Received: 25 February 2018 | Revised: 25 April 2018 | Accepted: 30 May 2018

DOI: 10.1111/jfpp.13712

ORIGINAL ARTICLE

WILEY Journal of Food Processing and Preservation Institute of Food Science + Technology ifst

Combination of sea tangle powder and high-pressure treatment as an alternative to phosphate in emulsion-type sausage

Haelim Lee[1] | Juhui Choe[1] | Hae In Yong[1] | Hyun Jung Lee[1] | Hyun-Joo Kim[2] | Cheorun Jo[1,3]

[1]Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University, Seoul, Korea

[2]Crop Post-harvest Technology Division, National Institute of Crop Science, RDA, Suwon, Korea

[3]Institute of Green Bio Science and Technology, Seoul National University, Pyeogchang, Korea

Correspondence
Cheorun Jo, Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea.
Email: cheorun@snu.ac.kr

Funding information
Rural Development Administration, Grant/Award Number: 011617

Abstract

A series of experiments were conducted to determine the possibility of using sea tangle powder (STP) and/or its combination with high pressure (HP) as substitutes for phosphate in emulsion-type sausages. The sausages with STP (1.5% and 3%) had similar cooking loss to sausages with 0.2% sodium pyrophosphate (PC), without a negative effect on overall acceptability, except for texture property, which was inferior to PC ($p < 0.05$). Instrumental texture properties of sausages containing 3% STP were improved by HP (100 MPa) treatment. Furthermore, the sausages treated with a combination of STP and HP showed similar or greater antioxidant and antimicrobial effect than that of PC. Therefore, a combination treatment of STP and HP could be used effectively as an alternative to phosphate in emulsion-type sausages because of their similar water holding capacity and instrumental hardness, and greater inhibition ability against lipid oxidation and bacterial growth compared with those of PC.

Practical applications

Meat industry has been challenged by the increasing consumer's demand for safe and high-quality meat and meat products. Particularly, the rapid increase of the concept of all-natural and clean-label makes manufacturers produce synthetic additive-free products. However, effective natural alternative for phosphate (synthetic additives) has not been introduced so far. Therefore, this study was conducted to evaluate the potential of combinational use of sea tangle powder and high pressure as an alternative to phosphate in emulsion-type sausage. Results revealed that this method was effective to replace the addition of phosphate and can be applied directly for industry.

1 | INTRODUCTION

Phosphate is one of the most widely used synthetic additives in meat products because of its beneficial effects, which include increasing the water-holding capacity (WHC) and improving cooking yield and texture properties (Pietrasik & Janz, 2009). Phosphate increases the WHC by: (a) an increasing the pH and ionic strength of the meat (Pietrasik & Janz, 2009); (b) dissociating and depolymerizing actomyosin cross-bridges (Trespalacios & Pla, 2007); and (c) enhancing the extraction of myofibrillar proteins (Xiong, Lou, Wang, Moody, & Harmon, 2000). Antioxidative and antibacterial activities of phosphate by chelating heavy metals are also reported (Lampila, 1993).

Over the last decade, consumers' perception of healthy foods has been growing, thereby increasing concerns over the safety of synthetic food additives. Consequently, numerous studies have been conducted to find alternatives to synthetic additives in processed

meat products (Alahakoon, Jayasena, Ramachandra, & Jo, 2015; Cho, Bae, & Jeong, 2017; Jarvis et al., 2012). As a result, certain synthetic additives, such as sodium nitrite, monosodium glutamate, and other synthetic preservatives, have been replaced by natural materials and introduced to the market successfully (Alahakoon et al., 2015). However, no effective alternative sources to phosphate in meat and meat products have been developed so far. Moreover, studies searching for natural alternatives to phosphate are relatively scarce.

The best candidate for a natural alternative to phosphate should enhance the texture and WHC, while minimizing adverse effects on the sensory property in meat products. Previous studies found that several natural sources (plum, persimmon, and sea tangle) have positive effects on the WHC and texture in meat and meat products (Jarvis et al., 2012; Kim, Jin, & Ha, 2008). Among them, sea tangle has water-retention and binding ability because it contains alginate, a major dietary fiber (Jiménez-Escrig & Sánchez-Muniz, 2000). Previous studies showed that addition of sea tangle powder enhanced the WHC and texture properties of breakfast sausages (Kim et al., 2010). It would enhance the flavor and antibacterial activity of phosphate-free final products because of the presence of glutamic acid and phenolic compounds (Ito & Hori, 1989).

High-pressure (HP) treatment, on the other hand, is being applied increasingly in the meat industry because of it beneficial effects, such as extending shelf-life by inactivating microorganisms, and enhancing texture property and WHC in meat products with minimal effects on the color, flavor, or nutritional value (Sun & Holley, 2010). HP causes these changes in WHC and texture properties by altering protein structures, which depends on the pressure levels (Lullien-Pellerin & Balny, 2002; Silva & Weber, 1993). According to previous studies, application of HP at <200 MPa generally provided better WHC or texture properties of meat products (Hong, Park, Kim, & Min, 2006; Sikes, Tobin, & Tume, 2009). However, HP without additional treatment is insufficient as a substitute for phosphate in meat product manufacturing (Trespalacios & Pla, 2007). In addition, adverse effects of HP, including lipid oxidation and discoloration, have been reported (Mariutti, Orlien, Bragagnolo, & Skibsted, 2008). Mariutti et al. (2008) stated that oxidation caused by HP treatment could be inhibited by the addition of an antioxidant. Therefore, it can be hypothesized that the combined application of sea tangle and HP might improve the WHC, texture properties, antibacterial activity, and inhibit oxidation caused by HP treatment of meat products.

Therefore, the aim of this study was to determine the suitability of the addition of sea tangle powder (STP) and/or its combination with HP as an alternative to phosphate addition in emulsion-type sausages. Two experiments were conducted to investigate: (a) the physicochemical and sensory properties of sausages with 3% STP compared with sausages with or without phosphate; and (b) the physicochemical quality and antibacterial and antioxidant activities against lipids and proteins of sausages prepared using different levels of STP (0%, 1.5%, and 3%) and HP (0.1, 100, and 200 MPa).

