A Dissertation for the Degree of Master

Effect of oyster mushroom (*Pleurotus ostreatus*) powder on emulsion-type sausage as a phosphate alternative

유화형 소시지에서 느타리버섯 분말의 인산염 대체 효과

August 2022

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Abstract

Effect of oyster mushroom (*Pleurotus ostreatus*) powder on emulsion-type sausage as a phosphate alternative

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This research evaluated the potentiality of oyster mushroom powder (OMP) as a phosphate alternative by improving emulsion stability of emulsion-type sausage. Sausage samples without phosphate (NC), with 0.2% sodium triphosphate (PC), and with 1 and 2% OMP (M1 and M2) were prepared. The OMP addition improved the physicochemical properties of sausage, effectively prevented lipid oxidation, and delayed the growth of aerobic bacteria during 28 days of cold storage. The M1 and M2 improved the emulsion stability similar to PC. M2 had the highest water holding capacity and apparent viscosity and the lowest cooking loss (P<0.05). The addition of OMP resulted in different textural characteristics from that of phosphate due to the formation of emulsion structures randomly

entrapped by filament-like components, which were derived from polysaccharides or the conjugates between polysaccharides and proteins. According to the results of this study, emulsion stability promoted by OMP was mainly due to the polysaccharides, which are involved in enhancing viscosity and steric hindrance. Considering the improved quality of sausages by the stabilized emulsion structure and long-term storage stability, OMP may be used as a phosphate substitute.

Keywords: Oyster mushroom, Phosphate alternative, Emulsion-type sausage, Polysaccharide.

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List of Abbreviations

a^*	:	Redness
$ABTS^+$	:	2, 2'-azinobis (3-ethylbenzothiazol ine-6-sulfonic acid) radical cation
ANOVA	:	Analysis of variance
b^*	:	Yellowness
CLSM	:	Confocal laser scanning microscopy
DDW	:	Distilled deionized water
DPPH	:	2, 2-diphenyl-1-picryl-hydrazyl-hydrate
EDTA	:	Ethylenediaminetetraacetic acid
FTIR	:	Fourier-transform infrared spectroscopy
HPP	:	High-pressure processing
IC50	:	The half maximal inhibitory concentration
L^*	:	Lightness
M1	:	1% OMP
M2	:	2% OMP

MDA	:	Malondialdehyde
NC	:	Negative control
OMP	:	Oyster mushroom powder
PC	:	Positive control
PU	:	Power ultrasound
SEM	:	Scanning electron microscopy
SHMP	:	Sodium hexametaphosphate
STPP	:	Sodium tripolyphosphate
TAB	:	Total aerobic bacteria
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
TSPP	:	Tetrasodium pyrophosphate
WHC	:	Water holding capacity
WMP	:	Winter mushroom powder

Chapter I.

Literature review

1.1. Phosphate as a food additive

1.1.1. Definition

Phosphates, the essential nutrient for human health, perform vital functions in the growth of cells and tissues, energy transfer, and signaling by involving in many metabolic pathways (Thangavelu et al., 2019). They are naturally present in the form of organic esters in foods such as cereals, eggs, meat, and potatoes. They have been served as acidulants, gel accelerants, and emulsifiers in food materials (Thangavelu et al., 2019).

Phosphates were classified into several types depending on their pH and solubility in water; TSPP, STPP, and SHMP (Alvarado et al., 2007). SHMP generally works very well in the near-neutral pH range, while TSPP and sodium triphosphate STPP work best in alkaline conditions (Long et al., 2011). Commonly, alkaline phosphates such as TSPP and STPP would be used to improve water holding capacity by enhancing pH away from the isoelectric point (Alvarado et al., 2007). In the meat industry, TSPP and STPP have been used in complex forms in processed meat because of their different functions; TSPP has a considerably high pH (about 10.2), and STPP has a high solubility in water (Long et al., 2011). Meanwhile, SHMP, a type of acid phosphate, could share most of the effects (retarding microbial growth,

reducing lipid oxidation, and stabilizing the meat color by chelating free divalent cations) of alkaline phosphates but decrease WHC (Cobos et al., 2015). Therefore, different types of phosphates could be used for various processed meat depending on the production process and formulation (Long et al., 2011).

1.1.2. Application for food materials

Phosphates could be added in the manufacturing step of processed foods such as coffee whiteners, baked foods, sliced cheeses, and processed meat products such as ham, sausages, and surimi (Lucina et al., 2017). It plays a role in increasing WHC, enhancing emulsifying capacity, and improving texture as an acidity regulator in food materials (Yong et al., 2020). Moreover, phosphates could extend the shelf life of foods by chelating divalent metal ions to retard oxidation and microbial growth (Choe et al., 2018).

Although these benefits of phosphate, dietary intake of phosphate needs to be controlled because excessive intake carries the risk of causing hyperphosphatemia, which is highly associated with cardiovascular disease (Pinton et al., 2019). Generally, the acceptable daily intake of phosphorus for a healthy adult is 40 mg/kg body weight per day (Younes et al., 2019). Therefore, the United States Department of Agriculture notes that phosphate concentrations do not exceed 0.5% by weight of the finished product. Several countries limit phosphate usage by legislation to 0.5%, although it is prohibited in others (Cobos et al., 2015). Meanwhile, in Korea, no restriction on adding phosphate to processed meat products, but it has been generally used at a level of about 0.2-0.3% (Kim et al., 2017).

1.1.3. Functions

1.1.3.1. Enhancing water holding capacity

Phosphate is used in manufacturing emulsified meat products as stabilizers and emulsifiers, the main function is to enhance the WHC by increasing the pH of the meat emulsion (Pinton et al., 2019; Thangavelu et al., 2019). It can change ionic charge distributions with sodium chloride and promotes myofibrillar protein isolation and dissolution (Long et al., 2011; Xu et al., 2022). Consequently, an increased level of swollen myofibrillar proteins can improve the emulsion stability, water, and fat retention capacity, and stabilize gel formation during cooking in meat products (Câmara et al., 2020; Pinton et al., 2019). These physical properties are improved by expanding the space by the repulsion between myofibrillar proteins inside the emulsified sausage. Based on this role, phosphate improves sensory quality, including cooking yield, texture, tenderness, and juiciness (Choe et al., 2018). The mechanism of these quality improvements by phosphate is as follows: i) Phosphates increase the pH of the meat product, causing it to move away from its isoelectric point; ii) Phosphates chelate Ca^{2+} ions, which form actomyosin complex. Then, actomyosin is inhibited from binding or becomes dissociated from actin and myosin, leading to an increase in the solubilization of meat proteins through depolymerization of thick and thin filaments (Long et al., 2011; Thangavelu et al., 2019).

1.1.3.2. Antioxidant effect

Lipid oxidation is one of the crucial factors which deteriorate meat quality (Jo, 1999). It can cause rancid off-flavor and the warmed-over flavor, thereby the consumer acceptance could be reduced. Phosphate can retard lipid oxidation of meat products through its chelating activity (Jeong et al., 2021). In meat products, lipid oxidation could be inhibited by chelating with divalent metal ions and proteins such as hemoglobin and phospholipids, catalyzing oxidation (Thangavelu et al., 2019). Therefore, phosphate could contribute to stabilizing meat color and preventing the generation of rancid off-flavors in the meat system.

1.1.3.3. Antimicrobial effect

One of the main functions of phosphate is to inhibit the growth of microorganisms to improve food safety (Lee et al., 2018). Phosphate can improve the storage stability of meat products due to its bacteriostatic effect against some gram-positive bacteria (Long et al., 2011). However, it is not considered a direct preservative and can only impart some desirable properties when combined with other additives (nisin, EDTA, sodium chloride, nitrite, erythorbate, etc.) (Long et al., 2011).

