°C) in an ice bath. Protein concentration was measured by the biuret method (Gornall, 1949) using BSA (bovine serum albumin; Sigma A39120) as standard.

Proximate composition

A total of 5 samples from each treatment were analyzed for moisture and crude protein content according to the AOAC method (1990) and crude fat content was determined as described by Folch et al. (1957).

Color and pH

Color was assessed on a sliced surface of cooked SLM using a Minolta Chromameter CR-300 (Minolta Co., Japan) standardized with a white plate (Y = 93.5, X = 0.3132, y = 0.3198). Three replicate measurements were taken and results were expressed as CIE (Commission International de l'Eclairage) L*a*b*. Furthermore, metric Chroma C* and metric hue-angle were calculated by the following formulae: $C^* = (a^{*2}+b^{*2})^{1/2}$ and hue = $\tan^{-1}(b^*/a^*)$. All samples (3 g) were homogenized using a poly-tron homogenizer (T25basic, IKA, Malaysia) with distilled water (27 ml) and then pH measured using a pH-meter (MP230, Mettler Toledo, Swiss).

Water-holding capacity (WHC) and gel hardness

The WHC was calculated for five replicates per treatment by Lianji and Chen (1989). Samples (10 g) of the batter were heated for 10 min in a water bath at 90°C. After heating, the samples were removed, cooled to room temperature, wrapped in cotton cheese cloth and centrifuged (9,000×g, 4°C) in 50 ml polycarbonate tubes (containing absorbent cotton wool) for 10 min. The cheese cloth was removed and the samples reweighed. Gel hardness was measured using a Sun Rheo Meter (CR-100D, Japan). A 5-mm diameter round plunger was used to penetrate the gel samples that had a length of 20 mm and a diameter of 12.7 mm. Gel hardness was reported as the height of the first compression force curve after 60% deformation (≒ 8 mm movement) expressed in values (Bourne, 1968). The Rheo meter had a 10 kg load cell, a crosshead speed of 120 mm/min and a full-scale load range of 1 to ~10 kg.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples for SDS-PAGE were obtained of the first supernatant and the final pellet when SLM was prepared. The first supernatant was used for sarcoplasmic fractions whereas the final pellet was used for myofibrils. SDS-PAGE was performed according to the method of Laemmli (1970). Molecular weights of protein bands were estimated using standard markers (062K9280, Sigma, USA; M-0630, Sigma, USA). The density of each band on a gel was measured with a gel documentation analysis system (EDAS 290, Kodak, Japan).

Statistical analysis

The surimi was manufactured 3 times for each treatment. Data were evaluated using SAS General Linear Models Procedures (SAS, 2001). Treatment means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Proximate composition, pH and color

SLM from SM had significantly ($p<0.05$) higher moisture content and lower crude protein content compared with PH and PM (Table 1). This was expected since the repeated water washing, the centrifugation and the lower density of the fat cause the fat to float off and be removed. Although pH of PH was higher than that of PM and SM, there were no significant differences in pH among SLMs from all muscles (Figure 1). No difference in pH between SLMs might be due to addition of 3% NaCl, 0.5% TPP and

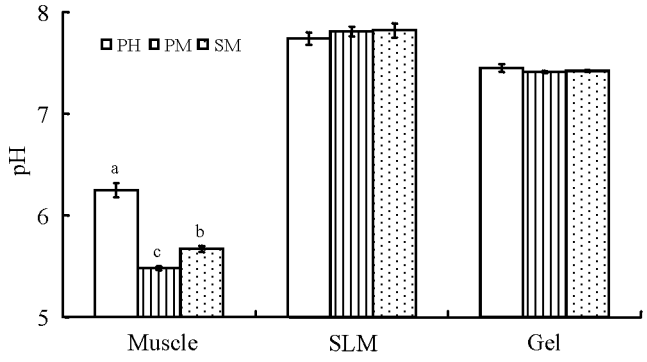

Figure 1. Changes in pH of muscle, surimi-like material (SLM) and gel during SLM processing from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs. [a, b, c] Means±SD with different superscripts within a bar differ significantly ($p<0.05$).

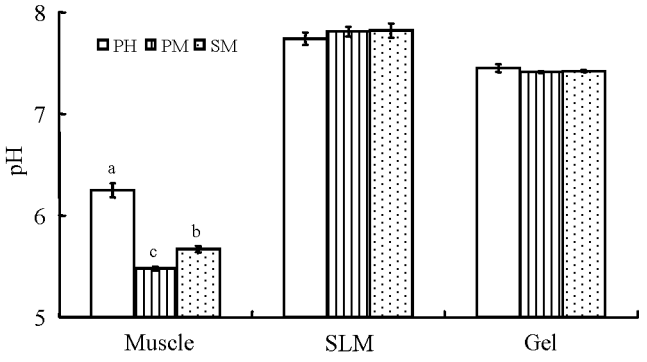

Figure 2. Values of gel firmness of surimi-like materials from heart (PH), *psoas major muscle* (PM) and *semimembranosus muscle* (SM) of pigs. [a, b, c] Means±SD with different superscripts within a variable differ significantly ($p<0.05$).

