A Thesis

For the Degree of Master of Science

Combined Effect of Natural Sources and High-pressure Treatment as an Alternative to Phosphate in Emulsion-type Meat Product

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Contents

page

List of Tables

1. Materials and Methods

2. Experiment I

3. Experiment II

4. Experiment III

List of Figures

1. Materials and Methods

2. Experiment II

3. Experiment III

List of Abbreviations

NC	Negative control
PC	Positive control
STP	Sea tangle powder
HP	High-pressure
WHC	Water holding capacity
TPA	Texture profile analysis
STD	Standard marker
MHC	Myosin heavy chain
MLC	Myosin light chain
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
FE-SEM	Field emission scanning electron microscopy
DNPH	2, 4-dinitrophenylhydrazine
PRB	Pyrophosphate relaxing buffer
TCA	Trichloroacetic acid
MDA	Malondialdehyde
SEM	Standard error of the mean

Abstract

The objective of this study was to investigate the suitability of natural powders and high-pressure (HP) processing as an alternative of phosphate in meat products.
Three experiments were conducted as follow.

Experiment I: Meat batters with 1, 2.5, and 5% of different natural powders (plum, persimmon, leg bone extract, and sea tangle) were prepared to find a substitute for phosphate. Physico-chemical properties of the meat batters containing different levels of natural powders were compared to meat batter added with/without 0.2% sodium pyrophosphate (PC, positive control; NC, negative control). Meat batters containing different levels of natural powders showed lower pH value compared to PC ($P < 0.05$). There were no significant differences in cooking loss between NC and meat batters added with natural powders except treatment with sea tangle powder (STP). The cooking loss decreased with increase in addition level of STP in meat batter. In conclusion, the addition of 5% STP could be suggested as a substitute for phosphate in terms of WHC of meat batters. However, meat batter with 5% STP was not appropriate as an alternative in meat products due to its strong flavor. Based on results, the addition levels of STP adjusted to 1, 2, and 3% of STP. The addition of 3% STP effectively decreased cooking loss in meat batter among the treatments with STP. Therefore, the addition level (3% STP) was selected to evaluate the suitability as an alternative of phosphate in meat product.

Experiment II: Based on the results of experiment I, the 3% STP was added to emulsion-type sausages and then was evaluated the physico-chemical and sensory properties compared to sausages with (PC) /without (NC) 0.2% sodium pyrophosphate. The sausage samples added with STP had similar cooking loss values to PC ($P > 0.05$). The addition of STP significantly increased water holding capacity (WHC) and instrumental hardness in sausage samples compared to NC (*P*

< 0.05). However, there were no significant differences in gumminess, chewiness, and cohesiveness between NC and sausages containing STP. For scanning electron microscopic photographs, PC had thick protein binding structure was observed in all treatments. Thick of strand of PC was thicker than other treatments and more binding structures were formed in PC and sausage with STP compared to NC. No significant difference in juiciness of sensory evaluation was shown between sausages containing STP and PC. Treatment added with STP had significantly higher springiness, hardness, and overall acceptability than NC ($P < 0.05$). Overall, the addition of STP lead to great WHC ($P < 0.05$), while it was not effective on overall texture properties emulsion-type sausage ($P < 0.05$) compared to PC. Therefore, it is necessary to find how to improve the texture property of emulsion-type sausage added with 3% STP.

Experiment III: In present study, HP treatment [0.1 (1 atm), 100, and 200 MPa] was applied to meat batters added with different levels of STP (0, 1.5, and 3%) for improving the texture property. Parts of the meat batters treated with HP were used to determine the pH value and salt solubilized protein content. The other parts were cooked for physio-chemical analysis and microbiological safety analysis of cooked sausages. The pH value of sausage was significantly increased by simultaneous application of STP (1.5 and 3%) and HP treatment (200 MPa). The sausages added with STP and treated with HP showed similar cooking loss and WHC to PC. Sausage added with 3% STP and treated HP at 100 MPa showed similar hardness value compared to PC ($P > 0.05$) and higher value of gumminess and chewiness than other treatments except PC ($P < 0.05$). Salt-soluble protein composition was also affected by addition of STP. The addition of STP leads to decrease in myosin heavy chain (MHC) and actin and increase in proteins smaller than 30 kDa compared to NC and PC. Especially, HP treatment tended to increase slightly in small molecule protein. Antimicrobial effect was observed in sausages with 3% STP and HP treatment at 200 MPa. The combined effect of STP and HP treatment was presented in inhibition of lipid oxidation, protein oxidation in sausages

compared to PC and/or NC regardless of pressure levels.
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.