2 | MATERIALS AND METHODS

2.1 | Materials and experimental design

Pork hind leg meat and back fat were purchased from a local butcher (Seoul, Korea) and ground through a 6-mm plate (M-12S, Hankook Fujee Industries Co., Ltd., Hwaseong, Korea). Dried sea tangle powder (STP) (Hansalim, Seoul, Korea) was purchased from a commercial market.

2.1.1 | Experiment I: Effect of the addition of STP on emulsion-type sausages

Sea tangle powder (STP) and its addition level (3%) were determined based on the result of a preliminary study that 3% STP increased WHC of the meat batter. Ground meat was mixed with back fat, iced water, and additives in a silent cutter. The formulas of the three treatments (PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate and STP; and 3% STP, sausages with 3% sea tangle powder) are shown in Table 1. After emulsification, 100 g of each meat batter was obtained to measure the cooking loss. The remainder was stuffed in a collagen casing (2.5 cm diameter; NDX, Viscofan, Ceske Budejovice, Czech Republic). The sausages were vacuum-packaged in low-density polyethylene/nylon bags (25 × 30 cm), with an oxygen permeability of 22.5 mL/m^2/24 hr atm at 60% RH/25°C, and a water vapor permeability of 4.7 g/m^2/24 hr at 100% RH/25°C. The packaged sausages were cooked in a water bath at 80°C for 30 min until the internal temperature of the sausages reached 75°C and were then cooled for 30 min in iced water. The sausage samples were analyzed for their WHC, texture profile analysis (TPA), and sensory evaluation.

2.1.2 | Experiment II: Effect of STP, HP, and a combination of STP and HP treatment on emulsion-type sausages

In Experiment II, STP at different addition levels (0%, 1.5%, and 3%) was tested in combination with different pressure levels (0.1 [1 atm], 100, and 200 MPa). The effect of the application of STP and/or HP was compared with sausages containing 0.2% sodium pyrophosphate (PC) and those without sodium pyrophosphate, STP, and HP treatment (NC). Batters were manufactured following the formulas in Table 1. After emulsification, 100 g of meat batter from each treatment was sampled to measure the pH and protein solubility. The remainder was stuffed in the collagen casing (diameter, 2.5 cm; length, 18 cm; Viscofan). The sausages were vacuum-packed and transported to the Korea Food Research Institute (Seongnam, Korea) in a container with ice packs for HP treatment. The samples were placed in a pressure vessel, submerged in hydrostatic fluid medium (Quintus food processor 6; ABB Autoclave Systems, Inc., Columbus, OH, USA), and pressurized with 100 and 200 MPa for 5 min (15 ± 3°C). The non-pressurized samples (sausages with sodium pyrophosphate [PC] and those with STP [0%, 1.5%, and 3%] instead of sodium pyrophosphate at 0.1 MPa) were kept in a refrigerator during

TABLE 1 Formulation (%) of emulsion-type sausages for Experiment I and II

Ingredients	PC[1]	NC[1]	Sea tangle powder (%)	
			1.5	3
Pork meat	60	60	60	60
Back fat	20	20	20	20
Ice water	20	20	20	20
Total	100	100	100	100
Sodium chloride	1.2	1.2	1.2	1.2
Sodium pyrophosphate	0.2	–	–	–
Sea tangle powder	–	–	1.5	3
Egg white	1.5	1.5	1.5	1.5
Sugar	0.5	0.5	0.5	0.5
Spice mix	1	1	1	1
L-Ascorbic acid	0.05	0.05	0.05	0.05
Celery powder	2.3	2.3	2.3	2.3

[1]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate and sea tangle powder.

the treatment. Immediately after HP treatment, all samples were transported to the laboratory (Seoul, Korea) and cooked in a water bath at 80°C for 30 min until the internal temperature reached 75°C. The sausages were then cooled in iced water for 30 min and their physicochemical properties (cooking loss, WHC, TPA, lipid oxidation, and protein oxidation) and microbial safety were analyzed. The antioxidant activity against lipid and protein, and microbial safety, were analyzed at day 1 and 14 of refrigerated storage (4°C).

2.2 | Physicochemical analysis

2.2.1 | pH

Each meat batter (1 g) was homogenized with 9 mL of distilled water using a homogenizer (T10 basic, Ika Works, Staufen, Germany) for 30 s. The homogenized mixture was centrifuged at 2,265× *g* for 10 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea) and then filtered using a filter paper (Whatman No. 4, Whatman PLC., Maidstone, UK). The pH value of each filtered solution was measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.2.2 | Cooking loss

Three replicates of each raw sausage (approximately 100 g) were vacuum packaged with polyethylene bags for cooking. Samples were heated for 30 min at 80°C in the water bath and weighed after removing the water on the surface and inside the casing using a paper towel. The weight changes of sausages before and after cooking were calculated as the percentage weight loss of a sample.

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

2.2.3 | Water holding capacity (WHC)

The WHC was measured using a texture analyzer (TA1, AMETEK Lloyd instruments Ltd., Fareham, UK). Approximately 9 g of cooked sausage samples (25 × 15 mm, diameter × height) were placed on a filter paper (Whatman No. 4) and compressed at a test speed of 2.0 mm/s and trigger force of 127.4 N for 2 min. The water content was determined by drying 5 g of samples at 105°C for 16 h. The WHC was calculated as:

$$\text{WHC } (\%) = \frac{B - A}{B} \times 100$$

$$A = \text{Weight of sample (before compression - after compression)}$$

$$B = \frac{\text{Weightof sample before compression} \times \text{Water content}}{100}$$

2.2.4 | Texture profile analysis (TPA)

Three replicates were measured for the TPA of sausages for each treatment. The centers of the cooked sausage samples (25 × 15 mm, diameter × height) were compressed twice to 60% of their original height using a TA1 texture analyzer (AMETEK Lloyd instruments Ltd.) attached to a compression plate (70 mm in diameter) at a test speed of 2.0 mm/s and a trigger force of 1 N. The texture analysis was performed using the NexygenPlus™ software (AMETEK Lloyd instruments Ltd.), and the values of hardness, springiness, cohesiveness, gumminess, and chewiness were recorded. The hardness, gumminess, and chewiness were expressed using Newton (N).

