

Gel Color and Texture of Surimi-like Pork from Muscles at Different Rigor States Post-mortem

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ABSTRACT : Gels were made from surimi-like pork (SLP) made from muscles obtained at 1, 24 and 72 h post-mortem. The SLP from pre-rigor muscle had higher pH and moisture percentage compared to in- or post-rigor muscles. Also, SLP from pre-rigor muscle showed higher concentration of water-soluble protein that was washed out during the process. Gel from post-rigor muscle exhibited higher a^* and b^* value, and also resulted in higher Chroma and lower hue values. The dark color of gel from post-rigor muscle was related to higher concentration of sarcoplasmic protein in SLP and denser structure in the gel matrix. SDS-PAGE showed higher intensity of the phosphorylase in the sarcoplasmic protein fraction from pre-rigor muscle. Gel from post-rigor muscle showed higher hardness and sensory firmness, and the greater firmness was related to higher concentration of protein in SLP, and a compact network with smaller pockets in the gel matrix. (**Key Words** : Surimi-like Pork, Gel Color, Texture, Muscle Rigor States)

INTRODUCTION

Surimi is a term denoting the ground fish meat paste formed during the manufacturing process of the traditional Japanese surimi-based product 'Kamaboko'. Currently, surimi and surimi seafoods are also produced and consumed in many countries (Mansfield, 2003). The successful development of the fish surimi process and increasing market share of surimi-based seafoods throughout the world have led to studies aimed at applying the surimi process to red meat and poultry to develop new commodities. There has been considerable interest in manufacturing surimi-like products from chicken (Nowsad et al., 2000; Lesiów and Xiong, 2003; Lee et al., 2004a,b), sheep (Antonomanolaki et al., 1999), beef and pork (Park et al., 1996), and beef heart (Wang and Xiong, 1999). Reported studies have identified large variations in functional properties of myofibrillar proteins associated with muscle fiber types. Park et al. (1996) reported that myofibrils from beef or pork

formed gels with greater hardness than commercial fish surimi. Water-washed beef or pork also had higher levels of salt soluble protein than that of commercial fish surimi. These findings and our preliminary experiments suggest that the greater hardness of surimi-like pork might make it more suitable for textures similar to lobster compared to flesh of crab or shrimp.

The most important characteristics of surimi products are their elastic texture and appearance (Mansfield, 2003). Surimi gel quality, the elastic texture and white color mainly, can be influenced by many factors affecting protein structure. Severe proteolysis of myofibrillar proteins, caused by the endogenous proteinases in muscle, is directly associated with poor gel quality (An et al., 1996; Katayama et al., 2006). Salting of pre-rigor muscle is also known to promote maintenance of high binding quality (water and fat stabilization, texture development) during short-term refrigerated storage, presumably by solubilizing the protein prior to a tight association of actin and myosin (Park et al., 1993). However, there is a little information on effects of rigor states of porcine muscle on functionalities including texture and color of surimi-like pork. Therefore, the objective of this study was to investigate the gel properties of water washed protein made from pork muscle at different states of rigor.

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MATERIALS AND METHODS

Preparation of surimi-like pork and gel

The manufacturing process began with slaughtering market pigs at the Meat Plant of Gyeongsang National University, Jinju, Korea. A total of 10 pigs were slaughtered at 10 times. The whole *semimembranosus* muscle was obtained (above about 1 kg) by hot boning of each pork carcass. After removing external adipose tissue, the lean muscle was divided into three chunks. A pre-rigor sample (300 g) was prepared by dicing one of the three chunks into approximately 2×2×2 cm cubes and grinding through a 4.7-mm diameter orifice, followed by manufacturing into surimi-like pork (SLP) which will be described later in this section. The other two muscle chunks were stored in zippered plastic freezer bags at 4°C until processed into the SLP at 24 h (in-rigor) or 72 h (post-rigor) *postmortem*. The SLP was manufactured 10 times for each pork carcass (treatment).

