Effects of Non-meat Protein Binders and Acidification on the Efficiency of Cold-Set Pork Restructuring by High Pressure

Geun-Pyo Hong¹, Ji-Yeon Chun¹, Si-Kyung Lee, and Mi-Jung Choi*

Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Korea

¹Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, Korea

Abstract

We investigated the effects of non-meat protein binders combined with glucono- δ -lactone (GdL) on the binding properties regarding restructured pork prepared by high-pressure treatment. Soy protein isolate (SPI), casein (CS), whey protein concentrate (WPC), and egg white (EW) were used as non-meat protein binders and compared with the control (no binder) and with the κ -carrageenan (KC) treatment. The compression and depression rates were 2.3 and 37 MPa/s, respectively, and pressurization was conducted at 200 MPa for 30 min at 4°C. After pressurization, the physical properties (pH, water-holding capacity, color, tensile strength, and microscopic structure) of the sample were evaluated. The combination of pressurization with acidification enabled cold-set meat binding, and the binding strength of restructured pork was enhanced by the addition of non-meat proteins. Among binders, SPI demonstrated the best efficiency in binding meat pieces. Therefore, the present study demonstrated that the combination of acidification and pressurization processes with the utilization of non-meat protein binders has a potential benefit in meat restructuring.

Key words: non-meat protein, restructured meat, pressurization, acidification, meat binding

Introduction

It is well known that proteins undergo aggregation and gelation depending on environmental conditions such as pH and temperature. Protein gel formation is important for the texturization of semi-solid food products, including tofu, cheese, and sausages. Among proteins, myofibrillar proteins (MPs) are likely to be more labile in response to environmental changes than plant proteins, owing to differences in structure and thermal stability. Texturization of meat products is most depends on the gel-forming ability of MPs. To form an MP gel, various techniques have been introduced. Microbial transglutaminase-catalyzed cross-linking is one of major examples of non-thermal meat gelation (Chin *et al.*, 2009). Protein oxidation also enables cross-linking of proteins, thereby forming an irreversible polymer; however, the oxidative process coincides precisely with functional and nutritional losses of proteins (Xiong, 2000). Ngapo *et al.*

(1996) reported that a cold-set MP gel could be obtained by glucono- δ -lactone (GdL)-induced slow acidification.

High-pressure treatment, an analogue of thermal treatment, induces protein unfolding, thereby promoting protein gelation. For pressurization of fresh meat, the physical and structural changes of proteins manifests partial unfolding and denaturation, and meat protein denaturation affects the functional properties and color of meat. Alternately, pressure-induced structural changes in proteins (aggregation and gelation), namely cold-set meat restructuring or reforming, provide a possibility to bind meat pieces without thermal treatment. In a preliminary study, cold-set meat restructuring could not be achieved by pressurization at 200 MPa for 30 min, and the addition of non-meat protein binders was of no use to bind meat pieces unless the sample was subjected to thermal treatment (Hong *et al.*, 2006). Effective cold-set meat binding was achieved under the same pressure conditions by using κ -carrageenan (KC). On pressurization, KC formed a continuous network-like structure inside the salt-extracted MP network, and the simultaneous addition of GdL resulted in better integrity of the MP network structure, leading to high tensile strength in the restructured meat (Hong *et al.*, 2008).

*Corresponding author: Mi-Jung Choi, Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-450-3048, Fax: 82-2-455-1044, E-mail: choimj@konkuk.ac.kr

