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ARTICLE

Determining the Optimal Level of Natural Calcium Powders and Whey Protein Concentrate Blends as Phosphate Replacers in Cooked Ground Pork Products

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Abstract This study was conducted to investigate the effects of the addition levels of a phosphate replacer blend in ground pork sausages. The phosphate replacer consisted of 0.2% oyster shell calcium powder, 0.3% egg shell calcium powder, and 0.25% whey protein concentrate. Depending on the presence or absence of synthetic phosphate and the addition level of phosphate replacer, the following products were processed: control (+) (0.3% phosphate), control (–) (non-phosphate), 20AL (20% replacer), 40AL (40% replacer), 60AL (60% replacer), 80AL (80% replacer), and 100AL (100% replacer). The pH values of pork sausages increased ($p<0.05$) with increasing addition level of the phosphate replacer. When more than 40% of the phosphate replacer was added to pork samples (40AL, 60AL, 80AL, and 100AL), cooking loss was significantly reduced compared to both the control (+) and control (–). However, no significant differences were observed in the moisture content and CIE L* values between the controls and the treatments with a phosphate replacer. The control (+) and 100AL treatment had the highest ($p<0.05$) hardness, but the samples with the phosphate replacer were not significantly different in cohesiveness and springiness from the control (+). As addition level increased, the gumminess and chewiness of the products with the phosphate replacer increased, which were lower than those of the control (+). Therefore, more than 40% of a phosphate replacer may possibly substitute synthetic phosphate to improve product yields in ground pork sausages, although further studies may be needed for improving the textural properties of the final products.

Keywords ground pork products, oyster shell powder, egg shell powder, whey protein concentrate, phosphate replacer

Introduction

Phosphates are commonly used in the meat and poultry processing for product improvement (Lamkey, 1998). They are essential for increasing the water holding capacity of meat proteins, allowing them to bind and retain water, resulting in improved cooking yields and texture (Long et al., 2011; Sebranek, 2009; Xiong, 2005).

Further, phosphates also function as antioxidants, antimicrobials, and buffering agents (Petracci et al., 2013). Despite these multifunctional benefits, the use of phosphates has dropped in the last decade because of poor consumer perception associated with health risks (Kim et al., 2017; Petracci et al., 2013; Watanabe et al., 2016). Therefore, recent years has witnessed an increase in efforts to find phosphate substitutes from natural sources (Casco, 2013; Cho et al., 2017; Jarvis et al., 2012). Ruusunen et al. (2003) investigated the physical and sensory properties of low-salt phosphate-free frankfurters prepared by using amounts of modified tapioca starch, sodium citrate, and wheat bran at different salt and fat levels. They found that modified tapioca starch and sodium citrate can decrease the frying loss and improve water and fat biding in frankfurters. Lee et al. (2011) reported that pork sausages treated with 0.5% oyster shell powder had improved water holding capacity and cooking loss and had better textural properties than the control. Cho et al. (2017) determined the optimal ratio of oyster shell and egg shell calcium as synthetic phosphate replacers in pork products. They suggested that the combination of 0.2% oyster shell calcium and 0.3% egg shell calcium, rather than the addition of each calcium alone, could provide phosphate-free pork products with lower cooking loss and desirable textural qualities. Whey protein concentrate has been used in comminuted and emulsified meat products to increase water holding capacity and emulsion stability, improve textural and gelling properties, and enhance nutritional values (El-Magoli et al., 1996; Hughes et al., 1998; Xiong, 2009). Choi et al. (2014) had reported that the addition of 0.5% oyster calcium powder and 0.5% whey protein decreased the cooking loss and improved the texture profile in restructured hams. Cho and Jeong (2018) investigated the combined effects of natural calcium mixtures and various nonmeat ingredients to produce phosphate-free pork products. They suggested that a combination of natural calcium mixtures (0.2% oyster calcium and 0.3% egg shell calcium) with 0.25% whey protein concentrate or 0.25% collagen powder could be a suitable treatment. However, in order to potentially apply their combination as a phosphate replacer in the meat processing industry, a suitable level of addition in comparison with synthetic phosphate should be investigated.

Therefore, this study was conducted to identify the optimal level of a blend of natural calcium powders and whey protein concentrate in ground pork products, with the aim of industrial application.