Keywords: Phosphate, High-pressure, Sea tangle powder, Combination treatment

I. 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 and Janz, 2009). Phosphate increases the WHC by: i) an increasing the pH and ionic strength of the meat (Pietrasik and Janz, 2009); ii) dissociating and depolymerizing actomyosin cross-bridges (Trespalacios and Pla, 2007); and iii) enhancing the extraction of myofibrillar proteins (Xiong, 1999). Those extracted proteins influence on texture by gelation, emulsification, and meat binding (Xiong, 1999). 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 (Georgantelis et al., 2007; Nassu et al., 2003). Consequently, the demand for natural additives to synthetic additives has remarkably increased (Karabacak and Bozkurt, 2008) regardless of great effects of synthetic additives in recent years. Numerous studies have been conducted to find alternatives to synthetic additives in processed meat products (Georgantelis et al., 2007; Grossi et al., 2012; Jarvis et al. 2012; Karabacak and Bozkurt, 2008; Nassu et al., 2003). 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 texture and WHC, while minimizing adverse effects on the sensory property in meat products. Previous studies found that several natural sources have positive effects on WHC and texture in meat and meat product (Jarvis et al. 2012; Kim et al., 2008; Kim et al., 2010). Plum contains sorbitol and fiber which help retaining moisture, and antioxidant phenolic compound (Jarvis et al. 2015; Yıldız-Turp and Serdaroglu, 2010). The previous study showed that plum has potential as an alternative to phosphate since addition of 1.1% plum in marinate solution lead to similar WHC and tenderness in marinated chicken breast compared to meat samples added with sodium tripolyphosphate (Jarvis et al. 2012). Persimmon showed possibility to replace phosphate because of the antioxidative activity by tannin (Seo et al., 2000) and water-retention ability in pork patty (Kim et al., 2008). Sea tangle also has water-retention and binding ability because it contains alginate, a major dietary fiber (Jiménez-Escrig and Sánchez-Muniz, 2000). Previous study showed that addition of sea tangle powder enhanced WHC and texture properties of breakfast sausages (Kim et al., 2010) and would enhanced the flavor and antioxidative activity of phosphate-free final product because of the presence of glutamic acid and phenolic compounds (Ito and Hori, 1989). Bone extract could be expected to substitute phosphate in meat product because it contains little amount of pyrophosphate (Perkins and Walker, 1958). However, those natural sources (plum, persimmon, sea tangle, bone extract) have not been applied as an alternative to phosphate in meat product despite of their functions.

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 and Holley, 2010). Especially, enhancement in WHC and texture property resulted from gelation of proteins which caused by increase in solubility of myofilbrilar protein by HP processing (Colmenero, 2002) and it depends on

pressure levels (Lullien-Pellerin and Balny, 2002; Silva and Weber, 1993). According to previous studies, application of HP at less than 200 MPa generally provided better WHC or texture properties of meat products (Hong et al., 2006; Sikes et al., 2009). However, HP without additional treatment is insufficient as a substitute for phosphate in meat product manufacturing (Trespalacios and Pla, 2007). In addition, adverse effects of HP, including lipid oxidation and discoloration, have been reported (Mariutti et al., 2008). Mariutti et al. (2008) stated that oxidation caused by HP treatment could be inhibited by the addition of an antioxidant. Thus, 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 possibility of natural sources and HP processing in emulsion-type sausage as an alternative to phosphate. Three experiments were conducted to find optimum conditions for phosphate replacement. The effect of different level (1, 2.5, and 5%) of natural powder (plum, persimmon, leg bone extract, and sea tangle) in meat batter are screened and selected the best source based on the results of pH and cooking loss compared to sausage with or without phosphate (PC, positive control; NC, negative control) in Experiment I. After that the effect of a selected source [3% sea tangle powder (STP)] in emulsion-type sausage was investigated on physico-chemical properties and sensory evaluation compared to PC or NC (Experiment II). In Experiment III, HP [0.1 (1 atm), 100, and 200 MPa] were treated to meat batter containing 0, 1.5, and 3% of STP to improve the texture properties and the combined effect of STP and HP treatment on physico-chemical properties and antimicrobial activity against lipids and proteins of sausage was evaluated.

II. Materials and methods

2.1. Experimental design

A series of experiments was designed to replace phosphate with natural source (Fig. 1).

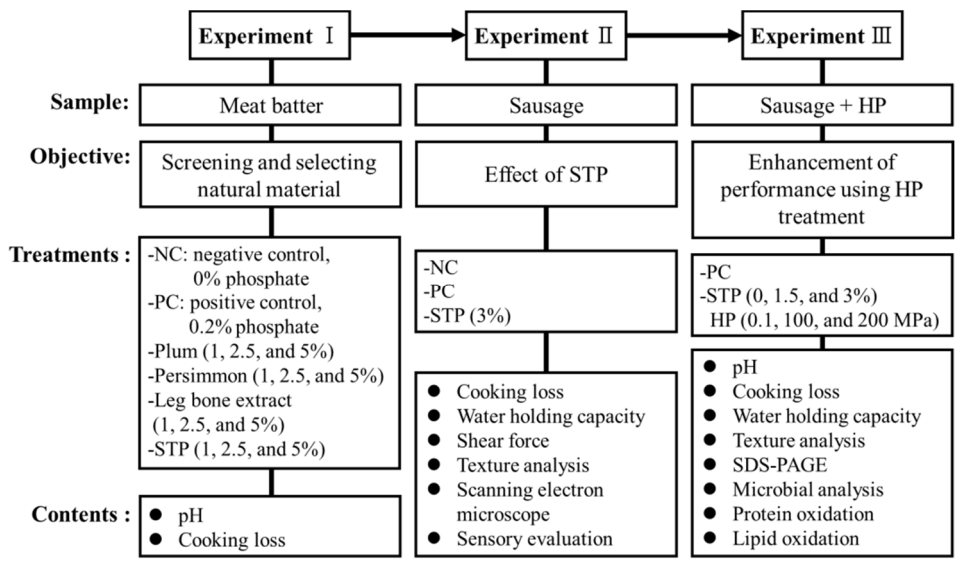

Fig. 1. Diagram illustrating the experimental procedure of the present study (STP, Sea tangle powder; HP, High-pressure)

2.1.1. Experiment I: Preliminary-test in the meat batter system

Pork hind leg meat and back fat were purchased from a local butcher (Seoul, Korea) and plum power (Sunsweet Ingredients, Walnut creek, California, USA), persimmon powder (Edentown FnB, Incheon, Korea), leg bone extract powder (Hwami, Incheon, Korea), and sea tangle powder (STP) (Hansalim, Seoul, Korea) were obtained as the candidate for alternative sources. Pork meat and back fat were grounded with a 6 mm plate (M-12S, Hankook Fujee Industries Co., Ltd.,

Hwaseong, Korea) and ground meat was mixed (CH180A, Kenwood, Havant, UK) with back fat, iced water, and additives as shown in Table 1. During the mixing, temperature was maintained below 13°C and meat batters with 0.2% sodium pyrophosphate (PC), without sodium pyrophosphate (NC), and NC with alternative powders (1, 2.5, and 5%) were manufactured.

