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The Effects of High Pressure and Various Binders on the Physico-chemical Properties of Restructured Pork Meat

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ABSTRACT : This study was carried out to investigate the effect of high pressure and the addition of non-meat proteins on the physico-chemical and binding properties of restructured pork. Pressurizations were carried out at up to 200 MPa and non-meat proteins used as a binder were isolated soy protein (ISP), sodium caseinate (SC), whey protein concentrate (WPC) and egg white powder (EWP). The pH values of all treatments were affected by the level of pressure. L*-value of all treatments increased significantly ($p<0.05$), while both a*-value and b*-value of all treatments showed a significant decrease ($p<0.05$) with increasing pressure level. Binders could contribute only additive effects on both pH and color of the treatments. It was found that high pressure improved the water binding capacities and binding strength of the treatments. Binders also improved the binding strength of restructured pork. However, SC and WPC had no effect on water binding properties under high pressure. These results indicate that the application of high pressure had more significant effect on restructuring meat than binders. (**Key Words :** High Pressure, Restructured, Non-meat Protein, Binding, Physico-chemical)

INTRODUCTION

The application of high pressure technology has become the subject of renewed interest in the food industry as it offers the opportunity to produce foods of high sensory and nutritional quality with extended shelf-life without the use of additives (Cheah and Ledward, 1996). Since tenderization of meat by high pressure was first proposed by Macfarlane (1973) on pre-rigor meat, numerical research dealt with the effect of high pressure both on quality of pre- or post-rigor meat and meat products, and on functional properties of food proteins were published. Generally, the functional properties of food proteins may be classified into three main groups: (a) hydration properties, dependent upon protein-water interactions which have an important bearing on wettability, swelling, adhesion, dispersibility, solubility, viscosity, water absorption, and water holding; (b) interfacial properties including surface tension, emulsion and foaming characteristics; and (c) aggregation and gelation properties, which are related to protein-protein interactions (Galazka et al., 2000).

Restructuring of meat products enables the use of less valuable meat components to produce high quality meat products at reduced cost (Tsai et al., 1998). Meat binding may be achieved through the formation of gels that set thermally (hot-set) or chemically (cold-set), while several cold-set binding systems have been developed, conventional restructured meat products depend on hot-set binding of myofibrillar proteins that are extracted from meat with the combined effects of salt, phosphate and mechanical action (Boles and Shand, 1998). Proteins derived from a variety of plant and animal resources have potential value as binders in restructured meat products. Many studies have evaluated the effect of non-meat proteins as binder in meat systems (Huang et al., 2001; Sharma et al., 2004). However, limited information is available regarding use of non-meat proteins as binders in meat systems manufactured under high pressure processing. Previous researches deal with non-meat proteins separately, and comparisons among researches are difficult to interpret.

Pressure is able to affect the protein structure, at the secondary, tertiary and quaternary levels, leading in general to protein denaturation, a very well documented field (Silva and Weber, 1993; Balny and Massons, 1993; Silva et al., 2001). Denaturation is a complex process involving intermediate forms such as the molten globule state (Masson and Clery, 1996) leading to non-reversible denaturation, depending on the rate of compression and on the extent of secondary structure rearrangements (Balny and

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Masson, 1993). Thus, the native structure of a protein, i.e. the conformation that displays biological activity, is the result of a delicate balance between stabilizing and destabilizing interactions, within the polypeptide chains (Lullien-Pellerin and Balny, 2002). The quaternary structure of protein, maintained by hydrophobic interaction, is the most sensitive to pressure. Moderate pressure below 150 MPa was found to favor the dissociation of oligomeric proteins. At 150-200 MPa, oligomeric dissociation occurs lower than those at which unfolding of monomers is observed (Silva and Weber, 1993). Pressure above 200 MPa induces unfolding of proteins and reassociation of subunits from dissociated oligomers. Significant tertiary structure changes are observed beyond 200 MPa. However, reversible unfolding of proteins can occur at higher pressure (400 to 800 MPa), showing that the volume and compressibility changes during denaturation are not completely dominated by hydrophobic effects (Lullien-Pellerin and Balny, 2002).

Therefore, objectives of this study were to compare the effects of high pressure and binders on the physicochemical and binding properties of restructured pork meat.