Materials and Methods

Preparation and processing of pork sausage

Raw pork ham (*M. biceps femoris*, *M. semitendinosus*, and *M. semimembranosus*) was purchased from a local meat processor (Pukyung Pig Farmers Livestock Cooperatives, Korea) at 24–48 h postmortem. After removing subcutaneous and intermuscular fat and visible connective tissues from the fresh ham, lean pork meat was cut in squares of 4–5 cm, vacuum-packaged in nylon/PE film bags, and stored in a freezer at −18℃ until processing. Pork back fat was prepared in the same manner. A phosphate blend (mixture of sodium tripolyphosphate, tetrasodium pyrophosphate, and sodium hexametaphosphate) (Polymix-CS, SDBNI Co. Ltd., Korea) was used as the synthetic phosphate. The egg shell calcium (egg shell calcium 50, Essentron Co. Ltd., Korea), oyster shell calcium (oyster shell calcium 40, JK Biochem Co. Ltd., Korea) were purchased from commercial suppliers. According to the supplier information, these natural calcium powders are prepared from by-products generated during the processing of egg and oyster products, which are generally obtained by washing with water, drying or calcining between 800℃ and 1,000℃, and then milling. Oyster shell calcium used in this study contained 39.6% calcium and less than 0.1% magnesium, sodium, iron, and potassium. Egg shell calcium had 51.6% calcium, 0.4% magnesium, 0.2% sodium, and less than 0.1% iron and potassium (Cho et al., 2017). Whey protein concentrate (WPC, TOF Co. Ltd., Korea) was also prepared. The formulation of ground pork sausages with the calcium powders and whey protein concentrate is presented in Table 1.

Sample preparation was performed using a modified version of the protocol by Cho et al. (2017). The frozen pork ham and back fat were thawed at 2℃–3℃ for 24–36 h before processing. The thawed raw materials were ground in a chopper (TC-22 Elegant plus, Tre Spade, Italy) with an 8-mm plate and then again with a 3-mm plate. The control (–) was prepared by mixing ground pork meat and back fat with ice water and salt (1.5%). In the control (+), synthetic phosphate blend (0.3%) was added to the ground meat mixtures in a mixer (KitchenAid 5K5SS, Whirlpool Corp., USA) for 5 min. A blend of the phosphate replacer was prepared; it comprised 0.2% oyster shell calcium, 0.3% egg shell calcium, and 0.25% whey protein concentrate. Depending on the addition level (AL), the treatments were processed by adding 20% (20AL), 40% (40AL), 60% (60AL), 80% (80AL), or 100% (100AL) of the phosphate replacer blend prepared, along with ice water and salt (1.5%), to the ground pork meat and back fat (Table 1). The meat mixtures from each batch were stuffed into conical tubes (50 g each) using a stuffer (MOD.5/V Deluxe, Tre Spade, Italy). The stuffed tubes were centrifuged at 2,000×g for 10 min in a centrifuge (FELTA5, Hanil Scientific Inc., Korea) to remove air pockets. The tubes were then transferred to a 90℃ water bath (CB60L, Dong Won Scientific System, Korea) and cooked to an internal temperature of 75℃. Changes in temperature were monitored by inserting a K-type thermocouple attached to a digital thermometer (TES-1384, Ketech Scientific Instrument Co., Ltd., Taiwan) into the center of the samples. Immediately after cooking, the samples were cooled on ice slurry for 20 min and stored overnight at 2℃–3℃ before analysis. All experiments were performed in triplicate.

Determination of pH values

Five grams of the samples was weighed before and after cooking and homogenized with 20 mL of distilled water for 1 min in a homogenizer (DI 25 basic, IKA®-Werke GmbH & Co. KG, Germany). The pH was measured using a pH meter (Fisher Scientific Accumet AB150, Thermo Fisher Scientific, Inc., Singapore).

Determination of moisture content

The moisture content of the cooked products was measured using the drying method (AOAC, 2007).

Table 1. Formulations for ground pork sausages treated with a blend of natural calcium powders and whey protein concentrate

Materials (%)	Treatments[1)]						
	Control (+)	Control (–)	20AL	40AL	60AL	80AL	100AL
Ground pork	68.20	68.50	68.35	68.20	68.05	67.90	67.75
Back fat	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Ice	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Salt	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Phosphate blend	0.30	-	-	-	-	-	-
Oyster shell calcium	-	-	0.04	0.08	0.12	0.16	0.20
Egg shell calcium	-	-	0.06	0.12	0.18	0.24	0.30
Whey protein concentrate	-	-	0.05	0.10	0.15	0.20	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

1) The phosphate replacer consists of 0.2% oyster calcium powder, 0.3% egg shell calcium powder, and 0.25% whey protein concentrate. Control (+), 0.3% phosphate blend; Control (–), non-phosphate; 20AL, 20% replacer; 40AL, 40% replacer; 60AL, 60% replacer; 80AL, 80% replacer; 100AL, 100% replacer.

Determination of cooking loss

The cooking loss was determined using the following equation:

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

Instrumental color measurement

CIE L*a*b* values were measured on freshly cut surfaces of each cooked sample after cutting using a colorimeter (Chroma Meter CR-400, illuminant C; Konica Minolta Sensing Inc., Japan). The colorimeter was standardized against a white calibration plate (CIE L*=+94.90, a*=−0.39, and b*=+3.88).