Table 1. Formulation (%) of the meat batter for Experiment I

Ingredients	PC[1)]	NC[1)]	Treatment (%)		
			1	2.5	5
Pork meat	60	60	60	60	60
Back fat	20	20	20	20	20
Ice water	20	20	20	20	20
Total	100	100	100	100	100
Sodium chloride	1.2	1.2	1.2	1.2	1.2
Sodium pyrophosphate	0.2	-	-	-	-
Alternative powder[1)]	-	-	1	2.5	5

[1)]PC, meat batters with 0.2% sodium pyrophosphate; NC, meat batters without sodium pyrophosphate; Alternative powder (plum, persimmon, leg bone extract, and sea tangle).

2.1.2. Experiment II: Emulsion-type sausages containing sea tangle powder

Ground meat was mixed with back fat, iced water, and additives in a silent cutter depending on the formula of three treatments (PC, NC, and 3% STP) shown in Table 2. After emulsification, 100 g of each meat batter was obtained to measure the cooking loss. The remainder was stuffed in the collagen casing (2.5 cm diameter; NDX, Viscofan, Ceske Budejovice, Czech Republic). The sausages were vacuum-packaged with a low-density polyethylene/nylon bags (25 × 30 cm), with an oxygen permeability of 22.5 mL/m^2/24 h atm at 60% RH/25°C, and a water vapor permeability of 4.7 g/m^2/24 h 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 water holding capacity (WHC), texture profile analysis (TPA), microstructure, and sensory evaluation.

Table 2. Formulation (%) of emulsion-type sausage for Experiment II

Ingredients	PC[1)]	NC[1)]	Sea tangle powder (3%)
Pork meat	60	60	60
Back fat	20	20	20
Ice water	20	20	20
Total	100	100	100
Sodium chloride	1.2	1.2	1.2
Sodium pyrophosphate	0.2	-	-
STP	-	-	3
Egg white	1.5	1.5	1.5
Sugar	0.5	0.5	0.5
Spice mix	1	1	1
L-Ascorbic acid	0.05	0.05	0.05
Sodium nitrite	0.007	0.007	0.007

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

2.1.3. Experiment III: High-pressure (HP) treatment on emulsion-type sausage containing 3% STP

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] and the results were compared with sausages containing 0.2% sodium pyrophosphate (PC) and those without sodium pyrophosphate, STP, and HP treatment (NC). Meat batters were manufactured following the formulas in Table 3. 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, Ohio, 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 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).

Table 3. Formulation (%) of emulsion-type sausage for Experiment III

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.

2.2. Physico-chemical properties

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 values of each filtrated solution were measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.2.2. Cooking loss

In Experiment I and II, meat batters (30 g) were placed in petridish (60 × 15 mm) and vacuum-packaged with polyethylene bags, while stuffed meat batter in casing (approximately 100 g) was vacuum-packaged for cooking loss in Experiment III. Samples were heated for 30 min at 80°C in the water bath and weighed after cooling and removing the water on the surface and inside casing using paper towel. The weight changes of sausages before and after cooking were calculated as the percentage weight loss of a sample.

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

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:

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

A = Weight of sample (before compression-after compression)

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

2.2.4. Texture profile analysis (TPA)

Three replicates were measured for 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 with 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 NexygenPlusTM software (AMETEK Lloyd instruments Ltd., Fareham, UK), 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. Microstructure

Microstructure was analyzed using field emission scanning electron microscopy (FE-SEM) (SUPRA 55VP, Carl Zeiss, Oberkochen, Germany). Sausage samples cut into 5 × 5 × 2 mm and were fixed in Karnovsky's fixative at 4°C for 24 h. The fixed samples were washed with 0.05 M sodium cacodylate buffer for 10 min and it repeated three times. To post fixation, 2% osmium tetroxide and 0.1 M sodium cacodylate buffer (1:1, v/v) were added and placed the sample at 4°C for 2 h. The fixed samples were dehydrated with incremental concentrations of ethanol (30, 50, 70, 80, 90, and 100% for 10 min). Samples were critical point dried with CO_2, and mounted on aluminum sample holders, sputter-coated with platinum to 12 nm thickness and observed by FE-SEM with a magnification of 10000 ×.

2.2.6. Protein solubility

The salt solubilized protein content was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). 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., Incheon, Korea). 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/lane) were loaded onto 12.5% acrylamide gel with

a 4.5% stacking gel. After electrophoresis, the gels 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, California, USA) were used as molecular weight standards for SDS-PAGE. Stained gel images were captured using a ChemiDoc^{TM} XRS+ (Bio-Rad Laboratories Inc., Hercules, California, USA).