2.2.5 | Protein solubility

The salt solubilized protein content was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Each meat batter (1 g) was homogenized (Ika Works) in 0.6 M NaCl (1:4, v/v) at 425× *g* for 1 min at 4°C. The homogenate was centrifuged at 10,000× *g* for 30 min (4°C) (Micro 17TR, Hanil Co., Ltd.). The supernatant was put aside and the pellet was used for re-extraction using the same solution. After vortexing, the sample was centrifuged again. The two supernatants were pooled and used for analysis. Samples were mixed with SDS sample buffer and heated at 95°C for 10 min. Aliquots of protein (20 μg/μl) were loaded onto 12.5% acrylamide gel with a 4.5% stacking gel. After electrophoresis, the gel was stained with 0.1% coomassie brilliant blue R-250 in methanol:acetic acid:distilled water (3:1:6 by volume) for about 30 min. Destaining was performed in the same solution lacking coomassie brilliant blue R-250 for 1.5 h. Precision plus protein unstained standards (Bio-Rad Laboratories Inc., Hercules, CA, USA) were used as molecular weight standards for SDS-PAGE. Stained

gel images were captured using a ChemiDoc™ XRS+ (Bio-Rad Laboratories Inc.).

2.2.6 | Lipid oxidation

Malondialdehyde (MDA) was extracted from the sausage samples with acetonitrile as follows. A sausage sample (5 g) was homogenized using a homogenizer (T25, Ika Works) at 1,817× *g* for 1 min with 10 mL of deionized water and 50 μl of 7.2% 2,6-di-tert-butyl-4-methylpehnol (in ethanol). After homogenization, 500 μl of homogenate were transferred into micro-tube and 200 μL of 6 M NaOH solution were added for alkaline hydrolysis of protein-bound MDA. The tubes were heated in a water bath for 45 min at 60°C, before being cooled at room temperature for 10 min. Acetonitrile (1 ml) was added and the tube was vortexed. The tubes were centrifuged at 13,000× *g* for 10 min (HM-150IV, Hanil Co., Ltd.). Each supernatant was filtered using a 0.2-μm PVDF syringe filter (Whatman PLC.) and collected in a vial. MDA was analyzed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific Inc., Waltham, MA, USA). An Atlantis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) and 30 mM potassium phosphate dibasic (mobile phase, pH adjusted to 6.2 with phosphoric acid) were used. The flow rate of the mobile phase was 1.2 ml/min and the injection volume was 50 μl. UV/VIS detector was set to 254 nm and the column temperature was kept at 35°C. The concentration of MDA in a sample was calculated from the standard curve of 1, 1, 3, 3-tetraethoxypropane solution in 0.1 M hydrochloric acid, which was expressed in μM MDA/g meat sample.

2.2.7 | Protein oxidation

In order to evaluate the protein oxidation in the sausage samples, protein carbonyls were measured by derivatizing them with 2, 4-dinitrophenylhydrazine (DNPH), as described by Fagan, Sleczka, and Sohar (1999). Sausage (2 g) was homogenized with 15 ml of pyrophosphate buffer (pH 7.4; 2.0 mM $Na_4P_2O_7$, 10 mM Tris–maleate, 100 mM KCl, 2.0 mM $MgCl_2$, and 2.0 mM EGTA) using a homogenizer (T10 basic, Ika Works). Two equal aliquots of 0.2 ml each were taken from the homogenates and dispensed in prepared tubes. One of them was used to determine carbonyl content and the other was used as a protein blank. Chromophores from the sample were removed by washing the sample (2 ml) with 4 ml of HCl–acetone (3:100, v/v) twice, followed by washing with 2 ml of trichloroacetic acid (10%) twice. The samples were derivatized for 30 min with 4 ml of DNPH (10 mM) in HCl (2.0 N), and the protein blanks were prepared by adding 4 ml of HCl (2.0 N), instead of DNPH solution. Excess DNPH was removed by washing with 4 ml of trichloroacetic acid (20%) once, followed by five washes with 4 ml of HCl (10 mM) in ethanol–ethyl acetate (1:1, v/v). The pellets were solubilized in 4 ml of guanidine hydrochloride (6.0 M) and potassium dihydrogen phosphate (20 mM, pH 2.3) at 4°C for 24 hr. The absorbance of the protein blank was measured at 280 nm using a spectrophotometer (X-ma 3100, Human Co. Ltd., Seoul, Korea), and the amount of protein was calculated using a standard curve using bovine serum albumin. The carbonyl content was expressed as nmol carbonyls per mg protein, using an absorption coefficient of 22,000/M/cm at 370 nm for protein hydrazones.

2.2.8 | Sensory evaluation

For sensory evaluation, sausages were cut into the same size (25 × 10 mm, diameter × height) and cooked in a pan using a gas burner until the internal temperature of the sample reached 75C. The temperature was monitored using a digital thermometer (YF-160A Type-K; YFE, Hsinchu City, Taiwan) at the center of the meat sample. The samples were transferred to randomly coded dishes and water was served for mouth rinsing. Ten semi-trained panelists, who had experienced sensory evaluation of meat and meat product for at least 1 year, evaluated the cooked samples for color, flavor, taste, juiciness, springiness, texture, and overall acceptability by a 9-point hedonic scale (1 = dislike extremely, 5 = Neither like nor dislike, 9 = like extremely). The sensory evaluation was carried out three times independently for the replicates.