Muscle cubes prepared above were coarsely chopped for approximately 15 sec in a laboratory-scale silent cutter. After adding five volumes of ice-water, the coarsely chopped pork was further homogenized at 15,000 rpm for 3 min (Model AM-7, Nihouseiki Kaisha LTD, Japan). 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) and the supernatant containing fat and water-soluble proteins was discarded. The sediment was mixed with cold (4°C) water, the volume of which was a volume equal to that of the supernatant discarded in the previous step, homogenized again and filtered through a 500 µm mesh metal screen and centrifuged (15 min/2,220×g) again. The sediment was washed with 2.5 volumes distilled water and subjected to final centrifugation at 2,220×g for 10 min. The centrifuge bottles (500 ml) were inverted and placed in a cold room for 10 min to remove excess free water. The resulting sediment, water-washed pork that was now referred to as SLP, was next processed into the paste by adding NaCl, tripolyphosphate (TPP) and sorbitol to final concentrations (w/w) of 3%, 0.5% and 4%, respectively. The paste was stuffed into a 50 ml conical centrifuge tube (3 cm diameter, 15 cm height) then centrifuged at 2,220×g for 10 min for gas removal from the tube. After centrifugation, the paste was stored in a deep freezer at -60°C and all samples were evaluated at the same time after a week. Stored paste was kept overnight at 4°C then gels were prepared by heating the paste wrapped in 3 cm diameter casing in a 75°C water bath for 20 min.

Proximate composition and pH

A total of 10 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). All samples (3 g) were homogenized using a poly-tron homogenizer (T25basic, IKA, Malaysia) with distilled water (27 ml) and pH measured using a pH-meter (MP230, Mettler Toledo, Swiss).

Concentration of water-soluble protein

The concentration of water-soluble protein (WSP) lost during washing of muscle was determined. Muscles (50 g) were homogenized in 250 ml of cold distilled water (4°C) at 15,000 rpm for 3 min and centrifuged for 15 min at 2,220×g. The supernatant containing fat and WSP was filtered using filter paper (Whatman No. 1) to remove adipose tissues. The resulting solution of 1 mL was used to measure protein concentration by the biuret method (Gornall et al., 1949).

Free water content

To measure free water content of the SLP, samples (2 g) of SLP were placed in centrifuge tubes (Ultrafree-CL, Millipore, Japan) and heated in a water bath at constant 75°C for 30 min. After heating, the samples were removed, cooled to room temperature and centrifuged at 2,220×g at 4°C for 10 min. The intra tube was removed and the tube reweighed. Percent free water was calculated as ((weight of original SLP-weight of SLP after centrifugation)/(weight of original SLP))×100.

Color measurements

Color was assessed on the cut surface of the cooked gels 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 $\text{hue} = \tan^{-1}(b^*/a^*)$.

Gel firmness

Gel firmness was measured using a Sun Rheo Meter (CR-100D, Japan). A 5-mm diameter plunger was used to penetrate the gel samples previously cut to a length of 2 cm and diameter of 0.5 inches at a deformation rate of 120 mm/min using a 10 kg load cell. Gel hardness was determined as the height of the first compression force peak after 60% deformation expressed in grams (Bourne, 1968). Gel firmness was also evaluated by sensory panel test with a nine-person trained panel. Sensory firmness was defined as the amount of force required to bite through the sample with incisors. The firmness was evaluated by numerical scores up to 10, where 1 = very soft; 10 = extremely firm.

Table 1. Proximate composition and pH of surimi-like pork made from muscles at different times post-mortem

Treatment*	Moisture (%)	Protein (%)	Fat (%)	pH
Pre-rigor	88.97±0.18 ^a	11.41±0.51 ^b	<0.3	6.70±0.12 ^a
In-rigor	85.68±0.62 ^c	10.52±0.31 ^c	<0.3	5.90±0.11 ^b
Post-rigor	87.14±0.40 ^b	12.44±0.39 ^a	<0.3	5.66±0.11 ^c

^{a, b, c} Means±SD with different superscripts within a column are significantly different ($p < 0.05$).

* Samples of pre-, in- and post-rigor were taken at 1, 24 and 72 h post-mortem, respectively.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples for SDS-PAGE were obtained from the first supernatant and the final pellet when SLP was prepared. The first supernatant contained mostly sarcoplasmic proteins which were discarded during the process, while the final pellet contained mostly myofibrillar protein. SDS-PAGE was performed according to the method of Laemmli (1970). Protein solutions were mixed at 1:1 (v/v) ratio with the SDS-PAGE sample treatment buffer (0.125 M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 1% bromophenol blue) and heated at 100°C for 1 min in a heating block (Digi-Block[®] 5402, [®]Electrothermal, USA). The samples (1 mg/ml) were loaded on the gel made of 4% stacking and 15% separating gels and subjected to electrophoresis at a constant current of 10-20 mA per gel using a mini-gel electrophoresis unit (Might Small[™] SE 245, Hoefer Scientific Instruments, USA). After electrophoresis, the gels were stained with 0.1% Coomassie brilliant blue R-250 in 40% methanol and 7% acetic acid and destained with 40% methanol and 7% acetic acid. Molecular weights of protein bands were estimated using standard marker (062K9280, Sigma, USA; M-0630, Sigma, USA).