1.1.4. Consumer concerns on synthetic phosphate

Moreover, consumers' demand for safe and high-quality meat products has rapidly increased minimally processed, easily prepared, and ready-to-eat meat products combined with the novel concept of all-natural and clean-label (Jayasena et al., 2013). Consumers often perceive some meat products could be harmful to their health due to their composition. Although phosphate has a variety of functional properties in meat systems, consumers recently have negatively perceived the use of phosphate due to health concerns (Pinton et al., 2021). Excessive phosphate intake increases the risk of cardiovascular disease and mortality by accumulating serum phosphate levels through impaired phosphate excretion ability (Chin et al., 2020). Also, it can lead to bone disease by forming insoluble salts, which are responsible for disrupting calcium absorption (Pinton et al., 2019). Moreover, adding above a certain level of phosphate to meat products can cause a bitter taste, which harms sensory quality (Long et al., 2011). This objection to meat products can be alleviated by reducing the ingredients that consumer avoids, such as animal fat, sodium chloride, nitrite, and phosphate. However, by doing it, there is a problem that the physicochemical and sensory quality of meat products can deteriorate (Lee et al., 2018).

1.2. Strategies for phosphate replacement in meat industry

1.2.1. Technologies-based strategies

1.2.1.1. High-pressure processing

Recently, as a way to reduce or replace phosphate in processed meat products, HPP and ultrasound technologies have been used. HPP has been used worldwide to effectively maintain the original fresh taste, texture, and nutrition of meat products and secure microbial safety (Troy et al., 2016). It is a non-thermal treatment technology that applies a very high hydrostatic pressure of 300 to 600 MPa and mild temperatures (<45°C) to food (Pou et al., 2020). HPP treatment can help reduce the amount of phosphate added as it can improve WHC by assisting in the solubilization and extraction of myofibrillar proteins in meat (Thangavelu et al., 2019). Moreover, HPP can lead to structural changes in foods, such as cell deformation, cell membrane damage, and protein denaturation, improving mass transfer rates and solvent permeability (Pinton et al., 2021). These physicochemical changes induced by HPP can improve emulsion stability, cooking yield, textural properties, and oxidative stability (Thangavelu et al., 2019). O'Flynn et al. (2014) manufactured sausages with 0, 50, and 100% phosphate replacement using raw minced meat applied to HPP (150 or 300 MPa for 5 min). As a result, HPP with 150 MPa for 5 min on raw minced meat could successfully replace 50% of phosphate with the minimization of quality defects. HPP is an effective technology for improving meat quality, but there is a lack of studies applied to phosphate replacement. Therefore, research to assess the interaction of HPP and alternative ingredients as phosphate replacers in processed

meat is needed.

1.2.1.2. Power ultrasound

PU is another effective approach that could be applied to reduce the addition level of phosphate in meat products. It uses sound energy whose frequency ranges from human audible to microwave (20 kHz~10 MHz) for generating cavitation, a form of vibrational energy (Thangavelu et al., 2019). Cavitation generates many bubbles when they collapse, accompanied by intense physical forces, namely shock waves, microjets, turbulence, shear forces, and so on (Pinton et al., 2021). This phenomenon can affect the functional properties such as WHC and tenderness without altering the flavor, color, and nutritional quality of meat products (Pinton et al., 2019). Moreover, it leads to the formation of microjets that modify protein structure and improve the homogenization of additives in the meat system (Troy et al., 2016). Several studies have reported replacing additives such as sodium chloride and phosphate were added to meat products using PU technology (Barretto et al., 2020; Pinton et al., 2019). Pinton et al. (2019) investigated the treatment of 0, 9, and 18 min of PU with 25 kHz frequency for a phosphate replacement in meat batter. They reported that the PU exposure time of 18 min effectively compensates for quality defects (lipid oxidation and sensory properties) caused by up to 50% phosphate reduction in meat emulsion.

1.2.2. Ingredient strategies

1.2.2.1. Natural sources

The best candidate as a natural ingredient to substitute phosphate is to improve functional properties such as water binding, antioxidant, and antimicrobial activity during cooking and storage with minimizing adverse effects on the sensory profile of meat products. Natural functional ingredients such as winter mushroom, sea tangle, and chia powder could be used as phosphate alternatives (Choe et al., 2018; Lee et al., 2018; Câmara et al., 2020). Lee et al. (2018) reported that sea tangle powder could serve as a phosphate replacement in emulsion-type sausages due to L-arginine contained in sea tangle powder. Among the various types of natural ingredients, the mushroom has the potential to be used as a phosphate alternative due to its functional components such as proteins, polysaccharides, and dietary fiber (Kurt et al., 2018). According to Choe et al. (2018), the addition of more than 0.5% WMP significantly increased the pH of meat batter and inhibited lipid oxidation of sausage more than the phosphate. Similarly, Jeong et al. (2021) stated the cooking loss of beef patties with WMP was comparable to that with phosphate, possibly due to dietary fiber in WMP.

1.2.2.2. Non-meat proteins

Non-meat proteins such as isolated soy protein, whey protein, and casein protein have been evaluated as phosphate replacements for application in the meat industry (Kim et al., 2017). They can play a role in enhancers to compensate for the

deteriorated functionality of meat proteins induced by phosphate elimination in meat products and are mainly related to emulsifying capacity, gel network formation, texture, and sensory properties of meat products (Goemaere et al., 2021). Youssef et al. (2011) found that soy protein isolates increase moisture retention and emulsion stability and decrease cooking loss in the meat emulsion system. Meanwhile, even though the ingredients have demonstrated some ability to alternative phosphate, it can lead to deterioration of meat quality. For example, Sun et al. (2012) revealed that the incorporation of pea protein into meat products improved water binding properties but harmed textural properties. The water binding properties of the meat product can enhance in proportion to the added amount of the non-meat proteins, which may be accompanied by deterioration of the sensory properties (Kim et al., 2017).

1.2.2.3. Hydrocolloids

To achieve favorable meat quality in replacing phosphate, several hydrocolloids such as L-arginine, carrageenan, guar gum, and xanthan gum capable of improving WHC, cooking yield, emulsion stability, textural and sensorial properties have been investigated as phosphate replacer (Kim et al., 2014; Park et al., 2008). According to Kim et al. (2014), the addition of L-arginine significantly increased the pH of sausage, and the sensory properties of the 0.5% L-arginine and 0.5% phosphate groups were similar. Moreover, guar gum, carrageenan, and alginic acid significantly increased the WHC of the meat batter, of which carrageenan and guar gum showed

physicochemical properties and emulsion stability similar to those of phosphate (Park et al., 2008).

Table 1. Categorization of phosphate replacement strategies

Strategies		Methods	References
Technologies-based strategies	High-pressure processing	· High hydrostatic pressure treatment for 300 to 600 MPa and mild temperature (<45°C)	Pou et al. (2020)
	Power ultrasound	· Using sound energy whose frequency ranges from human audible to microwave (20 kHz ~ 10 MHz) for generating cavitation	Thangavelu et al. (2019)
Ingredient strategies	Natural sources	· Winter mushroom · Sea tangle · Chia powder	Choe et al. (2018) Lee et al. (2018) Câmara et al. (2020)
	Non-meat proteins	· Soy protein · Whey protein · Casein protein	Kim et al. (2017)
	Hydrocolloids	· L-arginine · Carrageenan · Guar gum · Xanthan gum	Kim et al. (2014) Park et al. (2008)

Chapter II.

Effect of oyster mushroom (*Pleurotus ostreatus*) powder on emulsion-type sausage as a phosphate alternative

This manuscript has been submitted to Meat Science and is now in review state.

2.1. Introduction

Emulsion-type sausage is a product in which fat is added to finely ground meat to form an emulsion and cooked to fix the structure, which can provide a good texture and flavor which meets the consumer's requirement through an appropriate cooking process (Lee et al., 2021; Peng et al., 2009). It is mainly consisted of an oil-in-water type emulsion by mixing protein, fat, and water. During its manufacture, the formation of emulsions is attributed to different factors, such as the content of main ingredients (muscle protein, water, and fat), the concentration of additives (sodium chloride, phosphate, and nitrite), and processing procedure (Santhi et al., 2017). Especially, the involved additives can extract myofibrillar protein and form a stable

emulsion structure by coating the surface of the cut fat globules (Kurt et al., 2018). Therefore, in the product, fat is dispersed in a continuous phase with dissolved proteins (Froning et al., 1970) and the role of different additives has been studied comprehensively.