2.2.9 | Microbial analysis

The number of total aerobic bacteria in sausage samples was analyzed for 14 days of storage at 4°C. Each sample (3 g) was blended with 27 mL of sterile saline (0.85%), using a lab blender (Bag Mixer® 400 P, Inter science, St. Nom la Bretèche, France) and diluted decimally with sterile saline solution. Total plate count agar (Difco Laboratories, Detroit, MI, USA) was used as the medium to enumerate the microorganisms. A 100-μl aliquot of each dilution was spread, in triplicate, on the plates. The plates were incubated at 37°C for 48 hr and the colonies were counted and expressed as the logarithm of the colony forming units per gram (Log CFU/g).

2.3 | Statistical analysis

One-way analysis of variance was performed with a completely randomized design using a General Linear Model. The statistical model included the fixed effects of phosphate content and treatment levels of STP and HP, and random effects of the replications. For, sensory data, panelist was included as a random effect. Significant differences among mean values were determined using Tukey's multiple comparison test in SAS Release 9.4. (SAS Institute Inc., Cary, NC, USA), with a confidence level of $p < 0.05$. All experimental procedures were conducted in triplicate, and mean values and *SE* of the mean were reported

3 | RESULTS AND DISCUSSION

3.1 | Experiment I

3.1.1 | Cooking loss, WHC, and TPA

The WHC, indicating water-retaining ability, is one of the factors that determine meat quality because it affects sensory properties in meat

products significantly (Van Oeckel, Warnants, & Boucqué, 1999). In the present study, lower cooking loss and higher WHC were observed for sausages containing STP compared with those of the sausages without sodium pyrophosphate (NC) ($p < 0.05$) (Table 2). Moreover, the cooking loss of sausages with 3% STP was similar to that containing 0.2% sodium pyrophosphate (PC), indicating that STP could enhance the WHC of phosphate-free sausages effectively. Several studies have shown that the WHC of meat products improved when STP was added because of the presence of dietary fiber, such as alginate (Jeon & Choi, 2012; Kim et al, 2010). Ruperez and Saura-Calixto (2001) observed that in general, seaweeds showed higher swelling and water retention capacity, which correlated positively with their alginate content.

Instrumental texture properties of the sausages were unaffected by the addition of STP, except for hardness (Table 2). Thus, there were no significant differences in gumminess, chewiness, and cohesiveness between NC and sausages with STP, while PC showed the greatest values in all texture properties, except for springiness. Similar observations were made in other studies, where the addition of seaweed, including sea tangle, to meat products improved their texture properties, especially hardness, mostly because of dietary fiber (Jeon & Choi, 2012; Kim et al, 2010). Generally, the addition of seaweeds containing dietary fiber enhances texture properties and WHC by forming a three-dimensional network and stabilizing the emulsion in meat products. Both soluble (e.g., alginate, fucans, and laminarans) and insoluble fibers (e.g., cellulose) from seaweed influence the extent of texture enhancement (Lahaye, 1991; Ruperez & Saura-Calixto, 2001; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997).

3.1.2 | Sensory evaluation

There were no significant differences in color, flavor, and juiciness in the sensory evaluation between PC and sausages with 3% STP (Table 3). NC showed the highest score for color among the treatments. The sausages containing 3% STP showed a higher score in springiness, hardness, and overall acceptability compared with those of NC ($P < 0.05$) but the three factors of the treatment had lower score than those of PC ($P < 0.05$). Taken together, the results indicated that although STP changed the color of the sausages, no adverse effect was found in overall acceptability compared with NC. Several studies reported that the addition of seaweed powder, including sea tangle, in meat products produced a color change, but did not influence their overall acceptability (Jeon & Choi, 2012; Kim et al, 2010). The addition of 3% STP in sausages was judged to result in similar juiciness to PC in this study. A similar result was observed whereby the addition of 1% seaweed enhanced the juiciness of pork patties, which was closely related to an increased WHC (Jeon & Choi, 2012). In other words, the increase in juiciness might be caused by greater WHC in sausages containing STP compared with that of NC.

From the results of Experiment I, the addition of STP enhanced WHC in emulsion-type sausage but the texture properties were inferior to the addition of phosphate on the basis of the results of instrumental and sensory evaluation. Therefore, HP was applied to improve the texture properties and to add other advantages including inhibition of microbial growth.

TABLE 2 Physicochemical traits of sausages containing 3% sea tangle powder in Experiment I

Traits	PC[1]	NC[1]	Sea tangle powder (3%)	*SEM*[2]
Cooking loss (%)	2.93^{b}	20.50^{a}	2.72^{b}	0.671
WHC (%)	85.11^{a}	51.31^{c}	81.49^{b}	0.573
Hardness (N)	108.03^{a}	39.49^{c}	59.39^{b}	4.344
Springiness	0.77	0.72	0.76	0.020
Gumminess (N)	40.59^{a}	6.70^{b}	13.95^{b}	3.391
Chewiness (N)	31.13^{a}	4.81^{b}	10.66^{b}	2.357
Cohesiveness	0.37^{a}	0.17^{b}	0.23^{b}	0.022

Notes. $^{a\text{-}c}$Values with different letters within the same row differ significantly ($p < .05$).
[1]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate and sea tangle powder.
[2]*SE* of the mean ($n = 9$).