2.2.7. Lipid oxidation

Lipid oxidation of sausage samples were measured following the method of Jung, Nam, and Jo (2016). 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, Staufen, Germany) 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., Incheon, Korea). Each supernatant was filtered using a 0.2-μm PVDF syringe filter (Whatman PLC., Maidstone, UK) and collected in a vial. MDA was analyzed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific Inc., Waltham, Massachusetts, 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.

2.2.8. Protein oxidation

Carbonyl content was measured for protein oxidation in the samples, and protein carbonyls were determined by derivatizing them with 2, 4-dinitrophenylhydrazine (DNPH), as described by Fagan et al. (1999). The sample (5 g) was homogenized in 25 mL of pyrophosphate relaxing buffer (PRB at pH 7.4; 2.0 mM $Na_4P_2O_7$; 10 mM Tris–maleate; 100 mM KCl; 2.0 mM $MgCl_2$; 2.0 mM EGTA) by using a homogenizer (T25). Chromophores from the sample were removed by washing the homogenated sample (1 mL) with 10 mL of HCl-acetone (3:100, v/v) twice and were centrifuged at 2,265 × g for 15 min (Union 32R). After washing, 2 mL of PRB was added and 500 μL of each sample was divided into two test tubes to determine carbonyl content and protein concentration. One for carbonyl content was derivatized for 30 min with 500 μL of 10 mM DNPH in 2.0 N HCl, and the other for protein concentration was prepared by adding 500 μL of 2.0 N HCl, instead of DNPH solution. After that, 500 μL of 30% trichloroacetic acid (TCA) was added and kept in ice for 10 min. Supernatant was removed after centrifuge at 10,000 × g for 10 min (HM-150Ⅳ). In case of sample for carbonyl content was washed with 1 mL of 20% TCA once, followed by 3 washes with 2 mL of ethanol–ethyl acetate (1:1, v/v) to remove excess DNPH. The pellets of two test tubes (carbonyl content and protein concentration) were solubilized in 2 mL of 6.0 M guanidine hydrochloride and 20 mM potassium dihydrogen phosphate (pH 2.3) at 4°C for 24 h. Before measuring of absorbance, samples were centrifuged at 10,000 × g for 10 min (HM-150Ⅳ). Carbonyl contents and protein concentration were measured at 380 nm and 280 nm respectively using spectrophotometer (DU 530, Beckman, Instruments Inc., Fullerton, California, USA). The amount of protein was calculated using a standard curve that was prepared using bovine serum albumin (Sigma-Aldrich, St. Louis, Missouri, USA). The carbonyl content was calculated using an absorption coefficient of 22,000 $M^{-1} \cdot cm^{-1}$ at 380 nm for

the formed hydrazones. The carbonyl content was reported as nmol carbonyl per mg protein.

2.3. 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 75°C. 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.

2.4. Microbial analysis

Total aerobic bacteria were analyzed for 14 days of storage at 4°C. Each sausage 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 decimally diluted with sterile saline solution. Total plate count agar (Difco Laboratories, Detroit, Mich, USA) was used as a medium for enumeration of microorganisms. A 100-μL aliquot of each dilution was spread, in triplicate, on the appropriate medium plates. The plates were and incubated at 37°C for 48 h. After incubation, the colonies were counted and expressed as log CFU/g.

2.5. Statistical analysis

One-way analysis of variance was performed with a completely randomized design using the procedure of General Linear Model. The statistical model included

the fixed effect of STP content, HP treatment, and phosphate content and random effect 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, North Carolina, USA) with the confidence level of $P < 0.05$. Mean values and standard errors of the mean were reported. All experimental procedures were conducted in triplicate with two observation numbers except for sensory analysis.

III. Results and discussion

Experiment I

The pH value and cooking loss of meat batter were affected by addition of various natural sources (Table 4). As expected, the highest pH value was observed in meat batter with 0.2% sodium pyrophosphate (PC) and Lampila (1993) also found that application of certain phosphates in meat leads to an increase in pH of muscle product. The pH values of meat batters containing plum and persimmon powder were significantly lower than that of meat batter without sodium pyrophosphate (NC) in all concentrations (1, 2.5, and 5%), while addition of leg bone extract powder and STP in meat batter showed similar ($P > 0.05$) or significantly higher pH value compared to NC. Previous studies found that increase in addition amount of plum and persimmon in meat patties lead to decrease of the pH value (Kim et al., 2008; Yıldız-Turp and Serdaroglu, 2010). Yıldız-Turp and Serdaroglu (2010) reported that the pH value decreased from 5.9 to 5.4 in beef patty with increasing amount of plum puree. Kim et al. (2008) also observed the decrease of the pH value in pork patty when 6% persimmon powder was added. Acidity of fruit probably influenced on pH value of meat batter. In contrast, the pH value of meat batters containing leg bone extract powder increased with increasing addition level ($P < 0.05$) which was due to presence of phosphate from leg bone (Perkins and Walker, 1958). There were no significant differences in pH values of meat batters regardless of addition amount of STP and it might be due to the pH values of STP itself (5.80). Similarly, Kim et al. (2010) found meat batter had pH values closer to pH value of STP ($P < 0.05$) with increasing addition of STP.