In general, commercial food proteins are mainly produced by spray drying, and the proteins show poor functionalities because of partial or complete denaturation. In meat product formulations, however, the use of denatured non-meat protein ingredients is a better way to improve the textural and rheological properties of the products, because structural unfolding of globular proteins manifests as a filamentous structure on re-heating (Bryant and MacClements, 1998; Feng and Xiong, 2002). Consequently, the filamentous structure of non-meat proteins can form a network with the MP network. These heterologous protein networks were formed only under thermal treatment, and not under pressurization at 200 MPa (Hong *et al.*, 2006). On the other hand, GdL is an effective protein-gelling ingredient, and some studies have reported that pre-heat-treated milk or soy proteins were more susceptible to acid gelation than their native forms (Bringe and Kinsella, 1990; Dybowska and Fujio, 1998). Thus, it was expected that the use of GdL combined with protein-based binders might eliminate the drawback of insufficient cold-set meat binding observed in our previous study (Hong *et al.*, 2006, 2008). The present study explored the effects of non-meat proteins combined with GdL on the efficiency of meat restructuring after high-pressure treatment.

Materials and Methods

Materials

Pork loin (*longissimus dorsi*) was obtained from 6 carcasses at 48 h post-mortem (pH 5.72±0.04). Meat was frozen at -30°C prior to sample preparation (within 3 weeks). Commercially produced non-meat proteins, including soy protein isolate (80.6% protein), milk casein (sodium salt, 90.8% protein), whey protein concentrate (81.3% protein), and egg white powder (82.2% protein), were donated by Sam-Hwa Asia Co. (Korea). κ -carrageenan (κ C) was purchased from Sigma-Aldrich Co (USA). All chemicals used in the present study were analytical grade.

Sample treatment

Two pork loins for each batch were thawed at 4°C for 48 h, and all visible fat and connective tissue was trimmed. Meat was cut into 1-cm cubes, and the cubes from 2 different loins were mixed together. The meat cubes (300 g per treatment) were hand-mixed with 0.5% NaCl and 0.3% sodium tripolyphosphate for 2 min, then 0.5% glucono- δ -lactone (GdL) and 1% binders were added and hand-mixed for 3 min (Table 1). Soy protein isolate (SPI),

Table 1. Formulation of restructured pork made with or without non-meat protein binders

Ingredients	Formulation (g/300 g batch)	
	Control	Treatments
Pork loin cubes	296.1	293.1
NaCl	1.5	1.5
Sodium tripolyphosphate	0.9	0.9
Glucono- δ -lactone	1.5	1.5
Binder ¹⁾	-	3.0

¹⁾Binders used in this study contained κ -carrageenan, soy protein isolate, casein, whey protein concentrate, and egg white.

casein (CS), whey protein concentrate (WPC), and egg white (EW) were used as non-meat protein binders and compared with κ -carrageenan as a non-protein binder. In the control (C), 1% binder was substituted with the same amount of meat cubes. The mixture was filled into fibrous casing (4.5 cm diameter) and vacuum-packed in a poly nylon pouch. The mixture was kept at 4°C for 24 h to allow sufficient acidification and pressurized using a lab-scale hydrostatic pressure system (1-L working volume) as described previously (Hong *et al.*, 2008). Ethanol was used as the pressure-transmitting medium. The compression and depression rates were 2.3 and 37 MPa/s, respectively, and pressurization was performed at 200 MPa for 30 min at 4°C. After pressurization, the physico-chemical properties of the sample were estimated without further storage. All procedures were repeated 3 times on different days (n = 3).

pH

The pH of the sample was determined using a pH-meter (pH 900, Precisa Co., Switzerland). Approximately 5 g of sample was homogenized with 20 mL of distilled water at 10,000 rpm for 2 min by using an SMT process homogenizer (SMT Co. Ltd., Japan), and the pH was determined in duplicate.