3.2 | Experiment II

3.2.1 | pH value, cooking loss, and WHC

No significant differences were observed in the pH values of meat samples with STP compared with those of NC (Table 4). Sausages treated with STP and/or HP had significantly lower pH values than that of PC. Meanwhile, the positive effects of STP and HP on cooking loss and WHC were observed in the sausages ($p < 0.05$), regardless of the levels of STP and HP used. According to Pietrasik and Janz (2009), phosphate increases the pH value of meat and induces an improved WHC. In the present study, the WHC of sausages with STP increased, showing similar values to

TABLE 3 Sensory analysis of sausages containing 3% sea tangle powder in Experiment I

Traits[1]	PC^{c}	NC^{c}	Sea tangle powder (3%)	*SEM*[3]
Color	6.20^{ab}	6.40^{a}	5.30^{b}	0.308
Flavor	6.50	5.77	5.60	0.350
Taste	6.80^{a}	5.10^{b}	5.43^{b}	0.291
Juiciness	6.28^{a}	4.67^{b}	5.43^{ab}	0.404
Springiness	6.97^{a}	2.77^{c}	4.80^{b}	0.311
Hardness	6.93^{a}	2.80^{c}	4.93^{b}	0.366
Overall acceptability	6.83^{a}	4.00^{c}	5.50^{b}	0.343

Notes. $^{a\text{-}c}$Values with different letters within the same row differ significantly ($p < .05$).
[1]9-point hedonic scale (1 = dislike extremely, 5 = Neither like nor dislike, 9 = like extremely).
[2]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate and sea tangle powder.
[3]*SE* of the mean ($n = 9$).

that of PC, despite of the relatively lower pH values of sausages with STP. This was probably related to the swelling and water retention ability of alginate from sea tangle (Ruperez & Saura-Calixto, 2001). In addition, the positive effect of each treatment on cooking yield and the WHC of the sausages was in the following order: combination of HP and STP ≥ addition of STP > HP treatment. The addition of STP and combined treatment of STP and HP resulted in a similar cooking loss and WHC to those of PC ($p > 0.05$). HP treatment led to a decrease in cooking loss of sausages compared with that in NC but not PC, regardless of treatment levels. HP treatment at 200 MPa had a similar WHC to that of PC, while HP treatment at 100 MPa had a similar WHC to that of NC ($p > 0.05$). Improved WHC in meat products by HP treatment has been reported (Hong et al., 2006). Grossi et al. (2012) explained that an increase in solubility of myofibrillar proteins by disruption of electrostatic interaction, hydrophobic interactions, and hydrogen bonding contributed to the enhancement of the WHC by HP. Overall, the combined effect of STP and HP on the cooking loss and WHC of sausages was greater compared with a single application of HP ($p < 0.05$) and similar to those of sausages containing STP ($p > 0.05$). Grossi et al. (2012) reported a synergistic effect of HP and fiber in low salt pork sausages with a significant improvement in the WHC.

3.2.2 | TPA

The addition of STP to sausages led to an increase in hardness compared with that of NC ($p < 0.05$), which was similar to the trend was observed in Experiment I (Table 5). A numerical increase (17% and 7% at 100 and 200 MPa, respectively) in the hardness of the sausages treated with HP was observed compared with NC, even though no significant difference was found between sausages treated with and without HP. This practical enhancement in texture properties could be caused by HP-induced improvement in meat gelation properties and emulsion stability, resulting from the altered solubility of swelled or fragmented proteins and changes in the binding ability among the proteins (Sun & Holley, 2010). Previous studies reported that HP treatment at 200 MPa improved texture properties and binding strength in comminuted meat products (Hong et al., 2006; Sikes et al., 2009). In addition, the individual application of 1.5% STP and HP (regardless of pressure levels) in sausages induced improved springiness and chewiness ($p < 0.05$). The addition of 3% STP in sausages did not improve the texture properties, except for hardness; however, the combined application of 3% STP and HP at 100 MPa increased the hardness, gumminess, and chewiness of the sausages significantly compared with those of NC. In particular, the combined application of 3% STP and HP at 100 MPa in sausages enhanced their hardness to levels comparable to that of PC ($p > 0.05$). In other words, in terms of texture properties, the combined application of HP (100 MPa) and STP (3%) in sausages might be an effective substitute for phosphate that minimizes the adverse effects on texture properties.

3.2.3 | Protein solubility

Changes of myofibrillar protein solubility are related to the meat quality and meat product (Marcos, Kerry, & Mullen, 2010). Thus, it is important to examine the effect of treatment with STP and HP on the myofibrillar protein solubility. As shown in Figure 1, SDS-PAGE patterns were unchanged whether the meat batter contained phosphate (Lane 1) or not (Lane 2). Similarly, Xiong et al. (2000) reported that the addition of phosphate caused no remarkable difference in chicken myofibrillar protein at an NaCl concentration of 1.2%. However, the protein band intensity changed when 3% STP was added to meat batter compared with PC and NC (Lane 3). The addition of 3% STP decreased the intensities of bands corresponding to

TABLE 4 pH, cooking loss, and water holding capacity (WHC) of sausages with sea tangle powder and treated at high-pressure in Experiment II

Treatment	Pressure (MPa)	pH	Cooking loss (%)	WHC (%)
PC[1]	0.1	6.23^{a}	2.44^{c}	78.53^{a}
NC[1]	0.1	6.02^{d}	17.95^{a}	62.35^{c}
0% STP[1]	100	6.02^{d}	11.06^{b}	69.57^{bc}
	200	6.06^{bcd}	10.22^{b}	73.38^{ab}
1.5% STP	0.1	6.06^{bcd}	2.20^{c}	77.73^{ab}
	100	6.06^{bcd}	4.50^{c}	73.40^{ab}
	200	6.09^{b}	2.50^{c}	75.37^{ab}
3% STP	0.1	6.05^{bcd}	1.84^{c}	81.39^{a}
	100	6.03^{cd}	3.59^{c}	78.65^{a}
	200	6.07^{bc}	3.18^{c}	81.30^{a}
SEM[2]		0.010	0.671	1.674

Notes. $^{a\text{-}c}$Values with different letters within the same column differ significantly ($p < .05$).

[1]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate, sea tangle powder, and high-pressure treatment; STP, sea tangle powder.

[2]*SE* of the mean ($n = 30$).