Among them, phosphate is a synthetic additive widely used in manufacturing emulsified meat products as stabilizers and emulsifiers, and its main function is to enhance the WHC by increasing the pH of the meat emulsion (Pinton et al., 2019; Thangavelu et al., 2019). It can change ionic charges distributions with sodium chloride and promotes myofibrillar protein isolation and dissolution (Long et al., 2011; Xu et al., 2022). Consequently, an increased level of swollen myofibrillar proteins may improve the emulsion stability and contribute to stabilizing gel formation during cooking in meat products (Câmara et al., 2020; Pinton et al., 2019). These physical properties are improved by the expansion of the space by the repulsion between myofibrillar proteins inside the emulsified sausage. Based on this role, phosphate plays a part in improving sensory quality, including cooking yield, texture, tenderness, and juiciness (Choe et al., 2018). In this respect, the role of phosphate in emulsified meat products has been considered to be significant in the meat industry. However, in recent times, consumers' negative perceptions of chemical synthetic food additives are increasing with the emergence of consumer trends such as health and sustainability. Therefore, various strategies such as high-pressure processing, power ultrasound, and adding natural sources are being found to replace phosphate in meat products (Yong et al., 2020). Recently, it has been reported that the addition of diverse natural heterogeneous ingredients containing

abundant polysaccharides and proteins such as mushroom, eggplant, and rice bran helps to improve the physicochemical and functional properties of emulsified meat products (Choe et al., 2018; Choi et al., 2011; Zhu et al., 2020). It has been reported that these materials can improve WHC, which is the main purpose of phosphate, and enhance emulsion stability by the interaction between polysaccharides and proteins (Guerrero et al., 2014; Li et al., 2021).

Oyster mushroom (*Pleurotus ostreatus*) is one of the most consumed edible mushrooms worldwide (Vargas-Sánchez et al., 2018). It has abundant protein and polysaccharide; in particular, polysaccharide contained in mushrooms is mainly composed of β-glucan (Tu et al., 2021). The conjugates between protein and polysaccharide formed in the emulsion system play an essential role in improving the emulsifying and gelation capacity (Kurt et al., 2018; Tu et al., 2021). Also, β-glucan of oyster mushroom is insoluble rather than water-soluble due to its unique structure (Gallotti et al., 2020), so it may cause different three-dimensional structural changes in emulsion system compared to other types of mushrooms. Thus, we hypothesized that oyster mushroom, unlike phosphate, will enhance emulsion stability by causing distinct changes of the structural bonding in meat system. To the best of our knowledge, there has been no scientific report on the mechanism of improving emulsion stability when OMP is added to meat product. The current study is aimed to investigate the potentiality of OMP as a substitute for phosphate in emulsion-type sausages by elucidating the mechanism for the improvement of emulsion stability by OMP.

2.2. Materials and methods

2.2.1. Formulation and processing of emulsion-type sausages

At 24-36 hr postmortem, the hind-leg pork and back fat were purchased from a local butcher shop (Seoul, South Korea), and the hot air-dried OMP (Dripship Co., Ltd., Daegu, South Korea) was purchased from a local market.

To manufacture the meat batter, the pork was ground using a grinder (MG51, Kenwood, Hampshire, UK) equipped with a 5 mm plate after removal of excessive visible fat and connective tissue. The ground pork (60%) was mixed with back fat (20%), ice (20%), and sodium chloride (1%) in a silent cutter (C4W, Sirman, Padova, Italy). To minimize the additives addition for clearer treatment effect, 1.0% NaCl was used which is the level that the quality defects of final meat products was not detected from a previous study (Kim et al., 2010). Then, sodium triphosphate or different levels of OMP were added following the formula for each of the four treatments: 1) NC: sausages manufactured without sodium triphosphate, 2) PC: sausages manufactured with 0.2% sodium triphosphate, 3) M1: sausages manufactured with 1% OMP, 4) M2: sausages manufactured with 2% OMP. The meat batters of four treatments were manufactured from each of 3 batches (n = 3), and the manufacturing process was conducted as previously described by Shin et al. (2022). Each meat batter was stuffed into a 25-mm diameter collagen casing (#240, NIPPI Inc., Tokyo, Japan), then the prepared sausage was cooked in a smoke chamber (Bastra 851C, Bayha Strackbein GmbH, Arnsberg, Germany) at 85°C until the internal temperature reached 73°C.

2.2.2. Physicochemical characteristics of emulsion-type sausage

2.2.2.1. Proximate analysis and pH

The moisture, crude protein, crude fat, and crude ash contents of sausage samples were determined using AOAC methods (2003). The moisture content was determined by the oven-drying method at 110ºC, and the crude protein content was measured by using the Kjeldahl method. The crude fat content was determined by the method of Folch et al. (1957) using a 2:1 ratio of chloroform and methanol, and the crude ash was measured using a furnace at 550°C.

To measure the pH value of sausages, the homogenate (T25 basic, IKA GmbH & Co. Staufen, Germany) prepared with 1 g of sample and 9 ml of DDW was centrifuged at 2,000 × g (Continent 512R, Hanil Co., Ltd., Incheon, Korea). After filtering the supernatant through filter paper (Whatman No. 1, Whatman PLC., Kent, UK), the pH value was measured using a pH meter (SevenGo2, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.2.2.2. Instrumental color analysis

The instrumental color of the sausage surface was measured using a colorimeter (CM-5, Konica Minolta Co., Ltd., Osaka, Japan), using standard Illuminant D65, a 10° standard observer, and an 8-mm measuring port. Before analysis, the instrument was calibrated with a standard black and white calibration plate (CM-A210, Konica Minolta Co., Ltd.). The results were recorded as CIE L^*, a^*, and b^* values.

2.2.2.3. Storage stability

2.2.2.3.1. Total aerobic bacteria count

The sausages (10 g) refrigerated (4 ± 1°C) for 0, 14, and 28 days were blended with 0.85% sterile saline (90 mL) for 2 min using a stomacher (BagMixer 400 P, Interscience, St. Nom la Bretèche, France). A series of decimal dilutions was prepared with sterile saline. Each diluent (100 μL) was spread on plate count agar (Difco Laboratories, Detroit, MI, USA). The agar plates were incubated at 37°C for 48 h and microbial counts were expressed as Log CFU/g.

2.2.2.3.2. TBARS and antioxidant activity

Five grams of sausage samples refrigerated (4 ± 1°C) for 0, 14, and 28 days was homogenized with 15 mL DDW and 50 μL of butylated hydroxytoluene (7.2%) for 30 s (T25 basic, IKA GmbH & Co. Staufen, Germany). Then, 1 mL of the homogenate was transferred to a 15 mL tube and 2 mL of a TBA/TCA solution (20 mM TBA in 15% TCA) was added. Subsequently, tube was heated at 90°C in a water bath for 30 min, and centrifuged at 2,090 × g for 15 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea). The absorbance of the supernatant was determined using a spectrophotometer (M2e, Molecular Devices, Sunnyvale, CA) at 532 nm. The TBARS value was expressed as mg malondialdehyde/kg sausage sample.

For measurement antioxidant activity, each sample (3 g) was homogenized (T25

basic, IKA GmbH & Co. Staufen, Germany) at 9,600 rpm for 30 s with 9 mL DDW. After centrifugation at 2,265 × g for 15 min, the supernatant filtered using filter paper (Whatman No. 1, Whatman PLC., Kent, UK) was used for measuring DPPH radical scavenging activity and $ABTS^+$ reducing activity. The DPPH and ABTS assays were performed according to Choe et al. (2020).