Texture profile analysis

After cutting the samples to a width of 2.5 cm perpendicular to the longitudinal axis, their hardness, springiness, cohesiveness, gumminess, and chewiness were measured using a texture analyzer (TA-XT2*i*, Stable Micro System, UK) equipped with a 50-mm-diameter aluminum cylinder (Bourne, 1978). The cross-head speed for measurement was 5 mm/s.

Statistical analysis

All experiments were performed in triplicate. Statistical analyses were performed using the generalized linear model (GLM) procedure of the SAS package program (SAS, 2013). The significance of the differences between the means was analyzed using Duncan's multiple range test ($p<0.05$).

Results and Discussion

pH values, moisture content, cooking loss, and instrumental color

Before and after cooking, the pH values of all treatments were higher ($p<0.05$) than the control (+), while the control (−) had the lowest pH values ($p<0.05$) (Table 2). The pH values increased ($p<0.05$) with the level of added phosphate replacer. These results may be due to the addition of egg shell calcium and oyster shell calcium powder, as reported in previous studies (Cho et al., 2017; Cho and Jeong, 2018). However, no differences ($p>0.05$) in the moisture content were observed between the controls and the treatments (Table 2). As expected, the cooking loss was highest ($p<0.05$) in control (–) (Table 2). Compared with control (+), treatments with more than 40% phosphate replacer (40AL, 60AL, 80AL, and 100AL) had lower cooking loss. Higher pH of the meat system can lead to better water retention, thereby improving cooking yield. In fact, this has been considered the primary function of added phosphates (Sebranek, 2009, 2015; Trout and Schmidt, 1983). In addition, there were no differences ($p>0.05$) in cooking loss between 60AL, 80AL, and 100AL treatments. Therefore, adding more than 40% phosphate replacer blend to pork products could be an effective method for reducing cooking loss. CIE L* values were not different ($p>0.05$) between the control (+) and the treatments (Table 2). However, the treatments showed significantly redder colors (higher CIE a* values; $p<0.05$) than the control (+). This may be due to the high pH of the phosphate replacer tested. According to Trout (1989), the increase in pH resulted in increased pinkness of the cooked meat products. However, the effects of the level of phosphate replacer blend on the CIE a* values were not consistent among the different treatments. The CIE b* values of 60AL and 80AL treatments were lower ($p<0.05$) than those of control (+) and

Table 2. pH values, moisture contents, cooking loss, and CIE color values of ground pork sausages treated with a blend of natural calcium powders and whey protein concentrate

Traits	Treatments[1)]						
	Control (+)	Control (–)	20AL	40AL	60AL	80AL	100AL
pH (uncooked)	6.42±0.01^{F}	6.23±0.02^{G}	6.51±0.00^{E}	6.73±0.01^{D}	6.95±0.01^{C}	7.16±0.03^{B}	7.43±0.02^{A}
pH (cooked)	6.69±0.01^{E}	6.60±0.02^{F}	6.57±0.02^{F}	6.80±0.02^{D}	6.96±0.02^{C}	7.19±0.03^{B}	7.53±0.02^{A}
Moisture content (%)	69.70±0.12^{A}	68.25±0.03^{A}	68.19±0.04^{A}	67.93±0.10^{A}	68.81±0.12^{A}	68.52±0.04^{A}	67.64±0.07^{A}
Cooking loss (%)	2.24±0.28^{C}	7.03±0.15^{A}	3.29±0.09^{B}	1.91±0.11^{C}	1.43±0.10^{D}	1.36±0.14^{D}	1.15±0.08^{D}
CIE L*	71.17±0.05^{ABC}	71.97±0.81^{A}	71.77±0.39^{AB}	70.46±0.14^{BC}	70.10±0.27^{C}	70.86±0.53^{ABC}	71.15±0.27^{ABC}
CIE a*	3.29±0.01^{D}	5.23±0.32^{C}	5.32±0.07^{BC}	5.17±0.07^{C}	6.33±0.16^{A}	5.84±0.15^{AB}	5.30±0.28^{BC}
CIE b*	8.95±0.06^{AB}	9.22±0.18^{A}	9.32±0.20^{A}	8.66±0.06^{B}	7.85±0.15^{C}	8.07±0.16^{C}	8.96±0.24^{AB}

[1)] The phosphate replacer consists of 0.2% oyster calcium powder, 0.3% egg shell calcium powder, and 0.25% whey protein concentrate. Control (+), 0.3% phosphate blend; Control (–), non-phosphate; 20AL, 20% replacer; 40AL, 40% replacer; 60AL, 60% replacer; 80AL, 80% replacer; 100AL, 100% replacer.
All values are presented as means±standard error of triplicates.
$^{A–G}$ Means within the same row with different superscript letters are significantly different ($p<0.05$).

control (–); however, 100AL treatment had a CIE b* value similar ($p>0.05$) to that of both the controls. This result was consistent with that of Cho and Jeong (2018), who found that CIE b* values of products treated with oyster shell calcium, egg shell calcium, and whey protein concentrate were not different from that of the control treated with phosphate.