Water binding properties

The moisture content of samples was determined in triplicate by the method described by the AOAC (1990). Expressible moisture was determined by applying centrifugal force. Approximately, 1 g of sample (M_{Meat}) was placed in a centrifuge tube along with gauze as moisture absorbent. After centrifuging at 1,000 g for 10 min using RC-3 centrifuge (Sorvall Co., Thermo Scientific, USA), absorbed moisture in the absorbent before (M_1) and after drying (M_2) was measured. The expressible moisture was expressed as a percentage of exudative loss over initial sample weight as follows. All measurements were carried

out in triplicate.

$$\text{Expressible moisture (\%)} = \frac{M_1 - M_2}{M_{\text{Meat}}} \times 100$$

Instrumental color

The sample was sliced to a thickness of 1 cm and kept at ambient temperature for 10 min. The color of the sample surface was randomly measured 6 times by using a color reader (CR-10, Konica Minolta Sensing, Inc., Japan) calibrated with a white standard plate ($L^* = 97.83$, $a^* = -0.43$, $b^* = +1.98$). The CIE L^* , a^* , and b^* values were determined as indicators of lightness, redness, and yellowness, respectively. The total color difference (ΔE) was calculated using the following equation on the basis of the color differences between treated samples and raw pork ($L^* = 48.9$, $a^* = 12.4$, $b^* = 9.7$).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Tensile strength

The sample was sliced to a thickness of 1 cm and cut into rectangular forms (1×1×4 cm) and was fixed at 1 cm from each end by using 3 pins connected to the plate and the plunger, respectively (Hong *et al.*, 2008). The force required to pull apart the sample was measured using a digital gauge (DPS-20, IMADA Co., Japan) at a head speed of 60 mm/min. The tensile strength of each treatment was determined 12 times.

Scanning electron microscopy

The sample was sliced to a thickness of 2 mm and cut into 1-cm squares. Sample was frozen at -75°C for 24 h and lyophilized at 0.6 torr for 48 h. The sample was gold-coated using an ion sputter (E-1010, Hitachi Science System Ltd., Japan), and the microstructure near the junction zone was observed using a scanning electron microscope (S-3000 N, Hitachi Science System Ltd., Japan) at $\times 200$ and $\times 1,000$ magnifications with an accelerating voltage of 25 kV.

Statistical analysis

A completely randomized block design was adopted, and the data were analyzed by the general linear model procedure by using the SAS program (ver. 9.1; SAS Institute Inc., Cary, USA). One-way analysis of variance (ANOVA) was conducted with the means of 3 different batches ($n = 3$), and the means were ranked by Fisher's Least Significant Difference (LSD) test when the main effect (treatment) was significant at the 95% level ($p < 0.05$).

Results and Discussion

pH and water-binding properties

As shown in Table 2, the final pH of the samples after pressurization was about 5.4–5.5, which was in accordance with our previous study (Hong *et al.*, 2008). All binder treatments resulted in a higher pH than that of the control ($p < 0.05$), with the exception of CS treatment. Nevertheless, variations in pH between treatments were small (< 0.04 pH unit), and the final pH of restructured pork might be mainly influenced by the addition of GdL (Ngapo *et al.*, 1996). It should be noted that pressurization increases the pH of the meat (Hong *et al.*, 2008). It is widely recognized that pH is crucial for the yield of the final meat product, and the lower the pH of the meat product, the greater the moisture loss (Aaslyng, 2002). Hong *et al.* (2008) reported that the moisture content of restructured pork prepared by pressurization at 200 MPa was similar at varying GdL concentrations, although the GdL concentration significantly influenced the water-holding capacity of the products. In the current study, the moisture content of restructured pork showed differences among treatments ($p < 0.05$), and the overall moisture content was 71–73%, compared to 73.2% of raw pork material (data not shown). Although the relationship between pressurization and the water binding ability of meat is not fully understood, it is widely accepted that pressurized meat shows better water-binding properties than untreated meat. According to the classical perspective, the water-binding ability of meat is dependent on the space between the muscle fibers, which is regulated by muscle pH (Hamm, 1986). Upon mild pressurization (~ 200 MPa), structural changes in proteins are driven by the penetration of water molecules into the cavities of the proteins (Boonyaratana-kornkit *et al.*, 2002). The penetration of water molecules