2.2.3. Emulsion characteristics of emulsion-type sausage

2.2.3.1. Water holding capacity

Each sausage sample (3 g) was chopped and placed onto a filter paper (Whatman No. 1, Whatman PLC., Kent, UK), and then centrifuged at 2,265 × g for 10 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea). WHC was calculated as follows: WHC (%) = (Moisture content – Released water) / Moisture content × 100.

2.2.3.2. Cooking loss

Cooking loss of sausages was determined in the cooking step of manufacturing sausages from the weight of the sausages stuffed with meat batter before and after cooking. The percentage of cooking loss was calculated using the following formula: Cooking loss (%) = (Weight before cooking – Weight after cooking) / Weight before cooking × 100.

2.2.3.3. Emulsion stability

The emulsion stability was determined according to the Shin et al. (2020). Absorbent cotton wool was laid on the bottom of a 50 mL tube, and a 5 × 5 cm, 25 mesh sieve was put on it. The batter (20 ± 0.5 g) was filled onto the mesh, and the lid was loosely put on to avoid the possible effect of vapor pressure. Subsequently, the tube was heated in an 85°C water bath (WB-22, Daihan Scientific) to a core temperature of 73°C, then cooled at room temperature for 12 h. After carefully removing the mesh with cooked batter from the tube, the water and lipid loss from the batter was calculated as the following Eq. 1 & 2:

$$Water\ loss\ (\%) = \frac{Separated\ water\ after\ heating\ (mL)}{Weight\ of\ batter\ before\ heating\ (g)} \times 100 \quad (1)$$

$$Lipid\ loss\ (\%) = \frac{Separated\ lipid\ after\ heating\ (mL)}{Weight\ of\ batter\ before\ heating\ (g)} \times 100 \quad (2)$$

2.2.3.4. Texture profile analysis

Sausage samples with a diameter 2.5 cm were cut into 2 cm height and were then analyzed using TA1 texture analyzer (AMETEK Lloyd Instruments Ltd., Fareham, UK). The samples were compressed twice to 60% of their original height (test speed of 2.0 mm/s, trigger force of 0.1 newton). The data were collected using the NexygenPlus™ software (AMETEK Lloyd instruments Ltd.), and the hardness (Newton, N), adhesiveness (g/sec), springiness (mm), cohesiveness (%), and chewiness (N) were recorded.

2.2.4. Physicochemical changes in emulsions

2.2.4.1. Apparent viscosity

Meat batter viscosity was evaluated using rotational viscometer (Advanced Rheometric Expansion System, Rheometric Scientific, Inc., Epsom, UK) equipped with parallel plates (Ø = 25 mm, gap 1.0 mm). The meat batter was analyzed in the range of shear rates of 0.1 to 600 s^{-1}, and the data presented only 0.1 to 10 s^{-1}, which showed a quite difference among the treatments. During the analysis, the temperature condition was maintained at 25°C.

2.2.4.2. Fourier-transform infrared spectroscopy

The sausage samples were prepared in powder form using a porcelain mortar after freeze-drying (PVTFD-10K, Ilshin, Korea). The lyophilized sausages were characterized by ATR-FTIR spectroscopy (Tensor 27, Bruker Optics GmbH, Ettlingen, Germany). FTIR spectra were recorded from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}, accumulating 20 scans per spectra.

2.2.5. Structural changes in emulsions

2.2.5.1. Scanning electron microscopy

Scanning electron microscopy was performed according to the procedure described by Shin et al. (2020). Briefly, the sausages were prepared by slicing with a razor blade at a long 0.5 cm and thickness 0.3 cm. The samples were then fixed for 24 h at 4°C in Carnoy fluid (60% ethyl alcohol, 30% chloroform, and 10% acetic acid, v/v), and then the dehydration process was carried out at 4°C using ethyl alcohol with increasing the concentration from 70% to 100%. The dehydrated samples were immersed twice in hexamethyldisilazane for 10 min each and dried in a fume hood. After drying, the samples were mounted on the aluminum stubs with the cross-section of the sausage facing upwards and then coated with a layer of platinum (EM ACE600, Leica Microsystem, North Ryde, NSW, Australia). Micrographs of the samples were obtained with a Zeiss Sigma field emission scanning electron microscope (AURIGA, Carl Zeiss Microscopy, Thornwood, NY).

2.2.5.2. Confocal laser scanning microscopy

The sausages were sliced into 0.3 cm thickness using the razor blade. Nile blue A sulfate was used as a fluorescent dye for protein and lipid at the concentration of 0.2% diluted with deionized water. The fluorescent dye, 0.1% Calcofluor white and 0.1% Nile red in isopropanol were used for polysaccharide and protein, respectively. A drop of staining dye was added to each sample and allowed to be absorbed into the sample at room temperature for 10 min. Especially, Nile red and Calcofluor white were sequentially used for double staining, and the excess of dyes

was washed away with deionized water. Each stained sample was carefully moved to a microscopic glass slide, and then the micrograph of the sample was obtained using a confocal laser scanning microscope (SP8X, Leica, Wetzlar, Germany). In the case of staining with Nile blue, the sample was excited with two laser beams at 488 nm and 633 nm for detecting protein (red) and lipid (green) phases. Emission spectra were collected from 500 to 650 nm for the lipid phase and 650 to 800 nm for the protein phase. Fluorescence of Nile red and Calcofluor white was observed using excitation (514 and 405 nm, respectively) and emission (605-640 and 410-480 nm, respectively) filters. Finally, the images were obtained overlaid.

2.2.6. Statistical analysis

This study was conducted with three separate batches (considered as replications, n = 3), and the results were statistically analyzed using the mixed models, with treatment as a fixed effect and random terms for batch. Also, in case of storage stability, storage day and its interaction with phosphate level were added as another fixed effect. Statistical analysis was performed using SAS software (SAS, Release 9.4; SAS Institute Inc., Cary, NC). Significant differences between mean values were identified with a significance level of $P<0.05$. The mean values and standard errors of the means were recorded.

2.3. Results and discussion

2.3.1. Physicochemical characteristics of emulsion-type sausage

2.3.1.1. Proximate composition and pH

As shown in Table 2, the addition of OMP significantly affected the moisture and crude protein content of sausages, while the crude fat and crude ash content did not change. The NC had the lowest water content, which became richer with increasing the levels of OMP; especially M2 reached a similar content to that of PC. It might suggest that the WHC of the sausages was enhanced when phosphate or OMP was added. Meanwhile, the NC had the highest crude protein content. Furthermore, although OMP is known to be rich in protein itself (Vargas-Sánchez et al., 2018), the addition of OMP did not significantly increase the crude protein content of M1 and M2. Therefore, it seems that M1 and M2 had relatively low crude protein content as they had higher water content compared to NC. Based on these results, it is assumed that OMP and phosphate, which is generally known as a moisture binder, can maintain moisture inside the sausages.

The PC had the highest pH in this study, followed by M2, M1, and NC ($P<0.05$, Table 2). In meat products, phosphate is commonly used to increase WHC by increasing pH and ionic strength and dissociating the bonds of actomyosin by chelating divalent metal ions (Gadekar et al., 2014). Therefore, the pH value of PC was higher than phosphate-free sausage (NC). Meanwhile, the addition of OMP significantly increased the pH of the sausage in the addition level-dependent manner. Cerón-Guevara et al. (2021) demonstrated that pH increased with increasing the

addition amount of OMP in liver pâté, which is good agreement with the result of this study. As previously reported, it might be a consequence of basic amino acids such as arginine and histidine in OMP (Chirinang et al., 2009).