MATERIALS AND METHODS

Materials and sample preparation

Porcine *M. longissimus dorsi* stored for 24 h at 4°C after slaughter was obtained from local abattoir and then was frozen at -18°C for 48 h. Formulae of restructured pork products were manufactured by the method of Tsai et al. (1998). Pork meat was trimmed of visible fat and connective tissue, and then ground through an Ø 8 mm plate. Ground pork was divided into five batches for a control (C) and four non-meat binders, included isolated soy protein (ISP), sodium caseinate (SC), whey protein concentrate (WPC) and egg white powder (EWP), respectively, and mixed with 10 g/kg salt, 3 g/kg sodium tripolyphosphate and 200 g/kg water. Each batch was mixed separately with 20 g/kg binder except for the control. The raw mixture was filled in fibrous casing (4 mm diameter), vacuum packaged with polyethylene bag and stored at 4°C refrigerator for 12 h.

Pressure treatment

High pressure treatments were performed in a high pressure unit manufactured by ourselves as described in our previous study (Hong et al., 2005). The compression fluid was ethylene glycol and pressurization was carried out at 100 and 200 MPa of pressure for 30 min at ambient temperature. After pressurization, all samples were treated thermally at 50°C for 60 min to allow palatable binding strength and to reduce the thermal denaturation of myoglobin, and cooled by running water for 10 min.

Product yield and moisture content

The product yields were determined by weight differences between before and after thermal processing. Moisture content was measured in triplicate by 102°C air drying method according to AOAC (1990).

pH measurement

pH measurements were carried out with a pH meter (Model 440, Corning, Schiphol-Rijk, the Netherlands) on 5 g of sample mixed with 20 ml of water and homogenized at 13,000 rpm for 1 min in a SMT process Homogenizer (SMT Co. Ltd., Tokyo, Japan).

Color measurement

Color measurements were taken with Color meter (JC801S, Color Techno System Co. Ltd., Tokyo, Japan) calibrated with a white standard plate (X = 97.83, Y = 81.58, Z = 91.51). CIE L*, a* and b*-value were determined as indicators of lightness, redness and yellowness, respectively. Three measurements were taken from each surface of the samples.

Water holding capacity

Water holding capacity was determined by the modified method of Pietrasik and Shand (2004). One gram of meat was weighed and placed in a centrifuge tube, along with gauze as absorbents. Samples were centrifuged for 10 min at 3,000 rpm in an automatic refrigerated centrifuge (RC-3, SORVALL Co., CA, USA) at 20°C. After centrifuging, meat was removed and weight centrifuge tube before and after drying. Water holding capacity was expressed as percentage of moisture content to meat.

Cooking loss

Cooking loss was determined by assessing the value of exudation after thermal treatment. Three pork samples from each treatment were weight before and after cooking at 75°C for 30 min, and expressed as percentage of initial weight.

Binding strength

After measurement of cooking loss, the force required to penetrate 10 mm restructured pork slices with a 9 mm diameter brass probe was determined using a digital gauge (DPS-20, IMADA Co., Toyohashi, Japan). Penetration force was expressed as binding strength. The measurement was conducted 12 replicates.

Experimental design and statistical analysis

Completely randomized design was adopted in designing the experiment. Five binders (C, ISP, SC, WPC and EWP)×3 pressure levels (0.1, 100 and 200 MPa) were analyzed by ANOVA using the SAS statistical program

Table 1. Effect of various levels of pressure and binders on pH value[1] of restructured pork meat

Binders[2]	Pressure level		
	0.1 MPa	100 MPa	200 MPa
C	6.08±0.00^{Ab}	6.10±0.01^{Aab}	6.11±0.02^{Aa}
ISP	6.05±0.01^{Bb}	6.06±0.00^{Cab}	6.07±0.01^{Ba}
SC	6.06±0.00^{Bc}	6.08±0.01^{Bb}	6.12±0.00^{Aa}
WPC	6.08±0.02^{Ab}	6.10±0.00^{Aab}	6.11±0.01^{Aa}
EWP	6.02±0.00^{Cb}	6.05±0.01^{Ca}	6.06±0.00^{Ba}

[1] Mean±standard deviation of three replicate determinations.
[2] C: control; ISP: isolated soy protein; SC: sodium caseinate; WPC: whey protein concentrate; EWP: egg white powder.
[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$).
[A-C] Means with different superscripts in the same column are significantly different ($p<0.05$).