4% sorbitol. Also, there was no significant difference in pH of cooked SLM gels from all muscles. This result suggested that pH was significantly ($p<0.05$) different for each muscle whereas there was no difference in final SLM because of the salt components added. The high pH of the washing solution raises the pH of the meat slurry, making the pigments and blood more soluble and, therefore, easier to remove, while it may also allow for easier extraction of myoglobin (Dawson et al., 1989). Yang and Froning (1992) also showed that a washing solution of elevated pH resulted in better lipid and pigment reduction.

There was a significant difference in color measurements of cooked SLM (Table 2). The cooked SLM of PH was darker than that of PM and SM. Gel of PH had significantly ($p<0.05$) lower L* and hue values, and higher b* and chroma values compared to gels of PM and SM. The results suggested that some of sarcoplasmic proteins, including heme pigments and enzymes, in PH were not excluded enough by the water-washing procedure, which resulted in their remaining in SLM. SDS-PAGE clearly showed many sarcoplasmic protein bands in the myofibrillar protein fractions of the PH sample (Figure 3). It is speculated that the sarcoplasmic proteins which remained in SLM would affect not only color measurements, but also functionality of cooked SLM gel.

Water-holding capacity and cooking loss

The cooked SLM of PH had a poor water-holding capacity (WHC) resulting in higher cooking loss (Table 3).

Table 3. Cooking loss and water-holding capacity of surimi like materials from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs

Treatment	Cooking loss %	Water-holding capacity %
PH	20.52 ± 0.46^{a}	67.63 ± 2.34^{c}
PM	17.33 ± 1.05^{b}	84.82 ± 3.11^{a}
SM	19.97 ± 0.72^{a}	74.30 ± 3.46^{b}

[a, b, c] Means±SD with different superscripts within a column differ significantly ($p<0.05$).

WHC of cooked SLM might be mainly a function of protein-protein interaction resulting in an open matrix, thereby allowing a higher proportion of total water to be immobilized than in meat proteins with strong protein interactions. The present data implied that cardiac muscle of porcine had strong protein interactions compared with skeletal muscles.

The increase of water holding capacity resulting from addition of salt and polyphosphate has been thoroughly reviewed (Tsai and Ockerman, 1981; Paterson et al., 1988; Chen et al., 2006). Addition of salt and phosphate into SLM dissociates actomyosin, reducing interactions of meat proteins and opening the protein matrix in the gel of cooked SLM. Although protein content in SLM of PH was higher than that of SM, moisture content in SLM was less in PH (Table 1) because of its poor WHC. The poor WHC probably affected gel-forming ability of cooked SLM of PH. Gel of PH had significantly ($p<0.05$) lower gel hardness compared with gels of PM and SM (Figure 2). Therefore these results suggested that proteins of cardiac muscle were not dissociated enough with salt and phosphate, which

Table 2. Color values of surimi-like materials from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs

Treatment	CIE values			Chroma	Hue
	L*	a*	b*		
PH	59.43 ± 1.80^{b}	-1.06 ± 0.16^{a}	11.03 ± 0.92^{a}	11.08 ± 0.91^{a}	95.53 ± 1.22^{c}
PM	73.68 ± 0.92^{a}	-3.70 ± 0.06^{b}	3.13 ± 0.18^{b}	4.84 ± 0.13^{b}	139.89 ± 1.64^{b}
SM	72.52 ± 1.41^{a}	-3.80 ± 0.07^{b}	-2.56 ± 0.20^{c}	4.58 ± 0.11^{b}	213.96 ± 2.26^{a}

[a, b, c] Means±SD with different superscripts within a column differ significantly ($p<0.05$).