Table 2. Proximate composition and pH of sausages with different concentrations of oyster mushroom powder

Item	NC	PC	M1	M2	SEM[1]
Moisture (%)	62.62[c]	64.43[a]	63.46[b]	64.20[a]	0.184
Crude protein (%)	14.70[a]	13.70[b]	13.70[b]	13.60[b]	0.270
Crude fat (%)	20.96	20.08	20.28	21.38	0.758
Crude ash (%)	0.90[b]	1.13[a]	1.07[ab]	1.00[ab]	0.078
pH	6.23[d]	6.46[a]	6.29[c]	6.30[b]	0.004

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

[a-d]Different letters within the same row indicate differences (P<0.05).

NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.1.2. Color

The addition of OMP decreased the L^*-value, while the a^*- and b^*-values increased (P<0.05, Table 3). Especially, the change of L^*-value by adding OMP might be due to the change of WHC. Hughes et al. (2014) reported that decreases in pH are accompanied by myofibrillar shrinkage, which might cause light scattering by driving fluid relocation. Although pH might not be a major factor affecting the increase in WHC, the lowest L^*-value of M1 and M2 may result from higher WHC (Further details were discussed in section 2.3.2.1). Given this, the significantly higher L^*-values in NC than in PC may be due to lower WHC caused by lower pH (Table 6). Our results are in good agreement with Cerón-Guevara et al. (2020) that the addition of OMP as a salt and fat substitute in frankfurter sausage reduced L^*-value and increased a^*- and b^*- values. However, these results were in contrary with the study by Banerjee et al. (2020), who stated that enoki mushroom addition increased L^*-value and decreased a^*-value of goat meat nuggets. Therefore, it could be interpreted that the color of meat products might be affected by the mushroom type and the concentration of adding flour or ingredients (e.g., water, fat, lean meat). Generally, meat redness is closely related to its antioxidant activity, that is, to inhibit the oxidation of oxymyoglobin to metmyoglobin (Zhan et al., 2007). We verified that the OMP had considerable antioxidant activity measured by $ABTS^+$ reducing and DPPH radical scavenging activity (Table 4). Furthermore, antioxidant activities were the highest when OMP was added to sausages among all treatments (Fig. 1). Therefore, these results may explain the highest a^*-value of M2 in our study. As mentioned above, M2 showed the highest b^*-value among all treatments. Generally,

the formation of yellow pigment in meat is occurred by the non-enzymatic browning reaction between the by-product of lipid oxidation and the amine group (Wang et al., 2021). However, OMP not only possessed superior antioxidant activity but also significantly decreased TBARS value by the addition of OMP throughout the 28 days of storage (Tables 4 and 5). Therefore, it is considered that the characteristic color of OMP, rather than lipid oxidation, had a major effect on the change of yellowness in sausage (Table 4).

Table 3. Color of sausages with different concentrations of oyster mushroom powder

Item	NC	PC	M1	M2	SEM[1]
CIE L^*-value	79.74[a]	77.92[b]	73.61[c]	73.47[c]	0.546
CIE a^*-value	3.45[b]	3.70[b]	3.52[b]	4.53[a]	0.199
CIE b^*-value	15.50[b]	15.84[b]	16.12[b]	16.92[a]	0.234

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

[a-c]Different letters within the same row indicate differences (P<0.05).

NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

Table 4. pH, color, and antioxidant activity of oyster mushroom powder

Property	Oyster mushroom powder[1]
pH	5.97 ± 0.00
CIE L^*-value	74.82 ± 0.06
CIE a^*-value	2.66 ± 0.02
CIE b^*-value	20.15 ± 0.04
IC_{50} of $ABTS^+$ reducing activity (g/kg)	43.22 ± 3.6
IC_{50} of DPPH radical scavenging activity (g/kg)	2.44 ± 0.41

[1]Mean±SD (n = 3).

IC_{50}, the half maximal inhibitory concentration.

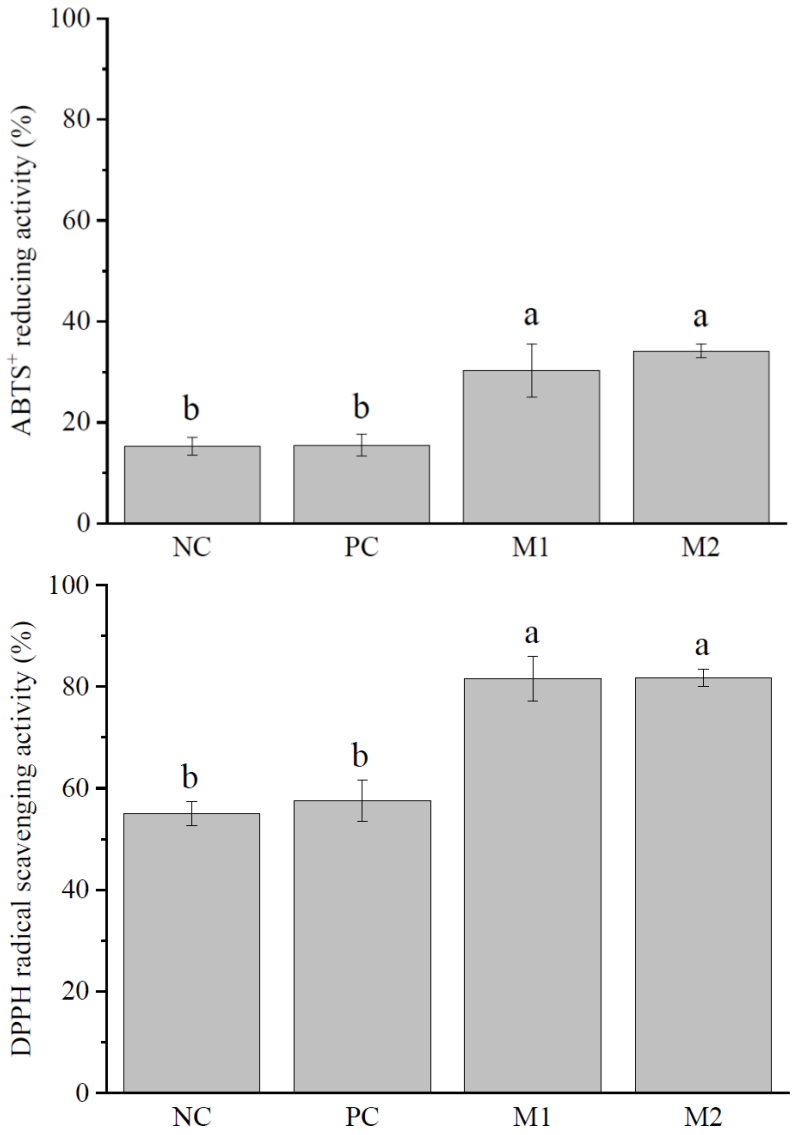

Figure 1. Antioxidant activity of sausages with different concentrations of oyster mushroom powder. [a,b]Different letters indicate differences within each analysis (P<0.05). NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.1.3. Storage stability

As shown in Table 5, the phosphate addition in sausages had the inhibiting activity for lipid oxidation at initial storage but was not effective during long-term storage for 28 days. After 28 days of storage, control groups (PC and NC) had a considerably higher MDA content, exceeding 2.0 mg/kg, which can be rejected due to off-flavor by consumers (Wereńska et al., 2022). Meanwhile, MDA content did not exceed this borderline when OMP was added in sausages; in particular, the addition of OMP 2% inhibited lipid oxidation most effectively on all storage days, which is even better than phosphate (Table 5). As previously reported, it might be a consequence of unique β-glucan structure in OMP, which had biological activities such as inhibiting lipid peroxidation and enhancing antioxidant enzyme activities (Bobek et al., 2001; Khan et al., 2017). Also, it can be verified in our results (Fig. 1 and Table 4), which showed high antioxidant activity in OMP or in sausages containing OMP. Thus, it is advantageous for long-term cold storage when phosphate is replaced with OMP, and among them, M2 is expected to better performance to maintain the quality of sausage.