Table 2. pH and water binding properties of restructured pork prepared with various binders, glucono- δ -lactone, and pressurization at 200 MPa for 30 min

Treatment ¹⁾	pH	Moisture (%)	EM (%) ²⁾
C	5.44±0.02 ^c	72.7±0.08 ^a	14.3±0.53 ^a
KC	5.51±0.02 ^{ab}	72.2±0.29 ^b	9.1±0.81 ^b
SPI	5.52±0.03 ^a	70.6±0.46 ^c	11.1±0.42 ^b
CS	5.47±0.03 ^{bc}	70.4±0.80 ^c	11.0±1.92 ^b
WPC	5.50±0.02 ^{ab}	71.7±0.60 ^b	9.9±0.38 ^b
EW	5.53±0.02 ^a	71.6±0.51 ^b	10.2±1.23 ^b

¹⁾C, control; KC, K-carrageenan; SPI, soy protein isolate; CS, casein; WPC, whey protein concentrate; EW, egg white.

²⁾EM, expressible moisture

^{a-c}Means with different superscripts within same column are significantly different ($p < 0.05$).

into the protein interior triggers the disruption of interactions maintaining or stabilizing the tertiary and quaternary structures of the proteins (Boonyaratankornkit *et al.*, 2002; Knorr *et al.*, 2006). As a result, proteins are partially unfolded (molten globule state) under high pressure; this presumably causes the high final pH and high water binding ability of meat.

All binder treatments resulted in lower moisture content than in the control ($p < 0.05$), probably resulting from the different formulations (with and without binders). The actual water binding ability of the protein binders was revealed by the measurements of expressible moisture; binder-treated meat had lower expressible moisture than control ($p < 0.05$). In addition, the moisture content of WPC- and EW-treated meat was higher than those of SPI- and CS-treated meat ($p < 0.05$), but not with KC treatment. In contrast to the poor hydration of thermal-treated SPI and SC (Shen, 1976; Vasbinder *et al.*, 2004) samples, those treated with WPC and EW were reported to retain hydration ability after thermal treatment (Campbell *et al.*, 2003; Vasbinder *et al.*, 2004), as evidenced by their different moisture contents after pressurization. However, binder types presumably had only a minor effect on water-binding properties since there were no differences in the expressible moisture with different binder treatments.

Tensile strength

The efficiency of meat restructuring relies on the binding strength (expressed as the tensile strength) of meat pieces. Cold-set meat binding could be achieved with 0.5% GdL (control), but the binding strength was 0.97 N; this value of tensile strength was not high enough to allow handling of the sample without having it break up easily into pieces (Fig. 1). Although it is difficult to quantify an acceptable tensile strength, Kuraish *et al.* (1997) suggested around 1.6 N ($\sim 80 \text{ g/cm}^2$) of tensile strength as an acceptable value, which would allow the handling of raw restructured meat. In the present study, all binder treatments resulted in a higher tensile strength than control ($p < 0.05$), but an acceptable tensile strength (ca 1.6 N) was achieved with the KC and SPI treatments. We have previously demonstrated the efficiency of KC as a cold-set meat binder upon pressurization (Hong *et al.*, 2008). In combination with acidification, however, SPI had better meat-binding ability than KC after depression ($p < 0.05$). It is well known that SPI is composed mainly of globulins and albumins, and that soy globulins (glycinin and β -conglycinin) are key proteins related to protein aggregation

Fig. 1. Effects of non-meat protein binders on the tensile strength of restructured pork prepared by pressurization at 200 MPa for 30 min. The binders used were κ -carrageenan (KC), soy protein isolate (SPI), casein (CS), whey protein concentrate (WPC), and egg white (EW). Means with different letters are significantly different ($p < 0.05$).

