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Effects of Nitrite and Phosphate Replacements for Clean-Label Ground Pork Products

Abstract

We investigated the effects of different phosphate replacements on the quality of ground pork products cured with sodium nitrite or radish powder to determine their potential for achieving clean-label pork products. The experimental design was a 2 × 5 factorial design. For this purpose, the ground meat mixture was assigned into two groups, depending on nitrite source. Each group was mixed with 0.01% sodium nitrite or 0.4% radish powder together with 0.04% starter culture, and then processed depending on phosphate replacement (with or without 0.5% sodium tripolyphosphate; STPP (+), STPP (−), 0.5% oyster shell calcium (OSC), 0.5% citrus fiber (CF), or 0.5% dried plum powder (DPP)). All samples were cooked, cooled, and stored until analysis within two days. The nitrite source had no effect on all dependent variables of ground pork products. However, in phosphate replacement treatments, the STPP (+) and OSC treatments had a higher cooking yield than the STPP (−), CF, or DPP treatments. OSC treatment was more effective for lowering total fluid separation compared to STPP (−), CF, or DPP treatments, but had a higher percentage than STPP (+). The STPP (+) treatment did not differ from the OSC or CF treatments for CIE L* and CIE a* values. Moreover, no differences were observed in nitrosyl hemochrome content, lipid oxidation, hardness, gumminess, and chewiness between the OSC and STPP (+) treatments. In conclusion, among the phosphate replacements, OSC addition was the most suitable to provide clean-label pork products cured with radish powder as a synthetic nitrite replacer.

Keywords: nitrite replacement, phosphate replacement, radish powder, pork products, clean-label

Introduction

As a curing ingredient in meat products, nitrite plays a key role in curing meat color, while conferring antimicrobial and antioxidant protection, and a curing flavor (Alahakoon et al., 2015¸ Pegg and Shahidi, 2000). Despite the benefits of nitrite in meat curing, increasing consumer awareness of health-related risks associated with synthetic food additives (Hur et al., 2015) has boosted the demand for ‘clean-label products,’ such as organic, eco-friendly, and synthetic additive-free products (Maruyama et al., 2021; Yong et al., 2021). In response to this need, the meat industry uses pre-conversion of nitrite from vegetable powders or nitrate-rich vegetable sources together with starter culture is applied to meat products (Jeong, 2016).

Celery juice powder, which is widely used, is a feasible alternative to synthetic nitrite. However, excessive addition of celery juice or powder affects the sensory properties of the products negatively (Alahakoon et al., 2015), and may cause allergic reactions (Ballmer-Weber et al., 2002). Therefore, other natural sources, such as vegetables, fruits, and their by-products, have been studied as alternative nitrite sources. Thus, Riel et al. (2017) found that the addition of parsley-extract powder to mortadella sausages produced a redness similar to that obtained by addition of synthetic nitrite. Similarly, Šojića et al. (2020) reported that a mixture of tomato peel extract and peppermint oil could be used for partial replacement of sodium nitrite in pork sausages. Moreover, testing different vegetable (Chinese cabbage, radish, and spinach) powders for nitrite substitution, Jeong et al. (2020) found that the use of radish powder conferred similar qualities to those obtained by the addition of synthetic nitrite, suggesting its potential as a substitute for synthetic nitrite. However, for 'clean-label' meat products, other challenges are faced, and solutions to replace synthetic phosphates are emerging in the meat industry (Thangavelu et al., 2019).

Phosphate is widely used for meat production because of its many functions, including increasing water-holding capacity, inhibiting lipid oxidation, and improving textural and sensory attributes (Long et al., 2011; Thangavelu et al., 2019). Recognized as a GRAS (Generally Recognized as Safe) substance by the FDA (Food and Drug Administration), phosphate can be added at a concentration of 0.5% or less of the final meat products (USDA-FSIS, 2015). With respect to replacing synthetic phosphates, the use of calcium powders from natural sources (Bae et al., 2017; Cho et al. 2017), polysaccharides (Meyer, 2018; Öztürk-Kerimoğlu and Serdaroğlu, 2019), amino acids (Kim et al., 2014), protein hydrolyzates (Shahidi and Synowieki, 1997; Vann and DeWitt; 2007), dietary fiber (Powell et al., 2019; Magalhães et al., 2020), and mushrooms (Choe et al., 2018), has been tested. Thus, Bae et al. (2017) reported that pork meat products containing oyster shell calcium had a texture similar to that of obtained upon sodium tripolyphosphate treatment. Fernández-Ginés et al. (2003) reported that Bologna sausages treated with citrus fiber had a cooking yield and emulsion stability similar to those of products added with sodium tripolyphosphate. Similarly, Jarvis et al. (2012) confirmed that chicken breast fillets marinated by combining plum powder and plum fiber showed similar quality characteristics to those obtained upon marinating with sodium tripolyphosphate. Although several studies have reported effective replacement of synthetic nitrite and phosphate, studies on the replacement of synthetic phosphate in naturally cured meat products with a vegetable powder have not been reported.

Therefore, in this study we compared oyster shell calcium, citrus fiber, and dried plum powder as candidate natural phosphate sources for phosphate replacement in meat products cured with either sodium nitrite or with radish powder as a natural nitrite alternative, aiming to contribute to the development of clean-label meat production.