Although M1 and M2 showed significantly higher number of TAB compared with control groups at day 0, they were within the acceptable range for consumption bellowed 6 log CFU/g (El Barbri et al., 2008). A higher number of TAB induced by OMP may be due to the surviving heat-resistant spore-forming bacteria during hot air-drying process of the oyster mushrooms (Cerón-Guevara et al., 2020, Table 5). A previous study documented that the amino acids, carboxylic acids, polypeptides, and sugar found in natural source can increase the survivability of bacteria during drying

(Chitrakar et al., 2019). Additionally, antioxidants contained in foods can positively affect the thermal stability of bacteria (Ghandi et al., 2013). On the other hand, after 14-28 days of storage, the growth of TAB was retarded by the addition of 1% or 2% OMP, and there was no significant difference with the control groups. It might be probably due to the inhibitory ability against bacterial growth which is attributed to the unique dietary fiber fraction of OMP (Gallotti et al., 2020). Therefore, the growth of TAB was controlled during 28 days of storage in M1 and M2 may be due to the antimicrobial activity in OMP.

Table 5. Storage stability of sausages with different concentrations of oyster mushroom powder

Item	Treatment	Storage (days)			PE[1]	*P* value		
		0	14	28		Phosphate level (P)	Storage day (S)	P*S
TBARS (mg MDA/kg)	NC	1.45^{Az}	2.12^{Ay}	2.61^{Ax}	0.094	< 0.0001	<0.0001	<0.0001
	PC	1.23^{Bz}	1.92^{Ay}	2.50^{Ax}				
	M1	0.46^{Cz}	1.52^{By}	1.95^{Bx}				
	M2	0.31^{Cy}	0.38^{Cy}	0.70^{Cx}				
Total aerobic bacteria (log CFU/g)	NC	1.76^{Bz}	3.40^{y}	5.43^{x}	0.189	0.0067	<0.0001	0.0001
	PC	1.59^{Bz}	3.31^{y}	5.22^{x}				
	M1	2.57^{Az}	3.17^{y}	5.53^{x}				
	M2	2.73^{Az}	3.17^{y}	5.23^{x}				

[1]Pooled standard error of means

[A-C]Different letters within the same column indicate differences ($P<0.05$).

[x-z]Different letters within the same row indicate differences ($P<0.05$).

NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.2. Emulsion characteristics of emulsion-type sausage

2.3.2.1. Water and fat binding properties

The WHC and cooking loss are closely related to consumers' willingness to pay by affecting the appearance of meat products (Lee et al., 2018). Among all treatments, WHC and cooking loss of NC had the lowest and highest values, respectively, due to the absence of phosphate (Table 6). However, both PC and M2 had the highest WHC, and M2 also had the lowest cooking loss (P<0.05). Moreover, in proportion to the amount of OMP added, the WHC increased and cooking loss decreased (P<0.05). These results were in line with the result of moisture content, which was the highest in PC and M2 and the lowest in NC.

Cooking loss, which could be influenced by the change of pH, were significantly higher in M2 than PC. However, the pH was higher in PC than that in M2 (P<0.05). It is widely accepted that phosphate affects the pH of the raw meat by moving it away from the isoelectric point. This increase in pH causes an electrostatic repulsion between meat proteins, which leads to an improving WHC (Thangavelu et al., 2019). Therefore, OMP may contribute to enhancing WHC by a different mechanism from that of phosphate. The improved WHC of sausages with OMP may be attributed to some components in OMP, such as protein, dietary fiber, and polysaccharides, rather than an electrostatic repulsion between proteins by increasing pH. Similarly, Jeong et al. (2021) reported the decrease in cooking loss of beef patties could be attributed to dietary fiber by the addition of winter mushroom powder.

After cooking, the stable entrapment of water and lipids in the matrix of meat products is one of the most crucial quality parameters for emulsion-type sausages

(Shin et al., 2022). As shown in Table 6, NC had the largest water and fat losses among all treatments due to the absence of phosphate (P<0.05). On the other hand, there was no significant difference between PC, M1, and M2, indicating that water and fat were stably bound in a meat emulsion system in these treatments. Liu et al. (2016) stated that the water and fat losses (i.e., phase separation) could be less in the more fine and uniform meat gel structure. Namely, a coherent emulsion structure was formed in PC, M1, and M2 due to the high emulsion stability contributed by different factors. In the case of PC, the loss of water and fat may be minimized because the ionic strength was increased by adding phosphate, leading to enhancing the hydration and swelling of salt soluble proteins (Xu et al., 2022). Likewise, it may be reasonable to suppose that a fine emulsion structure that well captures water and fat was formed in M1 and M2. However, this is probably not from the increased ionic strength that phosphate works but from some components present in the OMP.

Except for moisture, the mushroom mainly consists of protein and polysaccharides (Tu et al., 2021). Proteins have surface activity because of their amphiphilic properties, which enhance emulsifying capacity by adsorbing to interface between aqueous and lipid phases (Kurt et al., 2018). Meanwhile, polysaccharides classified as water-soluble and insoluble are known as well can act as a stabilizer in the emulsion system. Water-soluble polysaccharides increase water viscosity, and insoluble polysaccharides induce steric repulsion by adsorbing to the protein-formed interface in fat globules, which can contribute to increasing emulsion stability (Umaña et al., 2021). According to previous research, OMP contains considerable amounts of protein, soluble and insoluble polysaccharides (123, 17.8,

and 166 g/kg dry basis, respectively; Vargas-Sánchez et al., 2018). Moreover, β-glucan, which accounts for quite a large portion of polysaccharides in OMP (containing 141.82 g/kg dry matter basis), has various functional properties in the emulsion system (Khan et al., 2017). β-Glucan may be soluble or insoluble in water depending on its structure, but ones with a specific structure called 'pleuran' primarily distributed in oyster mushrooms are classified as water-insoluble polysaccharides (Bergendiova et al., 2011; Umaña et al., 2021). According to Manzi et al. (2000), it was reported that β-glucan of oyster mushroom has more insoluble fraction (62.2~72.9%) than soluble (27.1~37.8%).

From this point of view, the protein and polysaccharide in OMP improved the emulsion stability through different mechanisms, and we performed further analyses to find out which component majorly contributed to enhancing emulsion stability.

Table 6. WHC, cooking loss, and emulsion stability of sausages with different concentrations of oyster mushroom powder

Item	NC	PC	M1	M2	SEM[1]
WHC (%)	66.31[c]	74.41[a]	68.57[b]	74.06[a]	0.773
Cooking loss (%)	8.04[a]	3.38[b]	3.20[b]	2.45[c]	0.199
Water loss (%)	5.82[a]	1.48[b]	1.41[b]	0.93[b]	0.254
Fat loss (%)	0.36[a]	0.05[b]	0.03[b]	0.01[b]	0.019

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

[a-c]Different letters within the same row indicate differences (P<0.05).

NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.2.2. Textural properties

The addition of OMP resulted in different textural properties from those of phosphate, and the overall values were significantly decreased (Table 7). This may be a consequence of the hindrance of protein-protein interaction. Namely, it is believed that protein-polysaccharide interactions were formed inside the coherent protein matrix by the OMP polysaccharide, which caused the deterioration of textural properties in M1 and M2. These results are in agreement with the data previously reported that significant decrease of overall textural attributes (hardness, chewiness, and cohesiveness) in frankfurter sausages when replaced with salt and fat with OMP (Cerón-Guevara et al., 2020). Also, Choi et al. (2011) reported that the lower hardness of heat-induced gel can be attributed to the molecular interaction between proteins and hydrocolloids such as dietary fiber, pectin, and starch. Conversely, when the addition of OMP was increased from 1% to 2%, the hardness increased significantly. It might be due to the formation of more protein-polysaccharide interactions by OMP, leading to enhance hardness. Although polysaccharides in OMP can basically impair texture properties by interfering protein-protein interactions (Cho et al., 2020), the accumulated protein-polysaccharide bonds by addition of polysaccharide containing OMP can improve the texture properties (Banerjee et al., 2011). These results agreed well with Yang et al. (2020), who used polysaccharide nanoparticles from *Flammulina velutipes* as a stabilizer of emulsified sausage and resulted in the decrease of hardness up to 20% *Flammulina velutipes* concentration, whereas the hardness was increased above 20%. Taken together, it could be concluded that the protein-polysaccharide interaction developed inside the

compact protein-based structure leads to soft textural properties, whereas the enhancement of hardness can be expected as this bonding proportion increases. These changed textural trait induced by the addition of OMP could be effectively applied to types of meat products that require a creamy-like texture such as pâté and spreading meat (Cerón-Guevara et al., 2021; Freitas et al., 2012). We conducted further analyses to support the results of textural changes by confirming the binding properties between OMP components such as proteins and polysaccharides.