and thereby to gel formation. Soy globulins exhibit relatively high thermal stability; in particular, the presence of NaCl stabilizes the globulin structure against thermal treatment (Jiang *et al.*, 2010). On the other hand, globulins in commercial SPI were completely denatured, and denatured globulins are preferred to their native forms for gel formation under appropriate conditions (pH and temperature) (Feng and Xiong, 2002). Presumably, acidification-induced soy protein gelation gave rise to better binding of meat pieces under high pressure. CS and WPC treatments also improved the tensile strength of meat pieces compared with control ($p < 0.05$), although their impacts were lower than that of SPI ($p < 0.05$). In addition, no difference in tensile strength was observed between CS and WPC treatments. Roesch *et al.* (2004) reported that WPC was less susceptible to aggregation by GdL compared to SPI. As reported by Pelegrine and Gasparetto (2005), the majority of WPC consists of α -lactalbumin and β -lactoglobulin, and α -lactalbumin is the most stable milk protein against pH changes. These properties might result in better water-binding properties in restructured pork, but its contribution with GdL to meat binding appears to be limited compared to that of SPI. On the other hand, CS was reported to have better gelation properties than WPC, whereas the presence of NaCl suppresses the aggregation of CS, thereby showing no difference in tensile strength with WPC treatment (Lucey *et al.*, 1997). Even though it showed good thermal gelling ability (Campbell *et al.*, 2003), EW treatment showed the lowest binding strength among binder treatments ($p < 0.05$).

Weijers *et al.* (2006) noted that EW formed a weak and brittle acid-gel because of its fine-stranded microstructure and low degree of disulfide bonding; this is consistent with the present study. In the scope of our experiment, therefore, the results suggest that SPI is the best cold-set meat binder in the presence of GdL and with subsequent pressurization, and that the impact of protein binders on the tensile strength of restructured meat might be dependent on the applied processing conditions and regulated by GdL-induced acidification as well as pressurization.

Microstructure

Because meat binding is related to protein network formation in the junction zone, one can expect that the better the binding strength, the more intense the integrity of network structure. As shown in Fig. 2, a less intense protein network was exhibited in the control than in KC treatment, which showed a thin and intense network structure inside the junction zone. The results were in accordance with the binding strength; the more intense the network structure, the higher the tensile strength. Meanwhile, protein binders showed different characteristics than the non-meat proteins (KC), as they formed an amorphous continuum in the meat junctions. It is likely that these proteins filled the spaces between the meat pieces and formed a gel by acidification and pressurization. Therefore, observation of the microstructure did not guaranteed changes in the tensile strength of protein binder treatments, and the factors relating to the tensile strength in the meat

pieces were probably the characteristics of the protein gels. In general, SPI gel formation is compromised mostly because of balanced electrostatic interactions, hydrophobic interactions, and disulfide bonds. Comparing the different processing methods, the hydrophobic contribution of pressurization (~200 MPa) is not as high as that of thermal treatment, while pressurization promotes SH/SS interchanges among proteins (Messens *et al.*, 1997). Soy glycinin, which constitutes the majority of soy globulin, contains 8 cysteine residues per molecule, compared to 6 residues in ovalbumin and 5 residues in α -lactalbumin (Visschers and de Jongh, 2005). Consequently, the number of reactive residues of SPI might result in the highest tensile strength among the different treatments (Hong *et al.*, 2006). Meanwhile, the presence of NaCl in the formulation might influence the binding ability of CS and WPC in a different manner. Although CS treatment results in texturized milk proteins (Lucey *et al.*, 1997), electrostatic interactions are important for CS aggregation, and the presence of NaCl shields charged groups on the CS particles, decreasing the CS gel forming ability (Lucey and Horne, 2009). Pressurization might promote hydrophobicity and provide cold-set gelling ability of CS, but has limited impact compared to SPI. Alternately, NaCl enhanced WPC aggregation by enhancing hydrophobic interactions; thus presence of NaCl was appropriate to texturize WPC (Roesch *et al.*, 2004; Visbinder *et al.*, 2004). The different susceptibilities of CS and WPC to NaCl were likely resulted from the lack of any difference