(1996), and differences among the means were compared using Duncan's Multiple Range test. Each treatment had three replicate determinations. For binding strength measurement, however, each treatment had 12 replicates.

RESULTS AND DISCUSSION

pH

Table 1 shows the effect of high pressure and added binders on the pH of restructured pork meat. The pH values of all treatments were increased with increased level of pressure and ranged from 6.02 to 6.12. This is probably associated with the denaturation of some protein fraction which is postulated by Angsupanich and Ledward (1998). Although the mechanisms of protein denaturation differ between thermal processing and high pressure processing (Ma and Ledward, 2004), pressure possibly caused a decrement in available acidic groups in meat such as thermal processing (Tsai et al., 1998). In comparison among the treatments, the highest pH was obtained at WPC and the lowest at EWP. These differences remained relatively constant at all samples after high pressure processing. This result can be explained that the addition of binders could contribute additive effects on the changes in pH of samples, but could not prevent the exposure of basic groups or the loss of acidic ones during high pressure processing.

Color

The effect of various levels of pressure and binders on color of restructured pork meat showed in Table 2. L*-value of all treatments increased with increased pressure level ($p<0.05$). Both a* and b*-value of all treatments showed a significant decrease ($p<0.05$) at 100 MPa, as compared to atmospheric pressure. However, no significant differences ($p>0.05$) in a* and b*-value were found with increased pressure. Carlez et al. (1995) noted that L*-values of minced beef increased in the range 200-350 MPa due to globin denaturation or to heme displacement or release,

Table 2. Effect of various levels of pressure and binders on color[1] of restructured pork meat

Binders[2]	Pressure level		
	0.1 MPa	100 MPa	200 MPa
L*-value			
C	59.60±0.37^{BCc}	62.30±0.75^{Bb}	64.40±0.80^{Ba}
ISP	59.00±0.54^{Cc}	60.68±0.63^{Cb}	65.15±0.29^{Ba}
SC	63.30±0.50^{Ab}	64.33±0.40^{Ab}	66.28±0.91^{Aa}
WPC	59.30±0.50^{BCc}	63.48±0.57^{Ab}	64.53±0.53^{Ba}
EWP	60.05±0.70^{Bc}	62.45±0.70^{Bb}	64.60±0.61^{Ba}
a*-value			
C	9.13±0.38^{Aa}	8.35±0.21^{Ab}	8.15±0.37^{Ab}
ISP	8.40±0.84^{Aa}	6.88±0.05^{Bb}	6.60±0.16^{Bb}
SC	7.53±0.22^{Ba}	6.78±0.50^{Bb}	6.73±0.37^{Bb}
WPC	8.58±0.22^{Aa}	7.33±0.62^{Bb}	7.13±0.50^{Bb}
EWP	8.60±0.60^{Aa}	7.30±0.54^{Bb}	7.10±0.50^{Bb}
b*-value			
C	12.35±0.31^{Aa}	10.70±0.79^{BCb}	11.18±0.55^{ABb}
ISP	11.98±0.63^{Aa}	10.50±0.71^{Cb}	10.60±0.72^{Bb}
SC	12.50±0.57^{Aa}	11.85±0.13^{Ab}	11.68±0.10^{Ab}
WPC	12.43±0.57^{Aa}	11.43±0.39^{ABb}	11.18±0.36^{ABb}
EWP	12.60±0.26^{Aa}	11.33±0.19^{ABb}	11.15±0.76^{ABb}

[1] Mean±standard deviation of three replicate determinations.
[2] C: control; ISP: isolated soy protein; SC: sodium caseinate; WPC: whey protein concentrate; EWP: egg white powder.
[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$).
[A-C] Means with different superscripts in the same column are significantly different ($p<0.05$).

while a*-values decreased in the range 400-500 MPa due to oxidation of ferrous myoglobin to ferric metmyoglobin. However, they subjected all samples only for 10 min at each pressure. According to our previous study (Hong et al., 2005), not only pressure level but also holding time had effect on the meat color, although pressure intensity is more significant than holding time as described by Jung et al. (2003). They concluded that the changes in a*-value were correlated with metmyoglobin content. Metmyoglobin formation can be prevented by complete removal of oxygen through vacuum packaging with an oxygen scavenger, or by previous formation of nitrosylmyoglobin, as in processed brined products (Carlez et al., 1995; Goutefongea et al., 1995). Among the treatments, addition of binders preferred the additive effect on restructured pork. The treatments added binders showed a slightly higher L*-value and lower a*-value than control. This result could be corresponded to not only characteristic color of binders, but also moisture content. According to Hong et al. (2004), increase in moisture content of meat product resulted in lighter color, and agreed with the current study.