Cooking loss of meat batter was presented as a ratio (cooking loss of each treatment based on cooking loss of NC) in Table 4. Cooking loss is one of the

indicators for WHC of meat (Razminowicz et al., 2006) and is important factor because it affects to sensory quality such as appearance and juiciness (Aaslyng et al., 2003). PC shows relatively the lowest cooking loss among all treatments. The addition of natural powders in meat batter except for STP had similar cooking loss compared to NC regardless of addition levels, while cooking loss of meat batter with STP significantly lower than that of NC. In addition, a significant decrease was observed in cooking loss of meat samples with an increase of STP concentration. The WHC of meat depends on its pH value (Honikel et al., 1981). The closer pH value to isoelectric point of meat (5.4) increases the cooking loss because the most charged groups in protein are attracted to each other and result in a reduction of interstitial space at isoelectric point (Lusby et al., 1981). Thus, PC with the highest pH value showed the lowest cooking loss. In this study, the addition of STP in meat batter effectively decreased cooking loss regardless of pH value. A previous study reported decrease of cooking loss when 3% STP added in breakfast sausage (Kim et al., 2010). Previously it was shown that dried marine algae has great WHC due to the presence of dietary fiber which absorbs water about 20 times of their dry matter volume (Jiménez-Escrig and Sánchez-Muniz, 2000; Kuda et al., 1997).

Even though the lowest cooking loss was presented at meat batter added with 5% of STP, it is not appropriate as an alternative because of the strong flavor of sea tangle itself from the meat products. Thus, addition level of STP was rearranged to 1, 2, and 3%. The results of pH value (5.88) and cooking loss (27.95%) indicated that addition of 3% of STP is suitable because it has similar value to that of PC (data was not shown).

Table 4. pH and cooking loss of each meat batter that containing individual alternative sources in Experiment I

Treatment	Concentration (%)	pH	Cooking loss[3)]
PC[1)]		6.03^{a}	0.21^{f}
NC[1)]		5.69^{d}	1.00^{bc}
Plum powder	1	5.62^{e}	1.00^{bc}
	2.5	5.55^{ef}	1.02^{bc}
	5	5.48^{f}	1.03^{abc}
Persimmon powder	1	5.54^{f}	1.19^{a}
	2.5	5.40^{g}	1.14^{ab}
	5	5.22^{h}	1.13^{ab}
Leg bone extract powder	1	5.73^{cd}	1.10^{abc}
	2.5	5.75^{cd}	1.10^{abc}
	5	5.84^{b}	1.15^{ab}
Sea tangle powder	1	5.78^{bc}	0.94^{cd}
	2.5	5.76^{bcd}	0.80^{d}
	5	5.74^{cd}	0.58^{e}
SEM[2)]		0.014	0.033

[1)]PC, meat batters with 0.2% sodium pyrophosphate; NC, meat batters without sodium pyrophosphate

[2)]Standard error of the mean (n=12).

[3)] Cooking loss was expressed as the ratio of cooking loss in each treatment to that of NC.

[a-h]Values with different letters within the same column differ significantly ($P < 0.05$).

Experiment II

The WHC, indicating water-retaining ability, is one of the factors that determine meat quality because it affects sensory properties in meat products significantly (Pospiech and Montowska, 2011; Van Oeckel et al., 1999). In the present study, lower cooking loss and higher WHC were observed for sausages containing STP compared with those of NC ($P < 0.05$) (Table 5). Moreover, the cooking loss of sausages with 3% STP was similar to that of PC, indicating that STP could enhance the WHC of phosphate-free sausages effectively. Several studies have shown that the WHC of meat product improved when STP was added because of the presence of dietary fiber such as alginate (Jeon and Choi, 2012; Kim et al., 2010; Oh and Lim, 2011). 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 5). 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 and 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 and Saura-Calixto, 2001; Thebaudin et al., 1997).

Table 5. Physico-chemical traits of sausage containing 3% sea tangle powder in Experiment II

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

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

[2)]Standard error of the mean (n=9).

[a-c]Values with different letters within the same row differ significantly ($P < 0.05$).

The effect of STP on microstructure of sausages was observed using scanning electron microscopy (Fig. 2). Salt soluble myofibrillar proteins act as major components of comminuted meat matrix by forming interfacial protein film on the surface of fat globules. Thus, solubilization of salt soluble myofibrillar proteins is important to generate great quality of emulsion-type meat products (Gordon and Barbut, 1992). The marked circles in Fig. 2 indicated tread-like protein strands, which are known as connecting the protein matrix and some fat globules, usually seen on the surface of fat globules (Gordon and Barbut, 1992). In terms of extent formation and thickness of binding structure, PC showed the strongest protein binding structure (Fig. 2A) compared to other treatments. It may be caused by enhanced extraction of myofibrillar protein by phosphate (Xiong, 1999). Binding structure of sausage with STP (Fig. 2C) formed more strands in the structure compared to NC (Fig. 2B). According to previous study, the dietary fiber from seaweeds improved the physicochemical properties by affecting the matrix structure of the meat gel/emulsion system and emulsion stability in meat products (Cofrades et al., 2008; Kim et al., 2010). The result could suggest that WHC and texture properties are affected by extent of binding formation.