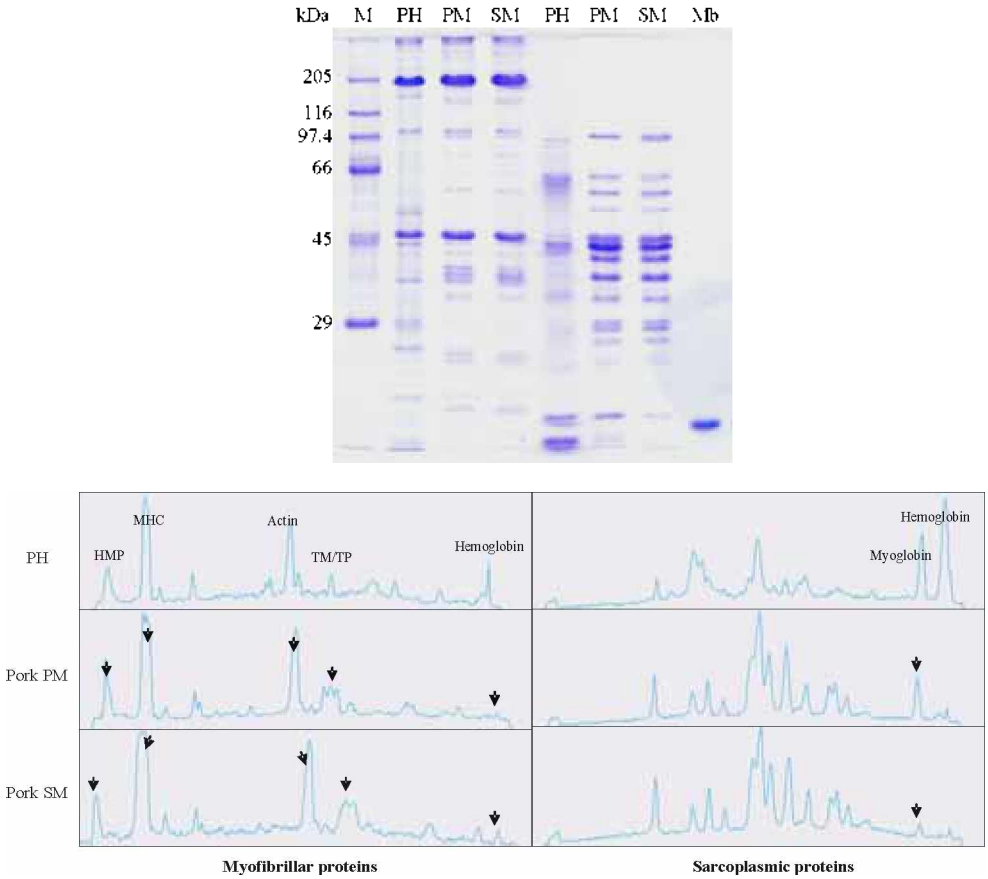

Figure 3. SDS-PAGE patterns (*upper*) and band intensity (*lower*) of myofibrillar and sarcoplasmic protein from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs. The myofibrillar (lanes 2-4) and sarcoplasmic (lanes 5-7) protein fractions (*upper*), which were defined as the sediment and supernatant after the first water-washing and centrifugation of the water-washed pork from heart, PM and SM of porcine. M and Mb denote protein molecular mass standards and horse myoglobin, respectively.

resulted in higher cooking loss compared with both skeletal muscles of PM and SM.

Gel hardness and protein fractions in SDS-PAGE

Gel of PH had significantly ($p<0.05$) weaker gel strength compared with gels of PM and SM (Figure 2). These results suggested that skeletal muscle was not only functionally excellent as raw material in the manufacture of surimi utilizing pork, but also its textural property showed it was more excellent than pig heart. Lan et al. (1995) reported that ultimate pH differences in fish, beef and pork muscles had effects on protein extractability, but that, on an equal protein basis, gelation properties differed among species. Also, differences in protein functionality depend upon species and fiber types (Xiong and Brekke, 1989; Samejima et al., 1992). Gel-forming ability differences among species observed by Lan et al. (1995) and Park et al. (1996) might be related to optimum pH for protein extractability and gelation, and to gel forming differences in the various myosin isoforms present (Lan et al., 1995).

It is well known that myofibrillar proteins play the most critical role during meat processing because they are responsible for cohesive structure and the firm texture of meat products (Xiong, 1997). The PH sample showed a significantly different SDS-PAGE gel pattern of myofibrillar and sarcoplasmic proteins compared to SM and PM samples (Figure 3, Table 4). Especially, in myofibrillar protein fractions, the bands of myosin and TM/TN

Table 4. Density analysis of myofibrillar protein fractions from heart (PH), *psoas major* muscle (PM) and *semimembranosus* muscle (SM) of pigs in SDS-PAGE

Treatment	HMP %	Myosin %	Actin %	TM/TP %
PH	8.26±1.10	21.18±0.82[b]	14.65±2.39	1.52±0 27[b]
PM	8.31±1.40	32 59±2 97[a]	15.23±0.59	9.43±0 60[a]
SM	9.06±0.86	31.18±2.87[a]	15.13±0.88	9.46±0.22[a]

[a, b] Means±SD with different superscripts within column differ significantly ($p<0.05$).
HMP: high molecular protein; TM/TP: tropomyosin/troponin.

(tropomyosin/troponin) had reduced staining intensity in PH samples, and some unidentified bands that were not in PM and SM samples were observed in PH samples. Samejima et al. (1992) suggested that tropomyosin and troponin bands were also detected, but only MHC and actin were analyzed quantitatively since tropomyosin and troponin are known to be relatively thermo-stable and have only a very minor role in gel/emulsion formation. SDS-PAGE indicated that the most notable difference between cardiac and skeletal muscles was the intensity of myosin and TM/TN bands. This result suggested that compositions of muscle protein in SLM had effects on gel properties of cooked SLM.

CONCLUSIONS

SLM made from cardiac muscle had a poor water-holding capacity and higher cooking loss compared to that made from skeletal muscles of pigs. Gels from skeletal muscles showed higher lightness whereas gel from cardiac muscle showed higher yellowness and dark color. The dark color and poor water-holding capacity of SLM from cardiac muscle might be due to lower contents of myosin, tropomyosin/troponin and some unidentified myofibrillar proteins. The data implied that gel properties of SLM from heart and skeletal muscle depended on protein composition, principally myosin and TM/TN.

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