Water binding properties

The product yields of treatments at atmospheric

Table 3. Effect of various levels of pressure and binders on product yield (%)[1] of restructured pork meat

Binders[2]	Pressure level		
	0.1 MPa	100 MPa	200 MPa
C	90.63±0.25^{Ba}	88.64±0.80^{Bb}	87.54±0.42^{Bc}
ISP	91.92±0.41^{Ba}	88.95±1.12^{Bb}	88.05±1.13^{ABb}
SC	91.76±0.83^{Aa}	90.96±0.50^{Aab}	89.75±1.15^{Ab}
WPC	91.66±0.10^{Aa}	90.62±0.24^{Ab}	89.13±0.06^{ABc}
EWP	92.47±0.23^{Aa}	90.88±0.79^{Ab}	89.06±0.65^{ABc}

[1] Mean±standard deviation of three replicate determinations.
[2] C: control; ISP: isolated soy protein; SC: sodium caseinate; WPC: whey protein concentrate; EWP: egg white powder.
[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$).
[A-C] Means with different superscripts in the same column are significantly different ($p<0.05$).

Table 4. Effect of various levels of pressure and binders on water binding properties[1] of restructured pork meat

Binders[2]	Pressure level		
	0.1 MPa	100 MPa	200 MPa
Moisture content (%)			
C	72.79±0.39^{Aa}	72.43±0.31^{Aa}	72.87±0.32^{Aa}
ISP	71.68±0.27^{Ba}	71.28±0.31^{Ba}	71.24±0.36^{Ca}
SC	72.79±0.26^{Aa}	72.44±0.11^{Aa}	72.44±0.08^{Ba}
WPC	72.68±0.16^{Aa}	72.67±0.24^{Aa}	71.55±0.15^{Cb}
EWP	72.43±0.13^{Aa}	71.53±0.18^{Bb}	71.35±0.07^{Cb}
Water holding capacity (%)			
C	79.44±1.78^{Bb}	81.68±1.27^{Bb}	87.26±0.78^{Aa}
ISP	83.03±1.37^{Ab}	85.24±1.37^{Aab}	87.69±1.21^{Aa}
SC	81.75±0.52^{ABc}	83.34±1.03^{ABb}	85.51±0.65^{Aa}
WPC	80.93±1.95^{ABb}	82.26±1.51^{ABb}	86.76±1.18^{Aa}
EWP	82.36±1.98^{ABb}	84.68±1.34^{ABab}	86.51±2.02^{Aa}
Cooking loss (%)			
C	29.09±0.59^{Aa}	25.67±0.10^{Ab}	19.22±0.88^{Ac}
ISP	24.93±0.70^{Da}	21.54±0.48^{Cb}	17.89±0.25^{Bc}
SC	26.03±0.72^{Ca}	23.27±0.76^{Bb}	20.21±0.51^{Ac}
WPC	27.43±0.54^{Ba}	23.71±0.66^{Bb}	20.00±0.68^{Ac}
EWP	26.52±0.27^{BCa}	21.31±0.34^{Cb}	17.72±0.14^{Bc}

[1] Mean±standard deviation of three replicate determinations.
[2] C: control; ISP: isolated soy protein; SC: sodium caseinate; WPC: whey protein concentrate; EWP: egg white powder.
[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$).
[A-C] Means with different superscripts in the same column are significantly different ($p<0.05$).