Fig. 2. Microstructure (200 $\times$) of the junction zone of restructured pork made without (control, C) and with κ -carrageenan (KC), soy protein isolate (SPI), casein (CS), whey protein concentrate (WPC), and egg white (EW). The scale bar indicates 100 μ m. The insert shows the microstructure at 1,000 $\times$ magnification.

in meat binding after pressurization. Eventually, it was reported that ovalbumin did not undergo denaturation by commercial spray-drying procedures (Campbell *et al.*, 2003), and Hayashi *et al.* (1989) noted that pressure-induced gelation of EW required at least 600 MPa. In the current study, acidification in the presence of NaCl reduced the structural stability of EW, subsequently resulting in molecular aggregation; however, the hydrophobic contribution was not as much as those of other protein-based binders (Ichikawa and Shimomura, 2007). The results demonstrate that protein binders, combined with acidification, have a potential advantage in pressure-induced cold-set meat restructuring, even though the binding efficiency is highly dependent on the protein source.

Color

Compared to the initial color of pork, all treatments showed higher L^* and b^* values and a lower a^* value, mainly due to acidification (Table 3). In general, both acidification and pressurization resulted in denaturation of meat proteins, and the meat had a significantly lighter appearance as a result (Hong *et al.*, 2008). It was identified that mild pressurization (<350 MPa) increased the a^* value of meat by reducing the metmyoglobin content (Jung *et al.*, 2003); this increase in a^* values was not observed with the different treatments after pressurization in this study, because of acidification. Among the treatments, the addition of protein binders influenced the color of restructured pork; in particular, CS and EW treatments showed the highest ΔE ($p < 0.05$). The unique color characteristics of different binders would cause differences in color with different treatments (Hong *et al.*, 2006). Finally, the results indicate that the use of protein binders contributed to the color of the final cold-set product; however,

these contributions were not pronounced compared to those caused from pressurization or acidification, since the control showed a relatively high ΔE (9 units).

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Table 3. CIE color of restructured pork prepared with various binders, glucono- δ -lactone, and pressurization at 200 MPa for 30 min

Treatment ¹⁾	L^*	a^*	b^*	ΔE ²⁾
C	57.4 $\pm$ 1.04 ^{ab}	11.3 $\pm$ 1.03 ^{ab}	10.1 $\pm$ 0.33 ^c	8.59 $\pm$ 1.10 ^{bc}
KC	54.8 $\pm$ 0.44 ^d	11.6 $\pm$ 1.07 ^a	10.5 $\pm$ 0.60 ^c	6.11 $\pm$ 0.42 ^c
SPI	56.6 $\pm$ 0.34 ^{bc}	9.9 $\pm$ 0.28 ^c	11.3 $\pm$ 0.54 ^b	8.32 $\pm$ 0.29 ^c
CS	57.8 $\pm$ 0.92 ^a	10.7 $\pm$ 1.02 ^{bc}	12.0 $\pm$ 0.60 ^a	9.42 $\pm$ 0.83 ^{ab}
WPC	55.9 $\pm$ 0.45 ^c	11.6 $\pm$ 1.00 ^a	10.1 $\pm$ 0.82 ^c	7.19 $\pm$ 0.52 ^d
EW	58.0 $\pm$ 1.73 ^a	10.3 $\pm$ 0.66 ^c	11.8 $\pm$ 0.16 ^{ab}	9.59 $\pm$ 1.73 ^a

¹⁾C, control; KC, κ -carrageenan; SPI, soy protein isolate; CS, casein; WPC, whey protein concentrate; EW, egg white

²⁾ ΔE , total color difference based on initial pork color ($L^* = 48.9$, $a^* = 12.4$, $b^* = 9.7$)

^{a-c}Means with different superscripts within same column are significantly different ($p < 0.05$).

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