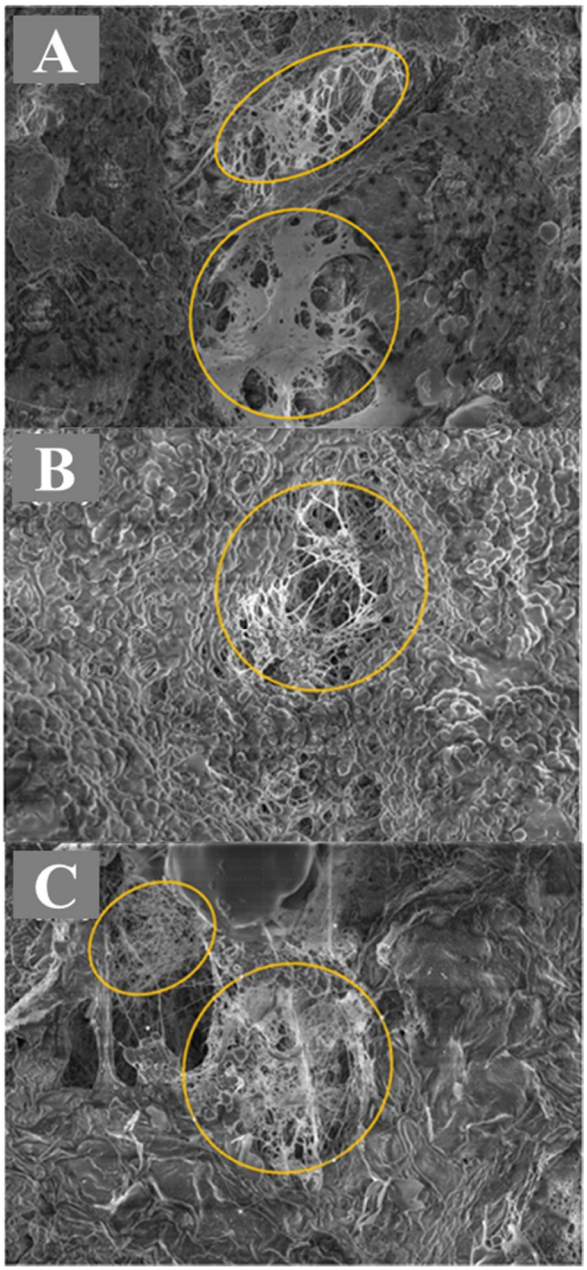

Fig. 2. Scanning electron photomicrographs of (A) PC, sausage with 0.2% sodium pyrophosphate; (B) NC, sausage without sodium pyrophosphate; (C) NC with 3% sea tangle powder

There were no significant differences in color, flavor, and juiciness in the sensory evaluation between PC and sausages with 3% STP (Table 6). 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 (Cofrades et al., 2008; Jeon and 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 and 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 II, the WHC was comparable, but the texture properties of sausages with STP should be enhanced by the substitution of phosphate on the basis of instrumental and sensory evaluations. Therefore, HP was applied to improve the texture properties and to add other advantages.

Table 6. Sensory analysis of sausage containing 3% sea tangle powder in Experiment II

Traits[3)]	PC[1)]	NC[1)]	Sea tangle powder (3%)	SEM[2)]
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

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

[2)]Standard error of the mean (n=9).

[3)]9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely).

[a-c]Values with different letters within the same row differ significantly ($P < 0.05$).

Experiment III

No significant differences were observed in the pH values of meat samples with STP compared with those of NC and results showed similar trend in Experiment I (Table 7). 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 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 and 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 $\geq$ 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 ($P > 0.05$). Several studies reported the HP treatment improved the WHC in meat products (Hong et al., 2006; Zheng et al., 2015). Grossi et al. (2012) explained that increase in solubility of myofibrillar proteins by disruption of electrostatic and hydrophobic interactions, and hydrogen bonding contributed to the enhancement in 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 significant improvement in the WHC.

Table 7. pH, cooking loss, and water holding capacity (WHC) of sausage added different concentration of sea tangle powder and treated high-pressure in Experiment III

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% sea tangle powder	100	6.02^{d}	11.06^{b}	69.57^{bc}
	200	6.06^{bcd}	10.22^{b}	73.38^{ab}
1.5% sea tangle powder	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% sea tangle powder	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

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

[2)]Standard error of the mean (n=30).

[a-d]Values with different letters within the same column differ significantly ($P < 0.05$).

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 II (Table 8). 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 and 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.

Table 8. Texture profile analysis of sausage added different concentration of sea tangle powder and treated high-pressure in Experiment III

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% sea tangle powder	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% sea tangle powder	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% sea tangle powder	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

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

[2)]Standard error of the mean (n=30).

[a-f]Values with different letters within the same column differ significantly ($P < 0.05$).

Changes of myofibrillar protein solubility are related to the meat quality and meat product (Marcos et al., 2010). Thus, it is important to examine the effect of treatment with STP and HP on the myofibrillar protein solubility. As shown in Fig. 3, 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 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) and actin and other proteins (Iwasaki et al., 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. 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.