Microstructure

The microstructures of intact muscle fiber, SLP, paste and gel were evaluated using a field emission scanning electron microscope (FESEM: XL30S, Philips, Netherland) after preparing the samples by a method reported previously (Carroll and Lee, 1981). The specimens of muscle, SLP, paste and gel were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.2 for 3 h at 4°C. The specimens were rinsed three times with 0.1 M PB for 15 min and fixed with 1% O₃O₄ in 0.1 M PB for 2 h at 4°C. Specimens were rinsed three times with 0.1 M PB for 15 min at 4°C, and the final rinse was conducted with distilled water. Dehydration of specimens was accomplished with an ethanol series. After dehydration, specimens were settled in acetone for 15 min and critical point dried using hexamethyl-disilazane (HMDS; Sigma H-4875) for 20 min at 4°C and then stored in desiccators. Specimens were mounted on a holder with double sided adhesive tape and sputter-coated with gold using an ion-sputtering device (JFC-1100E, JEOL, Japan). The accelerating voltage was 15

kV and the working distance was 25 mm. The specimens were photographed with magnification ×200, ×5,000, ×10,000 and ×10,000 for muscle fiber, SLP, paste and gel, respectively.

Statistical analysis

The surimi-like pork was manufactured 10 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 and pH

Proximate composition and pH of SLP are presented in Table 1. As expected, pH of SLP from pre-rigor muscle was significantly higher than from in- or post-rigor muscles, and in-rigor SLP showed significantly higher pH compared to post-rigor SLP ($p < 0.05$). Pre-rigor SLP had the highest moisture content followed by post-rigor SLP and then in-rigor SLP. The in-rigor SLP showed the lowest protein content whereas the highest protein content was observed in post-rigor SLP. Fat contents were not significantly different among samples, and all SLP exhibited below 0.3% of fat content.

The pH of SLP was strongly affected by muscle rigor states, and moisture and protein percentage were also affected. It is well known that the formation of lactic acid results in pH drop during the postmortem period and was responsible for reduction of reactive groups on proteins being available for water binding (Offer and Trinick, 1983). Therefore the higher moisture percentage of pre-rigor SLP might be due to its higher pH. Consequently, the differences in pH, moisture and protein content in SLP would be expected to affect gel-forming ability of SLP. The noticeable reduction in fat content (<0.3%) of SLP was similar to that reported by Park et al. (1996). This was probably due to destruction of adipose cells by homogenization and floatation during centrifugation.

WSP concentration and water content

Table 2 shows that concentration of WSP was significantly higher in pre-rigor and lower in in-rigor muscles ($p < 0.05$). Free water percentage of pre-rigor SLP was significantly lower and gel from post-rigor SLP had significantly lower moisture percentage ($p < 0.05$).

Table 2. Water soluble protein (WSP) of intact muscle and water contents of surimi-like pork and gel

Treatment*	Intact muscle WSP (mg/g)	Surimi-like pork free water (%)	Cooked gel moisture (%)
Pre-rigor	88.06±1.47 ^a	30.89±0.94 ^b	81.55±1.21 ^a
In-rigor	75.49±2.18 ^c	34.99±1.13 ^a	81.24±0.70 ^a
Post-rigor	79.64±1.31 ^b	35.04±0.70 ^a	77.65±0.34 ^b

^{a, b, c} Means±SD with different superscripts within a column are significantly different ($p < 0.05$).

* Samples of pre-, in- and post-rigor were taken at 1, 24 and 72 h post-mortem, respectively.