Materials and Methods

Preparation of radish powder and other materials

Fresh radishes (*Raphanus sativus* L.) grown in South Korea were purchased and randomly selected to manufacture radish powder. Radish powder was prepared after subsequent washing, homogenizing, drying, and powdering as previously described by Bae et al. (2020). Then, powdered samples were vacuum-packed and stored at −18°C until further use. To standardize the nitrate content (32,000 ppm) from each batch, the radish powder was mixed with maltodextrin (#186785579, ESfood, Gunpo, Korea) before processing the meat products.

A starter culture (Bactoferm® CS-300, CHR Hansen, Pohlheim, Germany) comprising *Staphylococcus carnosus* and *Staphylococcus carnosus* subsp., sodium nitrite (S225, Sigma-Aldrich, St. Louis, MO, USA), sodium tripolyphosphate (238503, Sigma-Aldrich, St. Louis, MO, USA) , sodium chloride (S-3160-65, Fisher Scientific UK, Loughborough, UK), sodium ascorbate (#35268, Acros Organics, Geel, Belgium), and dextrose (A16828, Thermo Fisher Scientific, Heysham, UK) were purchased from commercial suppliers. As alternatives to synthetic phosphate, oyster shell calcium (Glucan Corp., Jinju, Korea), citrus fiber (CF-100, Fiberstar, Inc., River Falls, WI, USA), and dried plum powder (#80276308572, Sunsweet Growers Inc., Yuba City, CA, USA) were obtained.

Preparation of ground pork products

Fresh pork ham and back fat were purchased from a local market. After trimming intermuscular fat and visible connective tissues, the lean pork meat and back fat were stored at −18°C until processing within one month. Frozen materials (total batch size of 35 kg per trial) were completely thawed and then ground using a chopper (TC-22 Elegnant plus, Tre Spade, Torino, Italy) equipped with a 3-mm plate. Ground mixtures were randomly divided into ten portions and assigned to two groups (five batches each) depending on the nitrite source (Table

1). First, 70% pork meat and 15% back fat were mixed for 3 min with 0.01% sodium nitrite or 0.4% radish powder and 0.04% starter culture in a mixer (5K5SS, Whirlpool, St. Joseph, MI, USA). Second, each group was processed depending on phosphate replacement, including with or without 0.5% sodium tripolyphosphate (STPP) or 0.5% phosphate replacement (oyster shell calcium; OSC, citrus fiber; CF, and dried plum powder; DPP). Other ingredients (1.5% sodium chloride, 1% dextrose, and 0.05% sodium ascorbate; total meat mixture basis) along with 15% ice/water were added to a mixer and mixed again for 7 min. The treatments were filled into 50 mL conical tubes. Five batches of sodium nitrite were placed in a refrigerator at 4°C for 1 h. The remaining five batches of radish powder and starter culture were stored in an incubator at 40°C for 2 h to allow the conversion of nitrate to nitrite. All samples were cooked to 75°C in a water bath (MaXturdy 45, Daihan Scientific, Wonju, Korea) at 90°C. Once cooking, the samples were cooled for 20 min in ice slurry and stored overnight at 2–3°C in the dark until analysis. Experiments were performed in triplicate, and all dependent variables were measured in duplicate.

Determination of pH and cooking yield

The pH was measured with a pH meter (Accumet AB150, Thermo Fisher Scientific, Inc., Singapore) after adding 25 mL of distilled water to a 5 g sample and homogenized (DI-25 basic, IKA®-Werke Gmb & Co. KG, Staufen, Germany). Five samples per each batch were weighed before cooking and after cooking and cooling overnight. Cooking yield was calculated as follows: [cooked sample weight/raw sample weight] × 100.

Total fluid, lipid, and water separation

Total fluid, lipid, and water separation of ground pork products was measured by the method described by Hughes et al. (1997) and Lee et al. (2008). Twenty grams of the uncooked meat mixture was placed into a 50-mL conical tube with a mesh. After weight measurement, the conical tubes filled with the samples were cooked for 30 min in a water bath at 75°C (CB60L, Dongwon Scientific Instrument, Busan, Korea), cooled for 20 min, and centrifuged at 500 × *g* for 5 min. Pellets and supernatants in the conical tubes were weighed before drying. The supernatant was dried for 18 h at 105°C using a dryer (ON-12GW; Jeio Tech Co. Ltd., Daejeon, Korea) and weighed again. The percentage total fluid separation (TFS), lipid separation (LS), and water separation (WS) were calculated using the following equations:

$$\% \text{ TFS} = \frac{\text{Weight of sample before cooking (g)} - \text{Weight of pellet after cooking and centrifuging (g)}}{\text{Weight of sample before cooking (g)}} \times 100$$

$$\% \text{ LS} = \frac{\text{Weight of dried supernatant (g)}}{\text{Weight of sample before cooking (g)}} \times 100$$

$$\% \text{ WS} = \% \text{ TFS} - \% \text{ LS}$$

Color measurements

After cutting the samples in the longitudinal direction, the cut surfaces of samples were measured for CIE (the International Commission on Illumination) L*a*b* values using a colorimeter (CR-400, 8 mm aperture, illuminant C, 2° standard observer; Konica Minolta Sensing Inc., Osaka, Japan) after calibrating the standard plate (L* 94.87, a* −0.39, b* 3.88). Two readings were recorded on each cut surface for each pork sausage immediately after cutting.