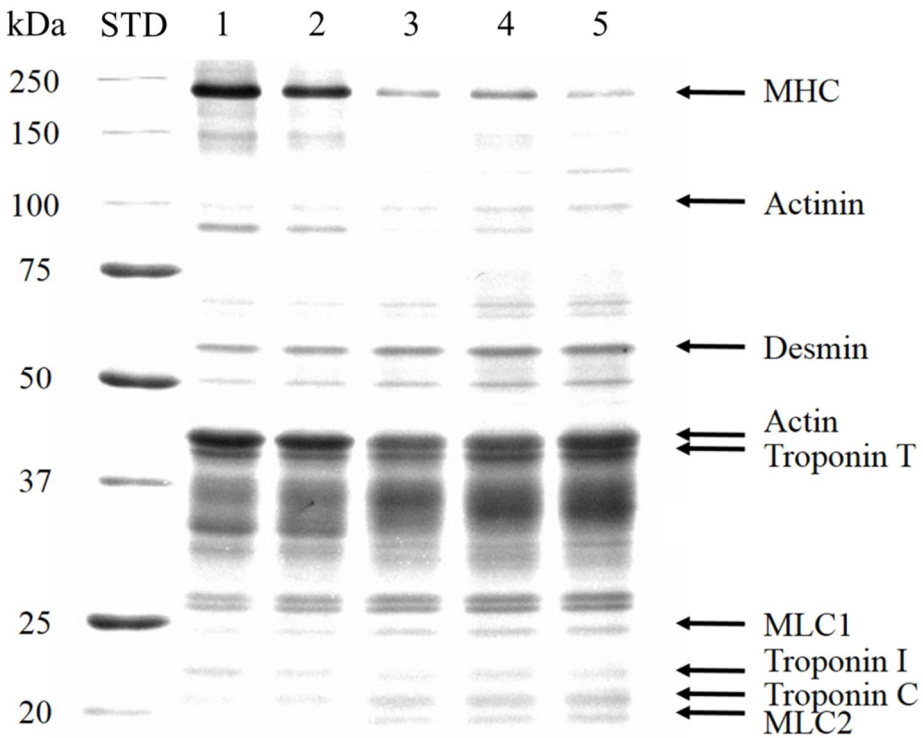

Fig. 3. SDS-PAGE of meat batter with 3% sea tangle powder (STP) that was treated by high-pressure (HP) in Experiment II. STD, Standard marker; Lane 1, meat batter with 0.2% sodium pyrophosphate (PC); Lane 2, meat batter without added sodium pyrophosphate, STP, and HP treatment (NC); Lane 3, NC with 3%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

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 9). The highest values and the lowest value for lipid oxidation were observed in the sausages treated with 3% STP and HP at 200 MPa and NC treatment at day 1 of refrigerated storage, respectively ($P < 0.05$). After 14 days, the development of lipid oxidation in PC 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 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 et al., 2004). Thus, it is considered that a combined application of STP and HP treatment would inhibit lipid and protein oxidation in sausages as improved the activities of bioactive components present in STP (Oh et al., 1998).

The highest and the lowest total aerobic bacteria 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 et al., 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).

Table 9. Changes in lipid oxidation (mg MDA/g), 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 III

	Storage days	PC[1)]	NC[1)]	Sea tangle powder (3%)			SEM[3)]
				0.1 MPa	100 MPa	200 MPa	
Lipid oxidation	1	34.73^{aby}	33.02^{by}	35.89^{aby}	36.09^{aby}	39.08^{ax}	1.149
	14	56.15^{ax}	46.20^{abx}	42.41^{bx}	43.41^{bx}	34.60^{by}	2.712
SEM[2)]		3.829	1.557	1.432	1.516	0.498	
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[2)]		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[2)]		0.189	0.326	0.118	0.082	0.210	

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

[2)] Standard error of the mean (n=6), [3)](n=15).

[a,b]Values with different letters within the same row differ significantly ($P < 0.05$).

[x,y]Values with different letters within the same column differ significantly ($P < 0.05$).

Ⅳ. Conclusion

Phosphate is a versatile additive in processed meat manufacturing. The addition of STP improved the WHC of phosphate-free meat products with no negative effect on overall acceptability in sensory evaluation; however, the texture properties were not satisfactory. The combination of HP (100 MPa) and STP (3%) improved the instrumental texture properties compared with a single application of STP. Moreover, the antioxidant and antimicrobial effects of the combined treatment were similar or greater than those of phosphate. Therefore, our results suggested that the combined application of STP and HP could substitute for phosphate in emulsion-type sausage effectively.

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Ⅵ. Summary in Korean

최근 건강에 대한 관심이 높아짐에 따라 합성 첨가물에 대한 우려가 나타나면서 합성 첨가물을 대체하기 위한 연구들이 꾸준히 진행되고 있다. 육제품에 사용되는 첨가제 중 인산염은 특히 육제품의 보수력, 조직감을 형성하는데 큰 역할을 하고 있는데 인산염의 경우 다른 첨가제에 비해 대체 연구가 적고 현재까지 적절한 대체 물질을 찾지 못하였다. 따라서 본 연구에서는 천연물 소재 분말(자두, 감, 사골 추출물, 다시마)와 초고압 처리를 이용해 유화형 소시지 내 첨가되는 인산염을 대체하고자 하였고 그에 따라 실험 I(소재 선정), II(대체 가능성 확인), III(대체 시 문제 보완)으로 나누어 연구를 진행하였다.

실험 I은 인산염 대체 소재와 적정 농도를 결정하기 위해 인산염 대체 가능성이 있는 천연물을 각 1, 2.5, 5%의 농도로 육반죽에 첨가하여 제조하였고 각 처리군의 pH와 가열 감량을 분석하였으며 그 결과를 0.2%의 인산염이 첨가된 양성 대조군과 인산염이 첨가되지 않은 음성 대조군의 결과와 비교해서 인산염 대체 효과를 확인 하였다. 천연물로 인산염을 대체한 모든 처리구에서 양성 대조군에 비해 유의적으로 낮은 pH와 높은 가열 감량을 나타냈다. 천연물로 인산염을 대체하였을 때, 대체적으로 음성 대조군과 유의적 차이를 보이지 않은 반면에 다시마 분말을 첨가는 음성 대조군에 비해 유의적으로 낮은 가열 감량이 확인되었고 다시마 분말 함량이 많아질수록 가열 감량이 줄어드는 것으로 확인되었다. 따라서 여러 대체제 중 5%의 다시마 분말을 첨가하였을 때 가열감량이 가장 효과적으로 감소하였지만 다시마 자체 풍미가 너무 심하게 나타났기 때문에 첨가 함량을 1, 2, 3%로 재조정하여 실험을 다시 진행하였고,

실험 결과 처리군 중 가장 낮은 가열감량을 나타낸 3% 다시마 분말 첨가가 인산염을 대체하기에 가장 적합한 것으로 판단 하였다.