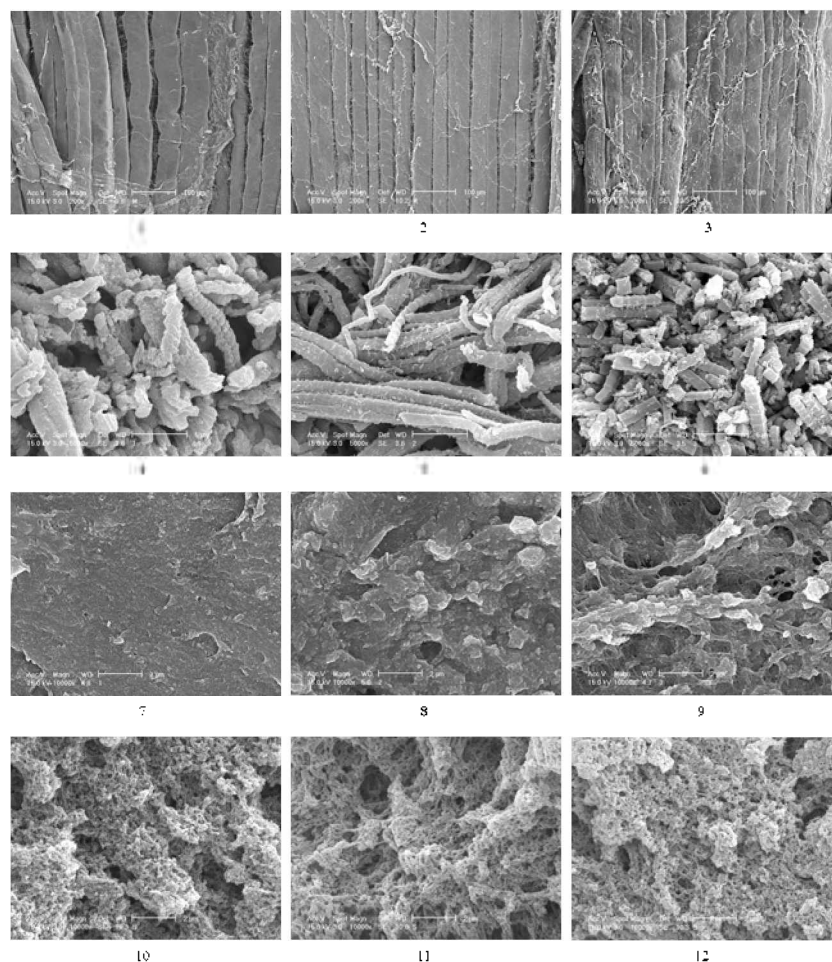


Figure 1. Photomicrographs of intact muscle, surimi-like pork (SLP), paste and gel from porcine *semimembranosus* muscle at different state of rigor. Photomicrographs 1-3: pre-rigor, in-rigor and post-rigor muscle, 4-6: SLP, 7-9: paste; 10-12: cooked gel, respectively.

Changes in concentration of WSP by rigor states were related to physical changes in microstructure of myofibrils post-mortem. It has been generally accepted that both the rate and extent of glycolysis are important factors affecting physical properties of porcine muscle (Joo et al., 1995). As shown in photomicrographs, appearance of myofibers was changed by times post-mortem (photographs 1-3, Figure 1). As myofibers entered full rigor they became firm to the point of stiffness at 24 h post-mortem, and as the process of enzymatic degradation and protein denaturation continued the myofibrils became less firm at 72 h post-mortem. Consequently the myofibrils were broken easily before and after completion of rigor mortis, and unbroken myofibrils

were observed in in-rigor SLP. Eventually, in relation to rigor state, more water containing proteins could be moved out when pre-rigor muscle was used to manufacture SLP. Furthermore, our electrophoresis result clearly showed that more proteins washed out from pre-rigor muscles compared to in- or post-rigor muscles (Figure 2). These photographs were sufficient evidence to explain why the concentration of WSP was higher in pre-rigor muscle but lower in in-rigor muscle.

Free water percentage of pre-rigor SLP was lower than of in- or post-rigor SLP. This was probably due to the higher pH of pre-rigor muscle, which resulted in increased water-holding capacity. Although there were significant