TABLE 5 Texture profile analysis of sausages with sea tangle powder and treated at high-pressure in Experiment II

Treatment	Pressure (MPa)	Hardness (N)	Springiness	Gumminess (N)	Chewiness (N)	Cohesiveness
PC[1]	0.1	104.75^{a}	0.70^{ab}	36.58^{a}	25.33^{a}	0.35^{a}
NC[1]	0.1	58.31^{e}	0.63^{b}	13.97^{c}	8.92^{f}	0.24^{b}
0% STP1	100	68.20^{cde}	0.76^{a}	17.17^{c}	13.03^{bcd}	0.25^{b}
	200	62.43^{de}	0.78^{a}	15.23^{c}	11.54^{cde}	0.24^{b}
1.5% STP	0.1	84.64^{bc}	0.77^{a}	17.63^{c}	13.50^{bc}	0.21^{b}
	100	75.58^{cd}	0.59^{b}	15.49^{c}	9.22^{ef}	0.21^{b}
	200	72.61^{cde}	0.71^{ab}	14.74^{c}	10.49^{def}	0.20^{b}
3% STP	0.1	79.33^{bcd}	0.60^{b}	16.73^{c}	10.10^{ef}	0.21^{b}
	100	93.65^{ab}	0.60^{b}	23.88^{b}	15.71^{b}	0.26^{b}
	200	71.04^{cde}	0.71^{ab}	15.93^{c}	11.23^{cdef}	0.22^{b}
SEM[2]		3.378	0.026	0.810	0.543	0.013

Notes. $^{a\text{-}c}$Values with different letters within the same column differ significantly ($p < .05$).
[1]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate, sea tangle powder, and high-pressure treatment; STP, sea tangle powder.[2]*SE* of the mean ($n = 30$).

myosin heavy chain (MHC) and actin, and increased the densities of bands corresponding to desmin and small molecule proteins under 30 kDa. In the HP-treated meat batter containing 3% STP (Lane 4 and 5), the changes in protein band densities were not significant; however, the intensities of some bands, including actinin, actin, and proteins less than 25 kDa, increased slightly compared with meat batter containing 3% STP. HP treatment in fibrous proteins of muscle led to increased solubility of myosin (Davis, 1981), actin, and other proteins (Iwasaki, Noshiroya, Saitoh, Okano, & Yamamoto, 2006), and caused disruption and dispersion into short filaments by swelling of myofibrils. The increased protein solubility by pressure-induced unfolding of soluble proteins improves the binding and gelling properties, which produce firmer structures (Sikes et al., 2009). As a result, a beneficial effect on the WHC of meat products was observed with reduced water loss after cooking, as shown in the present study (Table 4). However, myofibrillar protein solubility was affected more by the addition of STP than by HP treatment in this study. Further studies are needed to determine the reasons behind the changes in the protein solubility of meat batter caused by STP.

3.2.4 | Lipid oxidation, protein oxidation, and total aerobic bacteria

Lipid oxidation, protein oxidation, and total aerobic bacterial growth in sausages treated with 3% STP and/or HP (100 and 200 MPa) were determined for 14 days of refrigerated storage and compared with PC and NC sausages (Table 6). Generally, HP treatment cause oxidation of minced meat with increasing pressure and dramatic increase of oxidation has been reported over than 300 MPa (Cheah & Ledward, 1996). Similarly, no significant differences was observed between MDA level of PC and the sausages treated with 3% STP and HP at 200 MPa at day 1 in this study. After 14 days, the development of lipid oxidation in NC was notable; however, the sausages with added STP showed significantly lower values compared with that of PC ($P < 0.05$). Thus, it can be concluded that the addition of 3% STP and the combination of STP and HP treatment, regardless of pressure levels, decreased lipid oxidation significantly compared with that of PC during refrigerated storage ($p < 0.05$). There was no significant difference in protein oxidation among the treatments at day 1; however, significantly lower levels of protein oxidation were observed for the combination of STP and HP treatment (100 and 200 MPa) at day 14 of refrigerated storage compared with that of NC. Protein oxidation is linked with lipid oxidation and the both have similar radical chain reactions initiated by free radicals (Gardner, 1979). In the present study, a synergistic effect of STP and HP treatment was observed

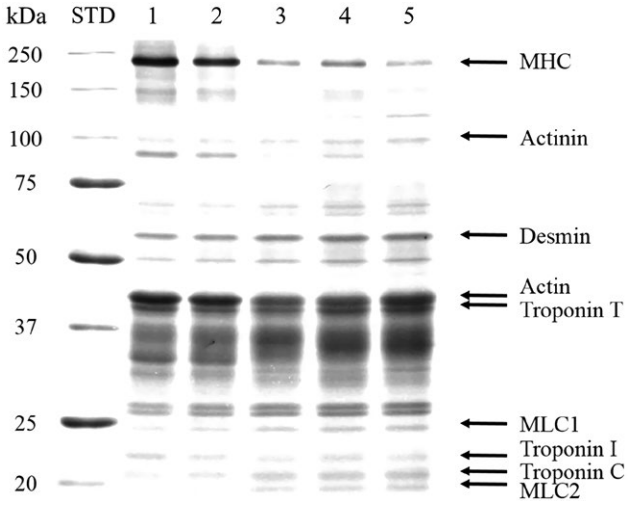

FIGURE 1 SDS-PAGE of meat batter with 3% sea tangle powder (STP) that was treated by high-pressure (HP) in Experiment II. Standard marker, STD; meat batter with 0.2% sodium pyrophosphate (PC), Lane 1; meat batter without added sodium pyrophosphate, STP, and HP treatment (NC), Lane 2; NC with 3% STP, Lane 3; NC with 3% STP and HP at 100 MPa, Lane 4; NC with 3% STP and HP at 200 MPa, Lane 5

TABLE 6 Changes in lipid oxidation (mg MDA/kg), protein oxidation (nmol carbonyls/mg protein), and total aerobic bacteria (Log CFU/g) of sausages containing 3% sea tangle powder and treated by high pressure during storage at 4°C in Experiment II