Nitrosyl hemochrome and 2-thiobarbituric acid-reactive substances (TBARS)

determination

Nitrosyl hemochrome in pork products was measured using the method described by Hornsey (1956). After extraction and filtration, absorbance of the filtrate was determined at 540 nm (A_{540}) using a spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). Nitrosyl hemochrome concentration (ppm) was calculated by multiplying absorbance (A_{540}) by 290. TBARS values was measured using the method described by Tarladgis et al. (1960). Briefly, after reacting malondialdehyde (MDA) in samples with 0.02 M 2-thiobarbituric acid (TBA) solution, absorbance of reactive substances was determined at 538 nm. The results were multiplied by a factor of 7.8 to calculate TBARS values (mg MDA/kg samples).

Texture profile analysis

After cutting the cross section of the samples to a thickness of 2.5 cm, the hardness, springiness, cohesiveness, gumminess, and chewiness of the samples (2.8 cm diameter) were measured using a texture analyzer (TA-XT2*i*, Stable Micro Systems, Surrey, UK) equipped with a 50-mm aluminum cylinder. Crosshead speed for the measurements was 5 mm/s and compression was 40% of sample thickness.

Statistical analysis

The experimental design was a 2 × 5 factorial design with two nitrite sources (sodium nitrite or radish powder) and five phosphate replacement treatments (with or without phosphate, oyster shell calcium, citrus fiber, or dried plum powder). All data were statistically analyzed using the PROC GLIMMIX procedure in the SAS software (version 9.4; SAS, 2012) to determine fixed effects for nitrite and phosphate replacement and their interactions. When significance ($p<0.05$) was determined, the least squares means were further separated using the LINES option in the same software.

Results and Discussion

The significance of nitrite sources (N), phosphate replacements (P), and their interaction was shown in Table 2. A two-way interaction (N × P) between the main effects was not found ($p>0.05$) for any dependent variables tested in this study. Therefore, the results for individual main effects are presented.

pH

Nitrite sources (N) did not affect ($p>0.05$) the pH of pork products (Table 2), indicating that there were no significant ($p>0.05$) differences in pH between sodium nitrite- and radish powder-treated pork products (Table 3). These findings agreed with those reported by Sindelar et al. (2007) and Yoon et al. (2021), who found that pH of meat products naturally cured with celery juice powder and white kimchi powder, respectively, did not differ from those of meat products cured with sodium nitrite. In contrast, phosphate replacements (P) was found to significantly ($p<0.001$) affect the pH of ground pork products (Tables 2). Thus, the OSC treatment had the highest ($p<0.05$) pH values, while the CF and DPP treatments had lower ($p<0.05$) pH values than either the STPP (+) or STPP (−) treatments (Table 3). In our preliminary test, the pH of OSC was 9.93, whereas those of CF and DPP ranged from 3.60 to 4.05. It is likely that organic acids, such as citric acid, quinic acid, and malic acid contained in citrus fiber and dried plum powder reduced the pH of the final products (Bae et al., 2014; Song et al., 1998).

Cooking yield

Cooking yield was not affected ($p>0.05$) by nitrite sources (N) (Tables 2 and 3). Conversely, Yoon et al. (2021) showed that pork sausages containing sodium nitrite showed a higher cooking yield than those containing white kimchi powder. However, Jeong et al. (2020) found no significant difference in cooking yield between pork products cured with various vegetable powders (Chinese cabbage, radish, and spinach) and sodium nitrite-added products, consistently with the findings reported herein. However, in this study, phosphate replacements (P) had a significant ($p<0.001$) effect on cooking yield of ground pork products (Table 2). Interestingly, the cooking yield in the OSC treatment was 96.81%, which did not differ significantly ($p>0.05$) from that of the STPP (+) treatment (98.45%) (Table 3). Similarly, Lee et al. (2011) found that emulsion-type pork sausages treated with 0.3% STPP showed similar cooking yield as those treated with 0.5% oyster shell powder. However, the CF and DPP treatments showed a lower ($p<0.05$) cooking yield than the STPP (+) treatment (Table 3). Dietary fiber from citrus fruits and sorbitol in dried plum powder have been introduced as good candidates for improving the water retention capacity of meat systems (Fernández-Ginés et al., 2003; Jarvis et al., 2015; Lundberg et al., 2014). However, the unexpected results for the CF and DPP treatments in this study might be attributed to the fact that the organic acids contained in CF and DPP had a negative effect on the cooking yield of ground pork products. Consistently, with regard to the effect of organic acids on meat products, Bae et al. (2021) reported that naturally cured sausages containing more organic acids showed a lower pH, thereby resulting in a lower cooking yield, which supports our findings.