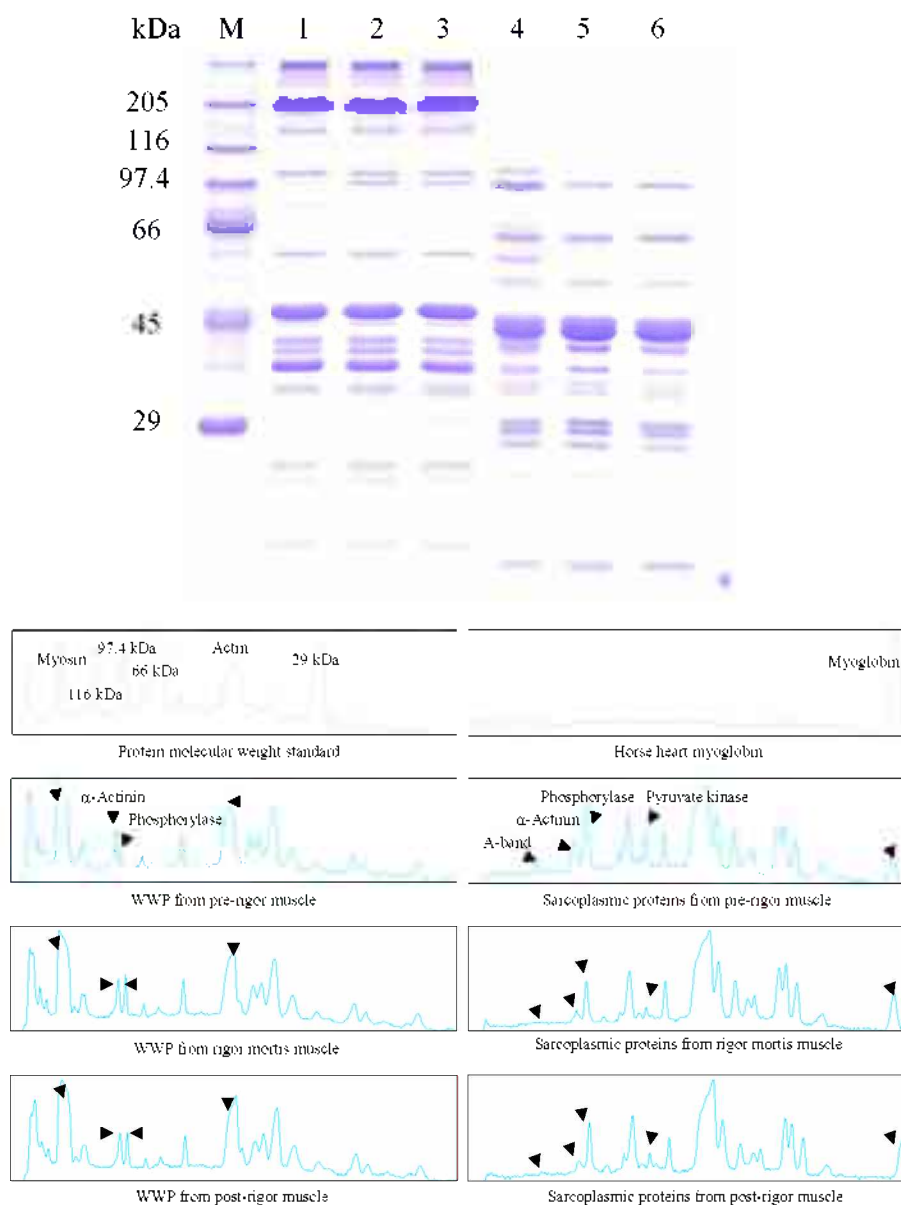


Figure 2. SDS-PAGE patterns (*upper*) and band intensity (*lower*) of myofibrillar and sarcoplasmic protein fractions of water-washed pork (WWP). The myofibrillar (lanes 1-3) and sarcoplasmic (lanes 4-6) protein fractions, which were defined as the sediment and supernatant after the first water-washing and centrifugation of the surimi-like pork which had been derived from pre-rigor (lanes 1 and 4), in-rigor (lanes 2 and 5) and post-rigor (lanes 3 and 6) *semimembranosus* muscle chunks. M and Mb denote protein molecular mass standards and horse heart myoglobin, respectively.

differences in pH between in-rigor and post-rigor muscles, our data showed no significant differences in free water percentage of SLP samples. The lower moisture percentage observed in gel from post-rigor muscle might be related to complex influences of lower pH of muscle, shrinkage and fragmentation of myofibrils and higher protein content in SLP.