Table 7. Texture of sausages with different concentrations of oyster mushroom powder

Item	NC	PC	M1	M2	SEM[1]
Hardness (N)	18.84[b]	32.27[a]	9.72[d]	11.42[c]	0.540
Adhesiveness (g/sec)	0.03	0.02	0.05	0.03	0.025
Springiness (mm)	0.87[b]	0.91[a]	0.91[a]	0.91[a]	0.011
Chewiness (N)	5.03[b]	11.44[a]	2.26[c]	2.37[c]	0.202
Cohesiveness (%)	0.31[b]	0.39[a]	0.26[c]	0.23[d]	0.010

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

[a-d]Different letters within the same row indicate differences (P<0.05).

NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.3. Mechanism for improving emulsification

2.3.3.1. Structural changes

2.3.3.1.1. Scanning electron microscopy

The SEM was performed to investigate the distinctive structures formed when OMP was added in emulsion-type sausages. The images of all treatments showed that fat globules were embedded in a three-dimensional emulsion system (Fig. 2). In particular, the fat globules in control sausages (NC and PC) were immobilized by surrounding a protein matrix (Fig. 2a and 2b). Among control sausages, PC showed a more cohesive gel matrix compared to NC, which could be interpreted by increasing protein crosslink by added phosphate in the meat system (Xu et al., 2022). In the presence of phosphate, the myofibrillar proteins were more solubilized or the ionic interactions between the phosphate group and the amino group were promoted. Meanwhile, when OMP was added to the sausage (M1 and M2), characteristic structures in which fat globules were randomly trapped by filament-like components were shown (Fig. 2c and 2d). These specific structures might be correlated to the interaction between water-insoluble polysaccharides in OMP and lipids or proteins in the meat system. Zhu et al. (2020) proved that the water-insoluble polysaccharides in eggplant flesh pulp were responsible for the filament-like structure in emulsions, enhancing emulsion stability by interrupting flocculation between droplets. Likewise, in the present study, M1 and M2 may have a uniform three-dimensional network formed by the filament-like structure in OMP, leading to significantly lower fat and cooking losses than NC (Table 6).

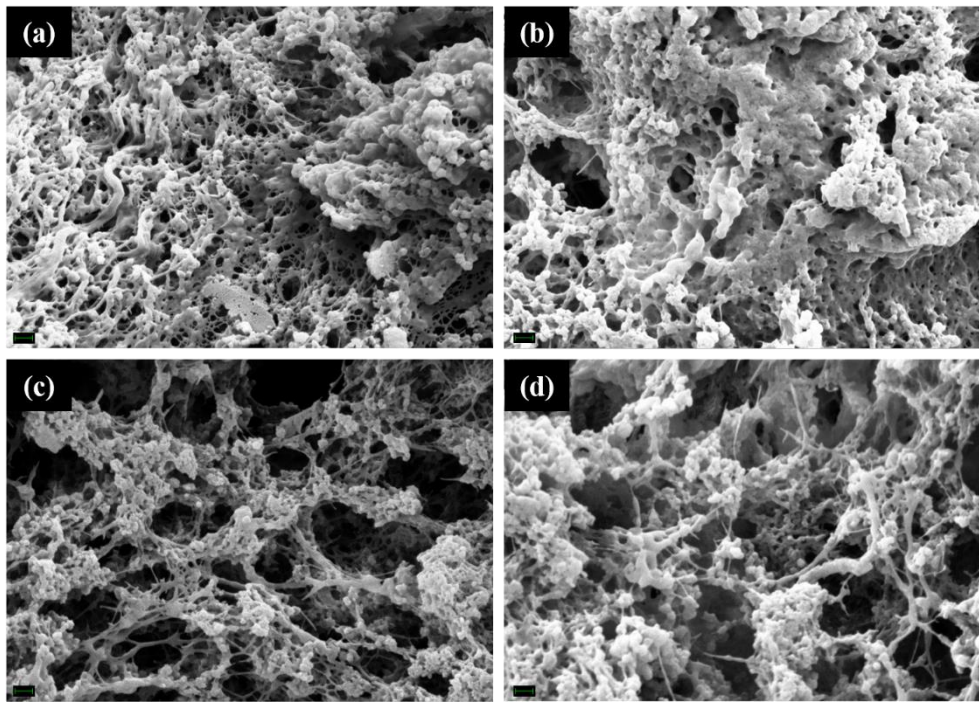

Figure 2. Scanning electron microscopy images of sausages with different concentrations of oyster mushroom powder at 50000× magnification. Bar, 0.2 μm. (a), NC; (b), PC; (c), M1; (d), M2. NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.3.1.2. Confocal laser scanning microscopy

The CLSM was taken to further study the effective emulsification components in OMP. As shown in Fig. 3, typical heated meat-emulsion structures in which fat globules (green) were entangled by the protein matrix (red) were observed in all treatments, indicating the protein derived from meat or OMP could act as an emulsion stabilizer onto the fat globules. Meanwhile, CLSM images stained with Calcofluor white clearly revealed the distribution of polysaccharides (blue) in network structures of sausages through fluorescence intensity (Fig. 3). In the case of the control groups, the blue fluorescence intensity between fat globules (red) was faint, whereas those of M1 and M2 were strong. Thus, it can be inferred that the polysaccharides in OMP mainly contribute to the formation of a stable emulsion structure in M1 and M2. Based on Figs. 2 and 3, it was likely that both polysaccharides and proteins in OMP were responsible for promoting emulsion stability. Possibly, pleuran, an insoluble fraction with a relatively large portion in OMP, might be attached to the interfacial surface of fat globules, which disturb flocculation and coalescence between them. Similarly, Umaña et al. (2021) stabilized oil-in-water emulsions with *Agaricus bisporus* by-product, mainly composed of insoluble material such as chitin and β-glucan, and reported that the primary stabilizing mechanism was the Pickering effect.

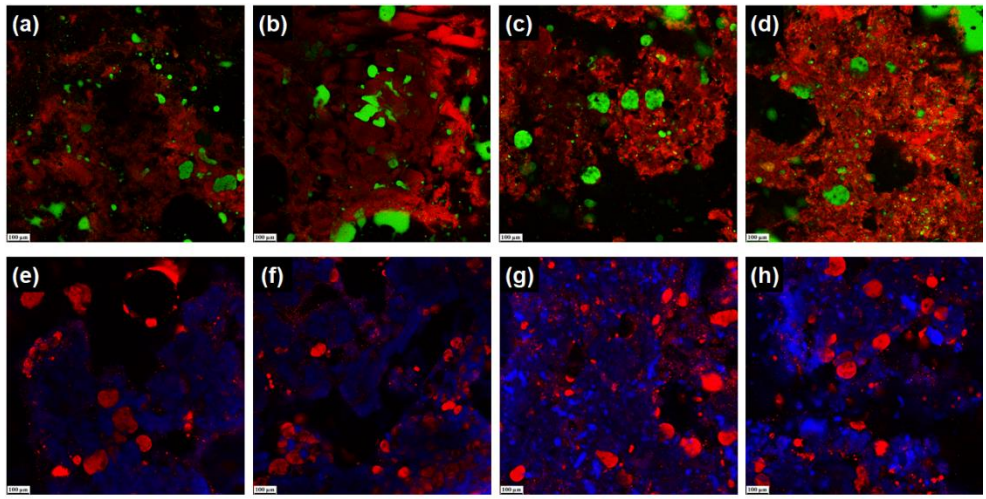

Figure 3. Confocal laser scanning microscopy images of sausages with different concentrations of oyster mushroom powder at 10× magnification. Bar, 100 μm. (a-d) Lipid phase, green; protein phase, red. (e-h) Lipid phase, red; polysaccharide phase, blue. (a, e), NC; (b, f), PC; (c, g), M1; (d, h), M2. NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.3.2. Physicochemical changes