Total fluid separation (TFS), lipid separation (LS), and water separation (WS)

Neither TFS, LS, nor WS of pork products were affected ($p>0.05$) by nitrite sources (N) (Tables 2 and 3). However, significant ($p<0.001$) phosphate replacement (P) effects were observed for TFS, LS, and WS in ground pork products (Table 2). The OSC treatment had a significantly ($p<0.05$) higher TFS than the STPP (+) treatment, but lower ($p<0.05$) than the STPP (−), CF, and DPP treatments (Table 3). Among the phosphate replacement treatments, the low TFS of the OSC treatment may be due to the increased water holding capacity owing to the high pH of OSC itself (Park, 2011). The highest TFS ($p<0.05$) was seen in the CF treatment. Since dietary fiber has a microporous structure, it can adsorb moisture and fat, but it is thought that the adsorbed components were discharged by strong physical forces such as centrifugation during the experiment (Lundberg et al., 2014; Wang et al., 2015). A lower TFS was observed with DPP treatment than with CF treatment in this study, likely because the high sorbitol content of DPP may affect its moisture binding ability (Javis et al., 2015). However, there were no significant differences ($p>0.05$) in LS between the OSC and STPP (+) treatments (Table 3). In addition, these treatments had a significantly ($p<0.05$) lower LS than the CF or the DPP treatments. Similar to the TFS results described above, the same trend was observed for WS (Table 3). The greatest WS ($p<0.05$) was observed in the CF treatment, likely due to the coalescence and agglomeration of fiber particles (Powell et al., 2019). Overall, our results suggest that, among the phosphate replacements tested in this study, OSC has the potential to substitute STPP in ground pork products in terms of water-holding capacity, regardless of the nitrite source.

CIE color

The nitrite sources (N) had no effects ($p>0.05$) on the CIE L* values of cooked products (Tables 2 and 4). Similarly, Choi et al. (2020) found that pork sausages cured with white kimchi powder obtained similar CIE L* values as those cured with sodium nitrite, although the type of vegetable powder used was different from that in this study. However, phosphate replacements (P) did significantly ($p<0.001$) affect CIE L* values of cooked products (Table 2). The OSC treatment did not differ ($p>0.05$) in CIE L* values from STPP (+) and CF treatments, but lower ($p<0.05$) than those in the STPP (−) treatment (Table 4). DPP treatment obtained the lowest CIE L* values ($p<0.05$). Similarly, Lee and Ahn (2005) observed that the inclusion of plum extract in turkey breast rolls resulted in reduced L* values.

The CIE a* values of products containing sodium nitrite and radish powder were 9.87 and 9.86, respectively, and were not significantly ($p>0.05$) affected by nitrite sources (N) (Tables 2 and 4). Similar results were obtained by Bae et al. (2020), who reported that pork products cured with radish powder showed CIE a* values similar to those with sodium nitrite. Yoon et al. (2021) also found that there were no significant differences in CIE a* values between pork sausages added with sodium nitrite and those added with white kimchi powder. Additionally, the main effect of phosphate replacement (P) on CIE a* values was significant ($p<0.001$; Table 2). The CIE a* values were lowest ($p<0.05$) for the DPP treatment and did not significantly ($p>0.05$) differ among treatments (Table 4). These CIE a* values were in agreement with those of Meyer (2018), who reported that the addition of plum concentrate as a phosphate replacement in whole muscle hams resulted in a decrease in redness.

Nitrite sources (N) did not affect ($p>0.05$) CIE b* values of ground pork products (Tables 2 and 4). Jeong et al. (2020) found that cooked meat products added with 0.4% radish powder showed similar CIE b* values to those with 150 ppm sodium nitrite, as shown in this study. Overall, our CIE color results suggest that radish powder is a useful alternative to synthetic nitrite for clean-label meat products. However, phosphate replacements (P) significantly ($p<0.001$) affected CIE b* values of ground pork products (Table 2). All phosphate replacement treatments significantly ($p<0.05$) increased the CIE b* values, compared to the STPP (+) treatment (Table 4). The impact of the addition of OSC on CIE b* of ground pork products was smaller, although significant. In contrast, DPP treatment showed the highest CIE b *values ($p<0.05$), probably due to the color of the endogenous pigments in the plant extract (Nowak et al., 2016; Riel et al., 2017). Thus, the use of DPP may have a negative effect on the color of ground pork products.

Nitrosyl hemochrome

Nitrosyl hemochrome, which provides a typical cured-meat color, is formed by the reaction of myoglobin with nitric oxide reduced from nitrite during cooking (Parthasarathy and Bryan, 2012). In this study, nitrite sources (N) had no effect ($p>0.05$) on nitrosyl hemochrome content in ground pork products (Tables 2 and 4), indicating that radish powder is a good candidate as a synthetic nitrite substitute for cured meat color. However, nitrosyl hemochrome content was significantly ($p<0.001$) affected by phosphate replacements (P) (Table 2). CF and DPP treatments had significantly ($p<0.05$) higher nitrosyl hemochrome contents than STPP (−), STPP (+), or OSC treatments, and the highest ($p<0.05$) nitrosyl hemochrome content was observed in DPP treatment (Table 4). As a decrease in pH can promote the rate of the curing reaction (Honikel, 2008), organic acids and polyphenols in CF or DPP may accelerate meat curing by lowering the pH or acting as a reducing agent (Ahmad et al., 2015; Terns et al., 2011). In contrast, the OSC treatment had the lowest ($p<0.05$) nitrosyl hemochrome content across all treatments, probably due to the high pH of OSC powder used in this study (Table 4). Nevertheless, in this study, there was no difference in CIE a* value in OSC treatment and other treatments except for DPP treatment. This may be because the high pH of OSCs limited the curing process or the denaturation of myoglobin (Honikel, 2008; Trout, 1989).