Gel color of SLP

All SLP samples from pre-, in- and post-rigor muscles exhibited a light, opaque and dough-like appearance. Upon

mixing the SLP with additive including salt, TPP and sorbitol, the paste became more translucent and sticky compared with the original SLP. However, after cooking of the paste, there were significant differences in color measurements among gel samples (Table 3). Gels made from in-rigor muscle had the highest lightness (L^*), while gels made from post-rigor and pre-rigor muscle had the same lightness. Gels made from post-rigor muscle were the least green ($-a^*$) and most yellow (b^*) of the samples, with this yellowness contributing to the higher saturation (C^*) and lower hue for this sample. Gels made from pre-rigor

Table 3. Color measurements of cooked surimi-like pork gel made from muscles at different times post-mortem

Treatment*	L*	a*	b*	Chroma	Hue
Pre-rigor	78.51±1.20 ^b	-2.48±0.54 ^b	1.95±0.80 ^c	3.16±0.40 ^c	159.61±9.49 ^a
In-rigor	80.30±2.04 ^a	-2.54±0.28 ^b	2.36±0.87 ^b	3.55±0.39 ^b	126.96±4.46 ^b
Post-rigor	78.75±1.15 ^b	-2.07±0.31 ^a	6.27±0.45 ^a	6.61±0.33 ^a	108.37±3.83 ^c

^{a, b, c} Means±SD with different superscripts within a column are significantly different ($p < 0.05$).

* Samples of pre-, in- and post-rigor were taken at 1, 24 and 72 h post-mortem, respectively.

Table 4. Gel firmness of cooked surimi-like pork gel made from muscles at different times post-mortem

Treatment*	Hardness (g/cm ²)	Sensory firmness**
Pre-rigor	186.18±11.93 ^b	6.3±0.6 ^b
In-rigor	175.83±22.31 ^c	6.7±1.2 ^b
Post-rigor	243.88±19.38 ^a	7.8±0.9 ^a

^{a, b, c} Means±SD with different superscripts within a column are significantly different ($p < 0.05$).

* Samples of pre-, in- and post-rigor were taken at 1, 24 and 72 h post-mortem, respectively.

** Sensory firmness was evaluated as; 1 = very soft, 10 = extremely firm.

muscle had the lowest chroma and highest hue. Data implied that gels made from post-rigor muscle showed significantly darker (i.e. more color and less vivid), whereas pre-rigor muscle showed significantly brighter (i.e. less color and more vivid) than those made from other rigor states of muscles.

It is speculated that the brighter color of gels made from pre-rigor vs. in- or post-rigor muscle resulted from a lower concentration of sarcoplasmic proteins in SLP. Although muscle washing would substantially remove water-soluble substances, fat particles and heme compounds, many enzymes in sarcoplasm could remain and combine with myofibrillar proteins during the post-mortem period (Toyoda et al., 1992). In sarcoplasmic protein fraction patterns of SDS-PAGE, intensity of myoglobin and hemoglobin effects on color were not different among muscles (Figure 2). However, the abundance of protein (97.4 kDa) which was phosphorylase (Joo et al., 1999) in the sarcoplasmic protein fraction was greater in pre-rigor vs.

in- and post-rigor muscle (Figure 2; lanes 4-6, Table 5), whereas the abundance of the adulterated phosphorylase was less in the myofibrillar protein fraction from the pre-rigor muscle (Figure 2; lane 1, Table 5). These results suggested that the brighter color of gels made from pre-rigor muscle was due to phosphorylase removal from SLP during the washing process.

It is also speculated that the dark color of gels made from post-rigor vs. in- or pre-rigor muscle resulted from a greater absorption of visible light by the denser myofibrillar matrix in the former (Figure 1; photo 12). Variation in muscle structure may affect light reflectance or light scattering and affect apparent color without any changes in pigment content (Wismer-Pederson, 1959). Light scattering in meat is caused by structural and myofibrillar proteins (MacDougall, 1986) and related to the size of scattering particle (Francis and Clydesdale, 1975). Inspection of the microstructure of the three muscles and gel preparations revealed that the *semimembranosus* muscle fibers of the porcine had different spatial characteristics (Figure 1; photos 1-3) and the gels had an amorphous protein particles structure (Figure 1; photos 10-12). Gel made from post-rigor muscle had smaller pockets with denser myofibrillar gel matrix, whereas gel from pre- or in-rigor muscle had larger pockets with sponge-like conglomerates of proteins. This structural change could lead to a higher concentration of particles with a different refractive index, and the higher concentration of particles might cause more light to be absorbed in the gel matrix. Consequently, gel made from post-rigor muscle had a darker appearance.