				Sea tangle powder (3%)			
	Storage days	PC[b]	NC[b]	0.1 MPa	100 MPa	200 MPa	*SEM*[2]
Lipid oxidation	1	0.07^{bcy}	0.07^{cy}	0.08^{bcx}	0.13^{ax}	0.11^{abx}	0.008
	14	0.12^{bx}	0.14^{ax}	0.06^{cy}	0.07^{cy}	0.06^{cy}	0.003
SEM[3]		0.004	0.004	0.003	0.008	0.007	
Protein oxidation	1	1.42	1.41	1.17^{y}	1.29	1.23	0.143
	14	1.63^{ab}	1.87^{a}	1.49^{abx}	1.28^{b}	1.23^{b}	0.120
SEM[3]		0.126	0.153	0.056	0.177	0.117	
Total aerobic bacteria	1	4.31^{ay}	4.21^{aby}	4.18^{aby}	4.01^{aby}	4.09^{b}	0.496
	14	6.05^{ax}	6.19^{ax}	5.04^{abx}	5.44^{abx}	4.62^{b}	0.283
SEM[3]		0.189	0.326	0.118	0.082	0.210	

Notes. Values with different letters (a, b) within the same row differ significantly ($p < .05$). Values with different letters (x, y) within the same column differ significantly ($p < .05$).

[1]PC, sausages with 0.2% sodium pyrophosphate; NC, sausages without sodium pyrophosphate, sea tangle powder, and high-pressure treatment; STP, sea tangle powder.[2]*SE* of the mean ($n = 15$).[3]*SE* of the mean ($n = 6$).

for the lipid and protein oxidation of sausages compared with PC or NC after storage. A previous study found that the application of HP treatment of a natural source containing bioactive components, such as carotenoid, tended to show a greater improvement in antioxidant activity compared with that of a non-pressurized one by extracting more bioactive components (Sánchez-Moreno, Plaza, de Ancos, & Cano, 2004). Thus, it is considered that a combined application of STP and HP treatment may inhibit lipid and protein oxidation in sausages as improved the activities of bioactive components present in STP.

The highest and the lowest total aerobic bacterial counts were observed in PC and sausages treated combined 3% STP and HP at 200 MP at both day 1 and 14 of refrigerated storage, respectively ($p < 0.05$). Moreover, the sausages with STP showed lower numbers of total aerobic bacteria compared with PC and NC at day 14. This result demonstrated the inhibitory ability of STP against bacterial growth, which agreed with a previous finding that sea tangle extracted by 70% and 90% ethanol showed antimicrobial effects against *Bacillus subtilis* and *Escherichia coli* (Oh, Oh, Kim, Lim, & Kim, 1998). The combination of 3% STP and HP treatment at 200 MPa showed a synergistic effect on the inhibition of microbial growth in the present study, even though it was noted previously that only HP great than 300 MPa could inhibit microbial growth (Smelt, 1998).

4 | CONCLUSION

The combinational use of HP (100 MPa) and STP (3%) improved the instrumental texture properties of phosphate-free emulsion-type sausage. In addition, it resulted in antioxidative and antimicrobial effect when compared with the addition of phosphate. Therefore, our results suggested that the combined application of STP and HP could be used as a substitute for phosphate in emulsion-type sausage.

ACKNOWLEDGMENTS

This work was supported by the Cooperative Research Program for Agriculture Science & Technology Development (Project No. 011617). Rural Development Administration, Korea.

ORCID

Juhui Choe http://orcid.org/0000-0003-4585-0327
Hae In Yong http://orcid.org/0000-0003-0970-4496
Hyun Jung Lee http://orcid.org/0000-0002-6891-8008
Hyun-Joo Kim http://orcid.org/0000-0002-4393-815X
Cheorun Jo http://orcid.org/0000-0003-2109-3798

REFERENCES

Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., & Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. *Trends in Food Science & Technology*, *45*(1), 37–49. https://doi.org/10.1016/j.tifs.2015.05.008

Cheah, P. B., & Ledward, D. A. (1996). High pressure effects on lipid oxidation in minced pork. *Meat Science*, *43*(2), 123–134. https://doi.org/10.1016/0309-1740(96)84584-0

Cho, M. G., Bae, S. M., & Jeong, J. Y. (2017). Egg shell and oyster shell powder as alternatives for synthetic phosphate: Effects on the quality of cooked ground pork products. *Korean Journal for Food Science of Animal Resources*, *37*(4), 571–578. https://doi.org/10.5851/kosfa.2017.37.4.571

Davis, J. S. (1981). The influence of pressure on the self-assembly of the thick filament from the myosin of vertebrate skeletal muscle. *Biochemical Journal*, *197*(2), 301–308. https://doi.org/10.1042/bj1970301

Fagan, J. M., Sleczka, B. G., & Sohar, I. (1999). Quantitation of oxidative damage to tissue proteins. *The International Journal of Biochemistry & Cell Biology*, *31*(7), 751–757. https://doi.org/10.1016/S1357-2725(99)00034-5

Gardner, H. W. (1979). Lipid hydroperoxide reactivity with proteins and amino acids: A review. *Journal of Agricultural and Food Chemistry*, *27*(2), 220–229. https://doi.org/10.1021/jf60222a034

Grossi, A., Søltoft-Jensen, J., Knudsen, J. C., Christensen, M., & Orlien, V. (2012). Reduction of salt in pork sausages by the addition of carrot fibre or potato starch and high pressure treatment. *Meat Science*, *92*(4), 481–489. https://doi.org/10.1016/j.meatsci.2012.05.015

Hong, G., Park, S., Kim, J., & Min, S. (2006). The effects of high pressure and various binders on the physico-chemical properties of restructured pork meat. *Asian-Australasian Journal of Animal Sciences*, *19*(10), 1484–1489. https://doi.org/10.5713/ajas.2006.1484

Ito, K., & Hori, K. (1989). Seaweed: Chemical composition and potential food uses. *Food Reviews International*, *5*(1), 101–144. https://doi.org/10.1080/87559128909540845

Iwasaki, T., Noshiroya, K., Saitoh, N., Okano, K., & Yamamoto, K. (2006). Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. *Food Chemistry*, *95*(3), 474–483. https://doi.org/10.1016/j.foodchem.2005.01.024