2-thiobarbituric acid-reactive substances (TBARS)

The TBARS values were not significantly ($p>0.05$) influenced by nitrite sources (N) or phosphate replacements (P) in ground pork products (Table 2). Regardless of nitrite sources, TBARS values of all cooked products were 0.12 mg MDA/kg (Table 4). These findings agreed with those reported by Magrinyà et al. (2016), who found that cooked cured sausages had similar TBARS values despite different nitrite treatment (sodium nitrite or vegetable powder). It is likely that nitrites reduced from nitrates contained in radish powder as well as antioxidants present in radish inhibited lipid rancidity (Ahn et al, 2019; Ozaki et al., 2021). Thus, adding radish powder may have a similar inhibitory effect on lipid oxidation as that which results from treatment with synthetic nitrite. Furthermore, TBARS values of ground pork products were not significantly (p>0.05) affected by phosphate replacements (P) (Tables 2 and 4). This result is supported by previous research on substitution for synthetic phosphates. Lee et al. (2011) reported that OSC had similar efficacy in inhibiting lipid oxidation as STPP in emulsion-type sausages. Powell et al. (2019) found that Bologna sausages treated with citrus fibers did not differ in TBARS value from those treated with STPP. Moreover, Nuñez de Gonzlaes et al. (2009) obtained similar TBARS values when dried plum concentrate or phosphate was added to boneless ham.

Textural properties

Nitrite sources (N) had no significant (p>0.05) effect on the textural properties of ground pork products (Tables 2 and 5). Our results were supported by Sucu and Turp (2018), who reported no difference in texture profile between nitrite- and beetroot powder-added Turkish fermented sausages. However, significant effects of phosphate replacements (P) on ground pork products was observed (p<0.001) only for cohesiveness and springiness (Table 2), whereas neither hardness, gumminess, nor chewiness were affected (p>0.05) by phosphate replacement. The STPP (+) treatment showed the highest cohesiveness and springiness (p<0.05), while cohesiveness and springiness of STPP (−), CF, and DPP treatments were lower (p<0.05) than those of STPP (+) and OSC treatments, but were similar to each other (p>0.05) (Table 5). Consistently with the findings reported herein, recently, Lee (2020) reported that the addition of OSC resulted in higher cohesiveness, springiness, and chewiness than restructured hams containing STPP, although, in our study, chewiness did not differ. Similarly, Powell et al. (2019) found that bologna sausages added with 0.5% citrus fiber had lower cohesiveness and springiness than those added with STPP but, in agreement with our results, hardness, gumminess, and chewiness were similar between them. However, Lee and Ahn (2005) reported that the hardness, cohesiveness, springiness, and chewiness of turkey breast rolls were not influenced by the addition of up to 3% plum extract, which partially agrees with our results. Consequently, the addition of OSC resulted in lower cohesiveness and springiness of ground pork products, compared to synthetic phosphate, although OSC can have a greater effect on the texture of the final products among the phosphate replacement treatments tested here, as observed by Bae et al. (2017) for various calcium powders.

Conclusion

Nitrite sources (sodium nitrite or radish powder) did not significantly affect the physicochemical or textural properties of ground pork products. However, most dependent variables were influenced by phosphate replacement treatment. The addition of oyster shell calcium maintained cooking yield and lipid separation, replacing sodium tripolyphosphate in the final products. In contrast, ground pork products with citrus fiber or dried plum power showed a negative effect on water and lipid binding ability. In particular, the addition of dried plum powder resulted in a difference in color in ground pork products compared to STPP (+) treatment. Pork products with oyster shell calcium showed textural properties relatively similar to those of products treated with sodium tripolyphosphate. Therefore, oyster shell calcium is suitable as a synthetic phosphate substitute for clean-label ground pork products when cured with radish powder.

References

Ahmad SR, Cokulakrichnan P, Giriprasad R, Yatoo MA. 2015. Fruit-based natural antioxidants in meat and meat products: A review. Crit Rev Food Sci Nutr 55:1503-1513.

Ahn SJ, Kim HJ, Lee N, Lee CH. 2019. Characterization of pork patties containing dry radish (*Raphanus sativus*) leaf and roots. Asian-Australas J Anim Sci 32(3):413-420.

Alahakoon AU, Jayasena DD, Ramachandra S, Jo C. 2015. Alternatives to nitrite in processed meat: Up to date. Trends Food Sci Technol 45:37-49.

Bae H, Yun SK, Jun JH, Yoon IK, Nam EY, Kwon JH. 2014. Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. J Appl Bot Food Qual 87:24-29.

Bae SM, Cho MK, Jeong JY. 2017. Effects of various calcium powders as replacers for synthetic phosphate on the quality properties of ground pork meat products. Korean J Food Sci Anim Resour 37:456-463.

Bae SM, Choi JH, Jeong JY. 2020. Effects of radish powder concentration and incubation time on the physicochemical characteristics of alternatively cured pork products. J Anim Sci Technol 62(6):922-932.

Bae SM, Gwak SH, Yoon J, Jeong J. 2021. Effects of lemon extract powder and vinegar powder on the quality properties of naturally cured sausages with white kimchi powder. Food Sci Anim Resour 41:950-966.

Ballmer-Weber NK, Hoffmann A, Wuthrich B, Luttkopf D, Pompei C, Qangotsch A, Kastner M, Vieths S. 2002. Influence of food processing on the allergenicity of celery: DBPCFC with celery spice and cooked celery in patients with celery allergy. Allergy 57:228-235.