pressure varied from 90.63 to 92.47% (Table 3). High pressure caused significant decrease ($p<0.05$) in product yield from 1 to 4%, which related with moisture content. Table 4 showed the effect of high pressure and binders on the water binding properties of restructured pork. Moisture contents of restructured pork ranged from 71 to 73%. High pressure treatment showed a slightly moisture loss and this loss possibly contributed to product yield of samples. In the current study, high pressure treatments were hold in pressure vessel at ambient temperature for 30 min, leading more moisture loss than atmospheric pressure, probably due to adiabatic heat generation (Kalichevsky et al., 1995). In contrast to product yield, high pressure treatments had higher water holding capacity than non-pressurized treatments. According to Macfarlane et al. (1984) pressure acted by disrupting divalent cation-protein bonds through electrostriction effect. Upon pressure release, the probability of salt bridges reforming would be reduced because of changes in protein conformation due to the treatment and to NaCl added. As a result, increases in water holding capacity and protein solubility could persist after pressure release, and agreed with the current study. The improvement in water holding capacity correlated with a decrease in cooking loss. Non-pressurized samples had significantly higher cooking loss ($p<0.05$) than high pressure treated samples. Among the treatments, the addition of binders showed an improvement in both water holding capacity and cooking loss at up to 100 MPa. However, no significant differences ($p>0.05$) in water holding capacity among the treatments were found at 200 MPa. Furthermore, treatments with SC and WPC did not differ significantly ($p>0.05$) in cooking loss with the comparison of C. No effects on functional properties of milk proteins at 200 MPa might be associated with excessive protein damage reflected as increased surface hydrophobicity, less protein-water interactions and thus lower water-binding properties (Uresti et al., 2004). The increase on surface hydrophobicity in pressure treated samples has been reported previously (Ishizaki et al., 1995). Ngarize et al. (2005) also noted that pressure-treated whey protein gel tended to lose water easily upon compression due to the presence of large aggregates with irregular pores as shown by transmission electron microscopy. In consequence, the addition of non-meat protein had no effect on the water binding properties at 200 MPa, probably due to increase in surface hydrophobicity (Ishizaki et al., 1995; Galazka et al., 2000) or protein cross-linking (Totosaus et al., 2002).

Binding strength

The effect of high pressure and added binders on the binding strength has shown in Table 5. The binding strengths of all treatments increased significantly ($p<0.05$) with increasing pressure level. This result indicated that the presence of more protein-protein interactions caused from high pressure might be responsible for the higher values of binding strength. The same results were obtained by Macfarlane et al. (1984) who found that pressure treatment at up to 150 MPa has been shown to also increase the binding achieved between meat particles in patties where they are subsequently cooked. They concluded that the increase in binding strength depended on the pressure level

Table 5. Effect of various levels of pressure and binders on binding strength (N)[1] of restructured pork meat

Binders[2]	Pressure level		
	0.1 MPa	100 MPa	200 MPa
C	3.75±0.54^{Bc}	6.98±1.06^{Bb}	8.59±1.18^{Ca}
ISP	4.88±0.64^{Ac}	9.41±1.29^{Ab}	11.49±1.05^{Aa}
SC	4.61±0.49^{Ac}	6.99±0.75^{Bb}	10.24±1.39^{Ba}
WPC	2.80±0.37^{Cc}	7.64±1.19^{Bb}	10.65±0.81^{ABa}
EWP	3.60±0.58^{Bc}	8.83±0.90^{Ab}	10.14±1.10^{Ba}

[1] Mean±standard deviation of 12 replicate determinations.

[2] C: control; ISP: isolated soy protein; SC: sodium caseinate; WPC: whey protein concentrate; EWP: egg white powder.

[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$).

[A-C] Means with different superscripts in the same column are significantly different ($p<0.05$).

and holding time, and NaCl concentration and pH of the patty. With the comparison of binders, WPC had significantly lower binding strength ($p<0.05$) than treatment at atmospheric pressure. Binding strength of all treatments, however, increased gradually and significantly higher ($p<0.05$) in all treatments added binders at 200 MPa than C. ISP showed the highest binding strength ($p<0.05$), probably due to eight cysteine residues and six disulfide bonds (Visschers and de Jongh, 2005). The result indicated that the application of high pressure up to 200 MPa combined with both thermal processing and added salt conferred the palatable binding properties on restructured meat products and that the addition of ISP had synergistic effect such as other meat products.

IMPLICATIONS

In general, the application of high pressure contributes to improve the functionalities of meat protein. In the current study, high pressure allowed the lower thermal processing than traditional restructured meat product. Low thermal processing combined with high pressure allowed fresh-like meat color which was one of the problems in hot-set restructuring, while they showed some discolorization induced by high pressure, treatments becoming pink. In addition, high pressure with thermal processing could achieve the palatable binding strength in restructured meat product. The addition of non-meat protein affected binding strength. However, milk proteins such as WPC and SC showed a poor water binding properties under high pressure. Therefore, these results indicate that the application of high pressure has more potential benefit than non-meat binders in restructured meat product, and further investigations are needed.

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