실험 II에서는 실험 I의 결과를 바탕으로 유화형 소시지 제조 시, 3% 다시마 분말의 인산염 대체 가능성을 확인하고자 하였고 3% 다시마를 첨가한 소시지의 물리화학적 특성(가열감량, 보수력, 전단력, 조직감, 미세 구조) 분석과 관능 평가 분석을 실시하여 양성 대조군과 음성 대조군의 결과와 비교하였다. 3% 다시마 분말을 첨가한 소시지는 가열 감량과 전단력에서 양성 대조군과 유의적 차이가 없었고 보수력과 견고성은 양성 대조군에는 미치지 못하였지만 음성 대조군 보다는 유의적으로 향상되었다. 반면, 탄력성은 처리군간에 유의적 차이가 없었고 심지어 검성, 씹힘성, 응집성은 음성 대조군과 유의적 차이가 나타나지 않았다. 주사전자 현미경으로 소시지의 미세구조를 확인한 결과 모든 소시지에서 실처럼 생긴 단백질 결합 구조를 확인 할 수 있었다. 양성 대조군에서 가장 굵고 많은 단백질 결합이 나타났으며 음성 대조군에 비해서 다시마 분말을 첨가한 소시지에서 실처럼 생긴 단백질 결합 구조가 더 많이 형성된 것을 확인되었다. 관능 평가 결과, 다시마를 첨가는 색, 풍미, 그리고 맛의 변화는 부정적인 영향을 나타내지 않았으며 다즙성은 양성 대조군과 비교해 유의적 차이가 없을 만큼 향상되었다. 하지만 탄력성, 견고성 및 종합적 기호도에서는 음성 대조군보다는 유의적으로 높았지만 양성 대조군의 조직감 특성만큼 향상되지 않았다. 따라서 유화형 소시지 제조 시 인산염을 대체하기 위해 다시마 분말을 첨가하는 것은 인산염의 보수력을 대체하기에는 충분하지만 조직감적 특성은 향상시킬 필요성이 있는 것으로 사료된다.

실험 III은 실험 II의 결과에 따라 다시마 첨가된 소시지의 조직감 형성을 돕기 위해 초고압을 처리하였으며 그에 따른 효과를 확인하기 위해 다시마 분말 0, 1.5, 3%가 첨가된 소시지 육반죽에 100 과 200 MPa의

초고압 처리 하였고 물리화학적 분석(pH, 가열 감량, 보수력, 조직감, SDS-PAGE)과 저장 실험(총 호기성 미생물, 단백질 산화, 지방 산화)을 통해 그 효과를 양성 대조군의 결과와 비교하였다. 소시지의 pH는 다시마 분말의 첨가와 초고압 처리를 동시에 하였을 때 음성 대조군에 비해 유의적으로 증가하는 것을 확인하였다. 한편, 초고압 처리에 따른 보수력 향상은 다시마 무첨가구에서만 나타났지만, 다시마 분말 첨가에 의해서는 가열 감량이 감소하고 보수력이 증가하여 양성 대조군과 유의적 차이를 보이지 않는 것을 확인하였으며 다시마 분말 첨가와 초고압에 의한 시너지 효과 또한 확인되었다($P < 0.05$). 조직감 측정 결과, 다시마 분말을 첨가하고 100 MPa을 처리하였을 때, 견고성이 NC에 비해 향상되는 것을 확인하였다. 처리군 중 3% 다시마 분말과 100 MPa을 처리한 소시지의 견고성이 양성 대조군과 유의적 차이가 없고 검성과 씹힘성은 양성 대조군을 제외한 모든 처리군에 비해 유의적으로 높게 나타나면서 조직감 특성이 다른 처리군들에 비해 양성 대조군 수치에 가장 가깝게 향상된 것으로 나타났다. 염용성 단백질 추출은 다시마 첨가와 초고압 처리에 의해 영향을 받았으며 다시마 분말 첨가에 의해 상대적으로 MHC와 actin이 감소하고 30 kDa 이하의 단백질이 증가하였고 초고압 처리에 의해서는 작은 단백질들이 상대적으로 증가하는 것이 확인되었다. 저장 실험 결과, 3% 다시마 분말의 첨가와 초고압(200 MPa) 동시 처리는 저장 14일 후 총 호기성 미생물의 성장이 PC에 비해 저해된 것을 확인하였고 3% 다시마 소시지의 지방 산화와 단백질 산화는 초고압 처리(100과 200 MPa)에 의해 각각 PC와 NC보다 유의적으로 산화의 진행이 감소된 것을 확인하였다.

결론적으로, 3%의 다시마 분말이 첨가된 유화형 육제품은 양성 대조군과 비슷한 보수력을 나타내며, 향상된 견고성, 단백질 용해도뿐만 아니라 지방 산화 억제를 보였기 때문에 인산염을 대체하기에 적합한 소재

라고 판단된다. 게다가 3% 다시마 분말과 초고압 동시 처리는 3% 다시마 분말이 첨가된 소시지에 비해 견고성, 검성, 씹힘성을 더욱 효과적으로 향상시켰고 뿐만 아니라, 양성대조군과 비교해 비슷하거나 더 뛰어난 향균 및 항산화 효과를 나타냄으로 저장성 향상이 기대된다. 따라서, 다시마 분말과 초고압 처리를 동시에 적용해 인산염 무첨가 육제품을 생산하는 것이 가능할 것으로 판단된다.