Cho MK, Bae SM, Jeong JY. 2017. Egg shell and oyster shell powder as alternatives for synthetic phosphate: Effects on the quality of cooked ground pork products. Korean J Food Sci Anim Resour 37:571-578.

Choe J, Lee J, Jo K, Song M, Jung S. 2018. Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. Meat Sci 143:114-118.

Choi JH, Bae SM, Jeong JY. 2020. Effects of the addition levels of white kimchi powder and acerola juice powder on the qualities of indirectly cured meat products. Food Sci Anim Resour 40:636-648.

Fernández-Ginés JM, Fernández-López J, Sayas-Barberá, Sendra E, Pérez-Alvarez JA. 2003. Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. J Food Sci 68:710-715.

Honikel KO. 2008. The use and control of nitrate and nitrite for the processing of meat product. Meat Sci 78:68-76.

Hornsey H. 1956. The colour of cooked cured pork. I.—estimation of the nitric oxide-haem pigments. J Sci Food Agric 7:534-540.

Hughes E, Cofrades S, Troy DJ. 1997. Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 30% fat. Meat Sci 45:273-281.

Hur SJ, Jang A, Jeong JY, Jo C, Chin KB, Lee GT. 2015. Misunderstanding and truths for controversy of carcinogenic substances in meat products. Food Sci Anim Resour Ind 4:7-22.

Jarvis N, Clement AR, O'Bryan CA, Babu D, Crandall PG, Owens CM, Meullenet JF, Ricke SC. 2012. Dried plum products as a substitute for phosphate in chicken marinade. J Food Sci 77:S253-S257.

Jarvis N, O'Bryan CA, Ricke SC, Crandall PG. 2015. The functionality of plum ingredients in meat products: A review. Meat Sci 102:41-48.

Jeong JY. 2016. Alternative curing technology in meat products. Food Sci Anim Resour Ind 5:77-84.

Jeong JY, Bae SM, Yoon J, Jeong DH, Gwak SH. 2020. Effect of using vegetable powders as nitrite/nitrate sources on the physicochemical characteristics of cooked pork products. Food Sci Anim Resour 40:831-843.

Kim YD, Lee JS, Park JH, Park DC, Jeon YS, In MJ, Oh NS. 2014. Application of L-arginine as a substitute for inorganic polyphosphate in pork sausage production. J Appl Biol Chem 57:171-174.

Lee EJ, Ahn DU. 2005. Quality characteristics of irradiated turkey breast rolls formulated with plum extract. Meat Sci 71:300-305.

Lee JH. 2020. Development of substitution techniques of the artificial additives for process of natural meat products. Ph. D. thesis, Chungbuck National Univ., Cheongju, Korea.

Lee JJ, Park SH, Choi JK, Kim JH, Lee SH, Choi SH, Choi YI, Jung DS. 2011. Effect of oyster shell powder on quality properties and storage stability of emulsion-type pork sausages. Korean J Food Sci Anim Resour 31:469-476.

Lee MA, Han DJ, Choi JH, Choi YS, Kim HY, Jeong JY, Paik HD, Kim CJ. 2008. Effect of hot air dried kimchi powder on the quality characteristics of low-fat sausages. Korean J Food Sci Anim Resour 28:146-153.

Long NHBS, Gál R, Buňka F. 2011. Use of phosphates in meat products. Afri J Biotechnol 10:19874-19882.

Lundberg B, Pan X, White A, Chau H, Hotchkiss A. 2014. Rheology and composition of citrus fiber. J Food Eng 125:97-104.

Magalhães IMC, Paglarini CS, Vidal VAS, Pollonio MAR. 2020. Bamboo fiber improves the functional properties of reduced salt and phosphate-free Bologna sausage. J Food Process Preserv 44:e14929.

Magrinyà N, Bou R, Tres A, Rius N, Codony R, Guardiola F. 2016. Use of tocopherol extract and different nitrite sources and starter cultures in the production of organic botifarra catalana, a cooked cured sausage. Food Sci Technol Int 12:221-234.

Maruyama S, Streletskaya NA, Lim J. 2021. Clean label: Why this ingredient but that one? Food Qual Prefer 87:104062.

Meyer WR. 2018. Effects of plum concentrate, potato starch, and rice starch as a phosphate replacement on quality and sensory attributes of whole muscle hams. MS thesis, Angelo State Univ., San Angelo, Texas, USA.

Nowak A, Czyzowska A, Efenberger M, Krala L. 2016. Polyphenolic extracts of cherry (*Prunus cerasus* L.) abd blackcurrant (*Ribes nigrum* L.) leaves as natural preservatives in meat products. Food Microbiol 59:142-149.

Nuñez de Gonzalez MT, Hafley BS, Boleman RM, Miller RK, Rhee KS, Keeton JT. 2009. Qualitative effects of fresh and dried plum ingredients on vacuum-packaged, sliced hams. Meat Sci 83:74-81.

Ozaki MM, Munekata PES, Jacinto-Valderrama RA, Efraim P, Pateiro M, Lorenzo JM, Pollonio MAR. 2021. Beetroot and radish powders as natural nitrite source for fermented dry sausages. Meat Sci 171:108275.

Öztürk-Kerimoğlu B, Serdaroğlu M. 2019. Powder/gelled inulin and sodium carbonate as novel phosphate replacers in restructured chicken steaks. J Food Process Preserv 43:e14243.