Jarvis, N., Clement, A. R., O'Bryan, C. A., Babu, D., Crandall, P. G., Owens, C. M., … Ricke, S. C. (2012). Dried plum products as a substitute for phosphate in chicken marinade. *Journal of Food Science*, *77*(6), S253–S257. https://doi.org/10.1111/j.1750-3841.2012.02737.x

Jeon, M. R., & Choi, S. H. (2012). Quality characteristics of pork patties added with seaweed powder. *Korean Journal for Food Science of Animal Resources*, *32*(1), 77–83. https://doi.org/10.5851/kosfa.2012.32.1.71

Jiménez-Escrig, A., & Sánchez-Muniz, F. J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, *20*(4), 585–598. https://doi.org/10.1016/S0271-5317(00)00149-4

Kim, H. W., Choi, J. H., Choi, Y. S., Han, D. J., Kim, H. Y., Lee, M. A., … Kim, C. J. (2010). Effects of sea tangle (*Lamina japonica*) powder on quality characteristics of breakfast sausages. *Korean Journal for Food Science of Animal Resources*, *30*(1), 55–61. https://doi.org/10.5851/kosfa.2010.30.1.55

Kim, I. S., Jin, S. K., & Ha, C. J. (2008). Improved quality properties of low-fat meat patties containing sweet persimmon powder during freeze storage. *Korean Journal for Food Science of Animal Resources*, *28*(2), 113–121.

Lahaye, M. (1991). Marine algae as sources of fibres: Determination of soluble and insoluble dietary fibre contents in some 'sea vegetables'. *Journal of the Science of Food and Agriculture*, *54*(4), 587–594. https://doi.org/10.1002/jsfa.2740540410

Lampila, L. E. (1993). Functions and uses of phosphates in the seafood industry. *Journal of Aquatic Food Product Technology*, *1*(3–4), 29–41. https://doi.org/10.1300/J030v01n03_04

Lullien-Pellerin, V., & Balny, C. (2002). High-pressure as a tool to study some proteins' properties: Conformational modification, activity and oligomeric dissociation. *Innovative Food Science & Emerging Technologies*, *3*(3), 209–221. https://doi.org/10.1016/S1466-8564(02)00045-0

Marcos, B., Kerry, J. P., & Mullen, A. M. (2010). High pressure induced changes on sarcoplasmic protein fraction and quality indicators. *Meat Science*, *85*(1), 115–120. https://doi.org/10.1016/j.meatsci.2009.12.014

Mariutti, L. R., Orlien, V., Bragagnolo, N., & Skibsted, L. H. (2008). Effect of sage and garlic on lipid oxidation in high-pressure processed chicken meat. *European Food Research and Technology*, *227*(2), 337–344. https://doi.org/10.1007/s00217-007-0726-5

Oh, C. K., Oh, M. C., Kim, S. H., Lim, S. B., & Kim, S. H. (1998). Antimutagenic and antimicrobial effect of ethanol extracts from sea-mustard and sea-tangle. *Korean Journal of Fisheries and Aquatic Sciences*, *31*(1), 90–94.

Pietrasik, Z., & Janz, J. A. M. (2009). Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. *Meat Science*, *81*(3), 523–532. https://doi.org/10.1016/j.meatsci.2008.10.006

Ruperez, P., & Saura-Calixto, F. (2001). Dietary fibre and physicochemical properties of edible Spanish seaweeds. *European Food Research and Technology*, *212*(3), 349–354. https://doi.org/10.1007/s002170000264

Sánchez-Moreno, C., Plaza, L., de Ancos, B., & Cano, M. P. (2004). Effect of combined treatments of high-pressure and natural additives on carotenoid extractability and antioxidant activity of tomato puree (*Lycopersicum esculentum* Mill.). *European Food Research and Technology*, *219*(2), 151–160. https://doi.org/10.1007/s00217-004-0926-1

Sikes, A. L., Tobin, A. B., & Tume, R. K. (2009). Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Innovative Food Science & Emerging Technologies*, *10*(4), 405–412. https://doi.org/10.1016/j.ifset.2009.02.007

Silva, J. L., & Weber, G. (1993). Pressure stability of proteins. *Annual Review of Physical Chemistry*, *44*(1), 89–113. https://doi.org/10.1146/annurev.pc.44.100193.000513

Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science & Technology*, *9*(4), 152–158. https://doi.org/10.1016/S0924-2244(98)00030-2

Sun, X. D., & Holley, R. A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of Food Science*, *75*(1), R17–R23. https://doi.org/10.1111/j.1750-3841.2009.01449.x

Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibres: Nutritional and technological interest. *Trends in Food Science & Technology*, *8*(2), 41–48. https://doi.org/10.1016/S0924-2244(97)01007-8

Trespalacios, P., & Pla, R. (2007). Synergistic action of transglutaminase and high pressure on chicken meat and egg gels in absence of phosphates. *Food Chemistry*, *104*(4), 1718–1727. https://doi.org/10.1016/j.foodchem.2007.01.077

Van Oeckel, M. J., Warnants, V. N., & Boucqué, C. V. (1999). Comparison of different methods for measuring water holding capacity and juiciness of pork versus on-line screening methods. *Meat Science*, *51*(4), 313–320. https://doi.org/10.1016/S0309-1740(98)00123-5

Xiong, Y. L., Lou, X., Wang, C., Moody, W. G., & Harmon, R. J. (2000). Protein extraction from chicken myofibrils irrigated with various polyphosphate and NaCl solutions. *Journal of Food Science*, *65*(1), 96–100. https://doi.org/10.1111/j.1365-2621.2000.tb15962.x

How to cite this article: Lee H, Choe J, Yong HI, et al. Combination of sea tangle powder and high-pressure treatment as an alternative to phosphate in emulsion-type sausage. *J Food Process Preserv*. 2018;e13712. https://doi.org/10.1111/jfpp.13712