Table 5. Composition ratio of myofibrillar and sarcoplasmic proteins in SDS-PAGE patterns from porcine *semimembranosus* muscle chunks taken before, during and after rigor mortis

Protein fractions	Pre-rigor	In-rigor	Post-rigor
Myofibrillar proteins (%)			
Myosin	12.89±2.44	11.92±2.36	13.16±2.76
α -Actinin	4.38±1.32 ^a	2.75±0.32 ^b	3.45±0.47 ^{ab}
Phosphorylase	1.65±0.32 ^c	3.20±0.78 ^b	4.11±0.48 ^a
Actin	10.85±1.51	8.78±0.54	10.40±2.58
Sarcoplasmic proteins (%)			
Protein A	1.11±0.24 ^a	0.47±0.08 ^b	0.43±0.13 ^b
α -Actinin	3.66±0.80 ^a	1.07±0.34 ^b	1.49±0.30 ^b
Phosphorylase	9.13±0.77 ^a	4.33±0.87 ^b	5.70±1.43 ^b
Pyruvate kinase	4.78±0.43 ^a	1.49±1.02 ^b	2.12±0.71 ^b
Myoglobin	2.85±0.40 ^b	4.06±0.21 ^a	4.50±0.71 ^a

^{a, b, c} Means±SD with different superscripts within column differ ($p < 0.05$).

Gel firmness of SLP

The mechanical and sensory firmness of gels are presented in Table 4. There was significant difference in hardness among gel samples from different states of rigor ($p < 0.05$). Gel from post-rigor muscle had the highest hardness followed by pre-rigor muscle and then in-rigor muscle. The panel also evaluated that gel from post-rigor muscle was more firm than that from pre- or in-rigor muscles ($p < 0.05$).

The gel-forming ability of surimi products is influenced by WHC, protein concentration, ultimate pH and heating condition of water-washed muscles (Lan et al., 1993). In this regard, the greater hardness of the gel derived from post-rigor vs. pre- or in-rigor muscle was likely to be related to the greater protein concentration in the former, because a higher-protein SLP usually resulted in a stronger and harder surimi gel (Park et al., 1996). Our data showed that mechanical hardness values of gel samples were well matched with protein concentrations of SLP shown in Table 1. Therefore, it was confirmed that the high concentration of proteins in SLP was basically responsible for firmness of gel.

The pH in relation to denser structure of the gel matrix would be considered as very important in the gel firmness. Especially, pH was an important factor influencing thermally induced protein change. Thermal transitions of myofibrillar tissues including myosin and actomyosin are dramatically influenced by pH (Xiong and Brekke, 1990). Our data also showed that the firmness of gel was related to pH of SLP and gel. The low pH with the high concentration of proteins in SLP from post-rigor muscle might affect micro-structural changes to denser gel matrix after cooking, resulted in higher hardness of gel.

On the other hand, salting of pre-rigor meat has been known to promote maintenance of high "binding" quality by solubilizing the protein prior to a tight association of actin and myosin (Hamm, 1981). Preliminary experiments in our research showed that the addition of 3% NaCl into SLP was essential for the production of good gel (Kang et al., 2006). In this study, the microstructures of SLP, paste and gel revealed different features with rigor states of porcine muscle. The SLP from pre-rigor muscle exhibited a fine paste of myofibrillar proteins solubilized, whereas SLP from post-rigor muscle showed a rough paste of myofibrillar proteins (Figure 1: photos 7-9). Probably this feature was due to differences in solubility of salt soluble protein with rigor states of muscle. Consequently, after cooking of the paste, gel from pre-rigor muscle had sponge-like conglomerates, whereas gel from post-rigor muscle had a compact network structure (Figure 1: photos 10-12). This compact network with smaller pockets in the gel matrix might explain the lower moisture content and firmness of

gel from post-rigor muscle (Table 2). These microphotographs implied that moisture percentage and firmness of SLP were affected by microstructure of the gel matrix.

CONCLUSIONS

Results of this study indicated that bright and white gel could be obtained when a pre-rigor muscle is used as starting raw material, whereas strong and firm gel can be obtained from a post-rigor muscle. However, post-rigor muscles have their own drawbacks. The post-rigor muscle can cause an undesirable dark color of the gel. The data implied that SLP made from pre-rigor muscle is the most useful to produce a bright gel, but SLP made from post-rigor muscle is useful to manufacture a firm SLP.

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