Textural properties

The textural properties of ground pork products treated with a blend of natural calcium powders and whey protein concentrate are shown in Table 3. The hardness of pork products increased ($p<0.05$) with the level of the added phosphate replacer blend. The 100AL treatment had the highest ($p<0.05$) hardness, which was similar ($p>0.05$) to that of the control (+). In addition, all treatments (except for 20AL) were harder ($p<0.05$) than the control (–). Lee et al. (2011) reported that emulsified sausages treated with oyster shell powder (0.15%, 0.3%, and 0.5%) had significantly higher hardness than the controls with or without phosphate. Cho et al. (2017) found that pork products formulated with oyster shell or egg shell

Table 3. The textural properties of ground pork sausages treated with a blend of natural calcium powders and whey protein concentrate

Traits	Treatments[1)]						
	Control (+)	Control (–)	20AL	40AL	60AL	80AL	100AL
Hardness (kg)	6.41±0.13^{A}	5.66±0.07^{DE}	5.42±0.10^{E}	5.72±0.14^{CD}	6.01±0.09^{CB}	6.04±0.11^{B}	6.33±0.55^{A}
Springiness	0.93±0.00^{A}	0.88±0.00^{B}	0.91±0.01^{A}	0.92±0.01^{A}	0.91±0.01^{A}	0.92±0.01^{A}	0.92±0.01^{A}
Cohesiveness	0.74±0.00^{A}	0.58±0.01^{B}	0.72±0.00^{A}	0.72±0.00^{A}	0.71±0.00^{A}	0.72±0.00^{A}	0.70±0.00^{A}
Gumminess (kg)	4.75±0.09^{A}	3.30±0.10^{E}	3.90±0.07^{D}	4.12±0.09^{CD}	4.28±0.05^{BC}	4.33±0.08^{BC}	4.45±0.06^{B}
Chewiness (kg)	4.43±0.09^{A}	2.92±0.09^{E}	3.55±0.06^{D}	3.79±0.08^{C}	3.92±0.05^{BC}	3.97±0.08^{BC}	4.08±0.07^{B}

[1)] The phosphate replacer consists of 0.2% oyster calcium powder, 0.3% egg shell calcium powder, and 0.25% whey protein concentrate. Control (+): 0.3% phosphate blend; Control (–): non-phosphate; 20AL: 20% replacer; 40AL: 40% replacer; 60AL: 60% replacer; 80AL: 80% replacer; 100AL: 100% replacer.
All values are presented as means±standard error of triplicates.
$^{A–E}$ Means within the same row with different superscript letters are significantly different ($p<0.05$).

calcium alone showed significantly lower hardness than the control with phosphate; the combination of both natural calcium powders was also effective in improving textural properties. In a recent study, Cho and Jeong (2018) suggested that the combined use of calcium powders and whey protein concentrate or collagen powder could improve the hardness of phosphate-free pork products. However, there were no differences ($p>0.05$) in springiness between the control (+) and the treatments, which had higher ($p<0.05$) springiness than the control (–). The cohesiveness of the samples showed similar trends as springiness. As gumminess and chewiness are secondary parameters influenced by the hardness (Bourne, 1978), they follow the general trends of hardness; our results were consistent with this (Table 3). Gumminess and chewiness of pork products increased with increasing levels of the phosphate replacer blend. All treatments had lower gumminess and chewiness than the control (+) and higher gumminess and chewiness than the control (–). Choi at al. (2014) had reported that adding 0.5% oyster shell calcium powder could increase chewiness and springiness of restructured pork hams, compared to controls treated with phosphate. Cho and Jeong (2018) obtained similar results for gumminess and chewiness in pork products treated with calcium powders and whey protein concentrate. Overall, the use of natural calcium powders and whey protein concentrate as phosphate alternatives could effectively improve some textural properties of the final products, although alternate treatments may be necessary to achieve desirable levels of gumminess and chewiness.

Conclusions

We found that the addition of different levels of a phosphate replacer blend formulated with natural calcium powders and whey protein concentrate had noticeable effects on the quality properties, including pH, cooking loss, hardness, springiness, and cohesiveness, of phosphate-free pork products. However, textural properties such as gumminess and chewiness of the products treated with the phosphate replacer blend were less desirable than those of the phosphate-treated controls. We conclude that using more than 40% of our phosphate replacer blend could provide final products with desirable qualities, although further investigations would be necessary for exploring methods for complementing the gumminess and chewiness.

Conflicts of Interest

The authors declare no potential conflict of interest.

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