Park SH. 2011. Effects of natural calcium powder addition on functional properties and storage characteristics of emulsion-type sausages. MS thesis, Chungbuck National Univ., Cheongju, Korea.

Parthasarathy DK, Bryan NS. 2012. Sodium nitrite: the “cure” for nitric oxide insufficiency. Meat Sci 92:274-279.

Pegg RB, Shahidi F. 2000. Nitrite curing of meat: The N-nitrosamine problem and nitrite alternatives. Food & Nutrition Press, Inc., Trumbull, CT, USA. pp 1-253.

Powell MJ, Sebranek JG, Prusa KJ, Tarté R. 2019. Evaluation of citrus fiber as a natural replacer of sodium phosphate in alternatively-cured all-pork Bologna sausage. Meat Sci 157:107883.

Riel GR, Boulaaba A, Popp J, Klein G. 2017. Effects of parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausage – Impact on microbiological, physicochemical and sensory aspects. Meat Sci 131:166-175.

SAS. 2012. SAS/STAT® software for PC. Release 9.4, SAS Institute Inc., Cary, NC, USA.

Shahidi F, Synowiecki J. 1997. Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications. Food Chem 60:29-32.

Sindelar JJ, Cordray JC, Sebranek JA. Love JA, Ahn DU. 2007. Effects of varying levels of vegetable Juice Powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of Ready-to-Eat uncured ham. J Food Sci 72:S388-S395.

Šojića B, Pavlića B, Tomovića V, Kocić-Tanackova S, Đurovićb S, Zekovića Z, Belovićc M, Torbicac A, Jokanovića M, Urumovićd N, Vujadinoviće D, Ivića M, Škaljaca S. 2020. Tomato pomace extract and organic peppermint essential oil as effective sodium nitrite replacement in cooked pork sausages. Food Chem 330:127202.

Song EY, Choi YH, Kang, KH, Koh JS. 1998. Free sugar, organic acid, hesperidin, naringin and inorganic elements changes of cheju citrus fruits according to harvest date. Korean J Food Sci Technol 30:306-312.

Sucu C, Turp GY. 2018. The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. Meat Sci 140:158-166.

Tarladgis BG, Watts B M, Younathan M T, Dugan L Jr. 1960. A distillation method for the quantification determination of malonaldehyde in rancid foods. J Am Oil Chem Soc 37:44-48.

Terns MJ, Milkowski AL, Rankin SA, Sindelar JJ. 2011. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. Meat Sci 88:311-318.

Thangavelu KP, Kerry JP, Tiwari BK, McDonnell CK. 2019. Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. Trends Food Sci Technol 94:43-53.

Trout GR. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J Food Sci 54:536–548.

USDA-FSIS. 2015. Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Directive 7120.1 Rev.31. United States Department of Agriculture. Food Safety and Inspection Service, Washington, DC, USA.

Vann DG, DeWitt CAM. 2007. Evaluation of solubilized proteins as an alternative to phosphates for meat enhancement. J Food Sci 72:72-77.

Wang L, Xu H, Tuan F, Pan Q, Fan R, Gao Y. 2015. Physicochemical characterization of five types of citrus dietary fibers. Biocatal Agric Biotechnol 4:250-258.

Yong HI, Kim TK, Choi HD, Jang HW, Jung S, Choi YS. 2021. Clean label meat technology: Pre-converted nitrite as a natural curing. Food Sci Anim Resour 41:173-184.

Yoon J, Bae SM, Gwak SH, Jeong JY. 2021. Use of green tea extract and rosemary extract in naturally cured pork sausages with white kimchi powder. Food Sci Anim Resour 41:840-854.

Table 1. Experimental design (2 × 5 factorial) to investigate the effects of nitrite and phosphate replacements for ground pork products

Samples	Nitrite sources[1]	Phosphate replacements[2]
1	Sodium nitrite	No sodium tripolyphosphate
2	Sodium nitrite	Sodium tripolyphosphate
3	Sodium nitrite	Oyster shell calcium
4	Sodium nitrite	Citrus fiber
5	Sodium nitrite	Dried plum powder
6	Radish powder	No sodium tripolyphosphate
7	Radish powder	Sodium tripolyphosphate
8	Radish powder	Oyster shell calcium
9	Radish powder	Citrus fiber
10	Radish powder	Dried plum powder

[1] Nitrite sources: Two different nitrite sources ($NaNO_2$ or radish powder) were used. Radish powder was added with a starter culture comprising *S. carnosus* and *S. carnosus* subsp.

[2] Phosphate replacement treatments: Samples prepared with or without 0.5% sodium tripolyphosphate or with one of three different synthetic phosphate replacements (0.5% oyster shell calcium: OSC, citrus fiber: CF, or dried plum powder; DPP).

Table 2. Significance of main and interaction effects on nitrite sources and phosphate replacements on physicochemical properties of ground pork products

Main and interaction effects[1]	Dependent variables[2]														
	pH	Cooking yield	TFS	LS	WS	CIE L*	CIE a*	CIE b*	Nitrosyl hemochrome	TBARS	Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Nitrite sources[3] (N)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phosphate replacements[4] (P)	**	**	**	**	**	**	**	**	**	NS	NS	**	**	NS	NS
N × P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Main and interaction effects: * = p<0.05, ** = p<0.001, NS = not significant.

[2] Dependent variables: TFS (total fluid separation), LP (lipid separation), WP (water separation), CIE L* (lightness), CIE a* (redness), CIE b* (yellowness), and TBARS (2-thiobarbituric acid reactive substances).

[3] Nitrite sources: Two different nitrite sources ($NaNO_2$ or radish powder) were used. Radish powder was added with a starter culture comprising *S. carnosus* and *S. carnosus* subsp.

[4] Phosphate replacement treatments: Samples prepared with or without 0.5% sodium tripolyphosphate or with one of three different synthetic phosphate replacements (0.5% oyster shell calcium; OSC, citrus fiber; CF, or dried plum powder; DPP).

Table 3. Effects of nitrite and phosphate replacements on pH, cooking yield, total fluid separation, lipid separation, and water separation in ground pork products

Main effects	pH	Cooking yield (%)	Total fluid separation (%)	Lipid separation (%)	Water separation (%)
Nitrite sources[1] (N)					
Sodium nitrite	6.25	93.39	11.14	1.01	10.13
Radish powder	6.25	93.02	11.24	1.03	10.21
SEM	0.01	1.27	0.47	0.11	0.36
Phosphate replacements[2] (P)					
STPP (−)	6.09^{C}	91.06^{B}	13.03^{C}	1.20^{B}	11.83^{C}
STPP (+)	6.32^{B}	98.45^{A}	5.08^{E}	0.25^{C}	4.83^{E}
OSC	6.79^{A}	96.81^{A}	6.83^{D}	0.42^{C}	6.41^{D}
CF	6.03^{D}	89.76^{B}	16.62^{A}	1.77^{A}	14.85^{A}
DPP	6.04^{D}	89.97^{B}	14.39^{B}	1.46^{AB}	12.93^{B}
SEM	0.01	1.38	0.56	0.14	0.43

[1] Nitrite sources: Two different nitrite sources ($NaNO_2$ or radish powder) were used. Radish powder was added with a starter culture comprising *S. carnosus* and *S. carnosus* subsp.

[2] Phosphate replacement treatments: Samples prepared with or without 0.5% sodium tripolyphosphate or with one of three different synthetic phosphate replacements (0.5% oyster shell calcium; OSC, citrus fiber; CF, or dried plum powder; DPP).

$^{A-E}$ Means within columns followed by different superscript letters are significantly different ($p<0.05$).

Table 4. Effects of nitrite and phosphate replacements on CIE color, nitrosyl hemochrome, and TBARS values in ground pork products

Main effects	CIE L*	CIE a*	CIE b*	Nitrosyl hemochrome (ppm)	TBARS (mg MDA/kg)
Nitrite sources[1] (N)					
Sodium nitrite	67.62	9.87	8.70	36.09	0.12
Radish powder	67.32	9.86	8.66	35.72	0.12
SEM	0.14	0.05	0.04	0.34	0.03
Phosphate replacements[2] (P)					
STPP (−)	68.67^{A}	10.15^{A}	7.67^{C}	36.71^{C}	0.14
STPP (+)	68.18^{AB}	9.93^{A}	6.25^{E}	33.66^{D}	0.13
OSC	68.02^{B}	9.93^{A}	6.75^{D}	32.91^{D}	0.13
CF	68.50^{AB}	10.03^{A}	8.50^{B}	37.82^{B}	0.10
DPP	63.97^{C}	9.27^{B}	14.23^{A}	39.71^{A}	0.09
SEM	0.21	0.08	0.04	0.42	0.03

[1] Nitrite sources: Two different nitrite sources ($NaNO_2$ or radish powder) were used. Radish powder was added with a starter culture comprising *S. carnosus* and *S. carnosus* subsp.

[2] Phosphate replacement treatments: Samples prepared with or without 0.5% sodium tripolyphosphate or with one of three different synthetic phosphate replacements (0.5% oyster shell calcium; OSC, citrus fiber; CF, or dried plum powder; DPP).

$^{A-E}$ Means within columns followed by different superscript letters are significantly different ($p<0.05$).

TBARS: 2-thiobarbituric acid reactive substances. MDA: malondialdehyde.

Table 5. Effects of nitrite and phosphate replacements on textural properties in ground pork products

Main effects	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
Nitrite sources[1] (N)					
Sodium nitrite	34.43	0.74	0.93	25.56	23.75
Radish powder	35.52	0.74	0.92	26.40	24.29
SEM	2.20	0.01	0.01	2.05	2.00
Phosphate replacements[2] (P)					
STPP (−)	34.95	0.72^{C}	0.91^{C}	25.23	22.99
STPP (+)	34.51	0.79^{A}	0.96^{A}	27.28	26.06
OSC	34.10	0.76^{B}	0.93^{B}	25.88	24.13
CF	36.67	0.71^{C}	0.90^{C}	26.78	23.81
DPP	34.65	0.72^{C}	0.91^{C}	25.23	23.12
SEM	2.29	0.01	0.01	2.12	2.07

[1] Nitrite sources: Two different nitrite sources ($NaNO_2$ or radish powder) were used. Radish powder was added with a starter culture comprising *S. carnosus* and *S. carnosus* subsp.

[2] Phosphate replacement treatments: Samples prepared with or without 0.5% sodium tripolyphosphate or with one of three different synthetic phosphate replacements (0.5% oyster shell calcium; OSC, citrus fiber; CF, or dried plum powder; DPP).

[A-C] Means within columns followed by different superscript letters are significantly different ($p<0.05$).