2.3.3.2.1. Apparent viscosity

The meat batter with 2% OMP (M2) had the significantly higher apparent viscosity than PC, followed M1 and NC (Fig. 4). It is widely accepted that the high apparent viscosity indicates improved emulsion stability of meat batter, which can be achieved by adding phosphate to create a dense protein structure with well-entangled fat droplets (Câmara et al., 2020; Choi et al., 2011). In our study, the addition of OMP to the formulation, especially M2, increased the apparent viscosity of the meat batters. As we confirmed above, this result might be attributed to the improved emulsion stability due to the filament-like structure formed by proteins and polysaccharides in OMP. Similarly, Veverka et al., (2018) reported that the apparent viscosity was increased with the increasing concentration of β-glucan, a type of polysaccharide isolated from the oyster mushroom in emulsion gel.

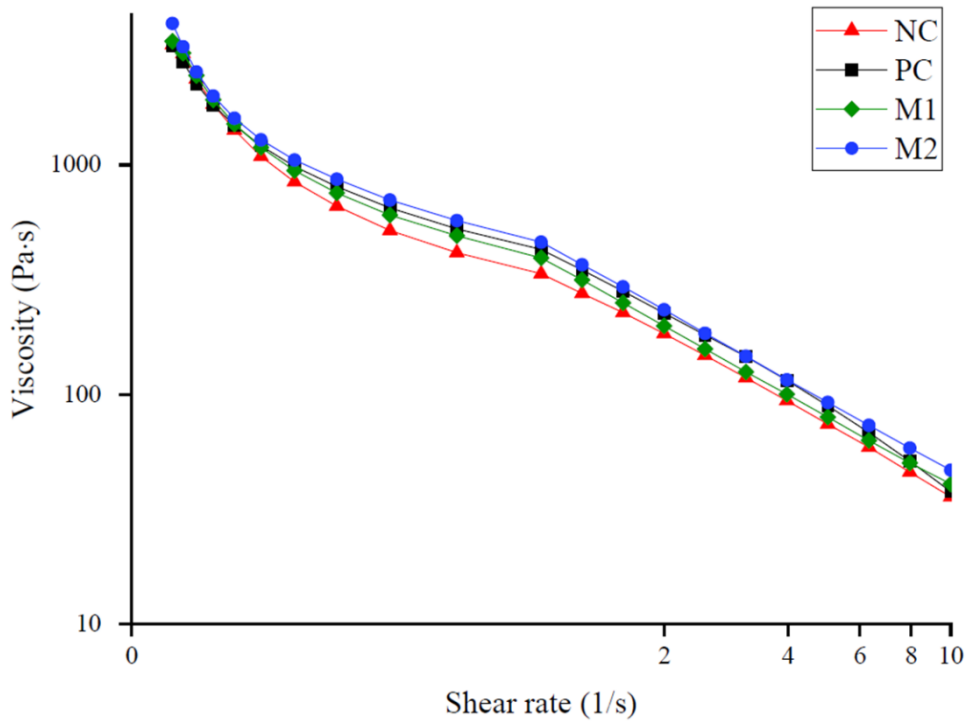

Figure 4. Apparent viscosity of meat batter with different concentrations of oyster mushroom powder. NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.3.3.2.2. Fourier-transform infrared spectroscopy

FTIR analysis was performed to verify the binding or interaction between the OMP components in the emulsion structure. FTIR spectral data in the range of 3000-2800 cm^{-1} was detected to analyze the lipid structure (Fig. 5a). The strong bands at 2918 and 2850 cm^{-1} result from the asymmetric and symmetric stretching vibrations of the acyl CH_2 groups, respectively (Herrero et al., 2011). It has been demonstrated that the increase in the intensity of these peaks implies the conformational order of lipid acyl chains diminished due to the insertion of hydrophobic groups in protein chains between the lipid chains (Herrero et al., 2011). Moreover, Herrero et al. (2014) documented that the downshift of two peaks indicates more oil-water or oil-carbohydrate interactions. However, in this study, the intensities of these two peaks did not show clearly strong when either phosphate or OMP was added to the sausages compared to NC. As we confirmed the above results in Table 6, adding phosphate or OMP can improve typical emulsion properties. Nevertheless, it is considered that the inconsistency with the earlier reports might be due to the difference in moisture content of sausages. Herrero et al. (2014) pointed out that the peak intensity in the range of 2800-3000 cm^{-1} can be low when the sample has an abundant water content, which may be responsible for the polysaccharide-water hydrogen bonding. Thus, the lipid-protein interaction was ample when phosphate or OMP was added to the sausages, but the difference in peak intensities was unclear due to high water content in PC, M1, and M2.

Fig. 5b shows a typical amide I spectra ranging from 1700-1600 cm^{-1} of sausages. This broad band provides information about the C=O stretching of amide

groups (Candoğan et al., 2021). These peaks in PC and M1 shifted down compared to NC. This result suggested that the content of β-sheet increased as protein in phosphate or OMP formed interfacial protein film onto fat globules, that is, the interaction between protein and lipid phase or absorbed proteins (Kurt et al., 2018; Zhao et al., 2020). Meanwhile, adding more than 1% OMP in sausage (M2) resulted in a decrement of these properties. It could be more protein-polysaccharide interaction in M2, and in this case, the intensity of the peak may decrease (Guerrero et al., 2014). Our result was in line with Kurt et al. (2018), who reported that the intensity of the amide I band broadened with the mushroom concentration increased but diminished above a certain level.

The spectral region ranging from 1300-400 cm^{-1} was collected for analyzing amide III band and polysaccharide structure in sausages (Fig. 5c). In amide III region, the peak at 1242 cm^{-1} and surrounding shoulders represents the structure of β-sheet, and their intensities were the highest and lowest in PC and NC, respectively (Anderle et al., 1987). As we confirmed above, the rich β-sheet structure reveals the interaction between protein and lipid, which is inferred to be more abundant in PC than in NC due to phosphate. In addition, spectral features in the 500-550 cm^{-1} region were related to S-S stretching vibration, which was most remarkable in PC (Jiang et al., 2022; Schmidt et al., 2013). It is probable that there is more cross-linking between myofibrillar proteins extracted by phosphate, thereby forming a stable structure in PC than in NC. In terms of polysaccharide structure, the IR feature in the region of 990-1200 cm^{-1} is assigned to COC and CC stretching vibrations, which displays the characteristics of polysaccharide (Novák et al., 2012). As shown in Fig. 5c, the peak

at 1095 cm^{-1} belonging to this region had the highest intensity in M2, followed by PC, M1, and NC. Novák et al. (2012) noted that the IR peaks near 1080 cm^{-1} was assigned to β-glucan structure, implying that M2 was composed of more β-glucans compared to other treatment in our study.

Based on our results, the mechanism of improvement of emulsion stability by adding OMP could be concluded as follows: 1) the forming interfacial surface of fat globules by proteins, 2) the increasing viscosity of continuous phase due to soluble polysaccharides, and 3) the construction of stable three-dimensional network structure by adsorbing insoluble polysaccharides to the interface of fat globules, leading to fat globules restraint and capture more water in the meat model system. Among these grounds, considering that the polysaccharide is a primarily constituents of OMP, water-insoluble ones such as pleuran, it can be concluded that emulsion stability is promoted by steric hindrance and Pickering effect (Bergendiova et al., 2011; Umaña et al., 2021).

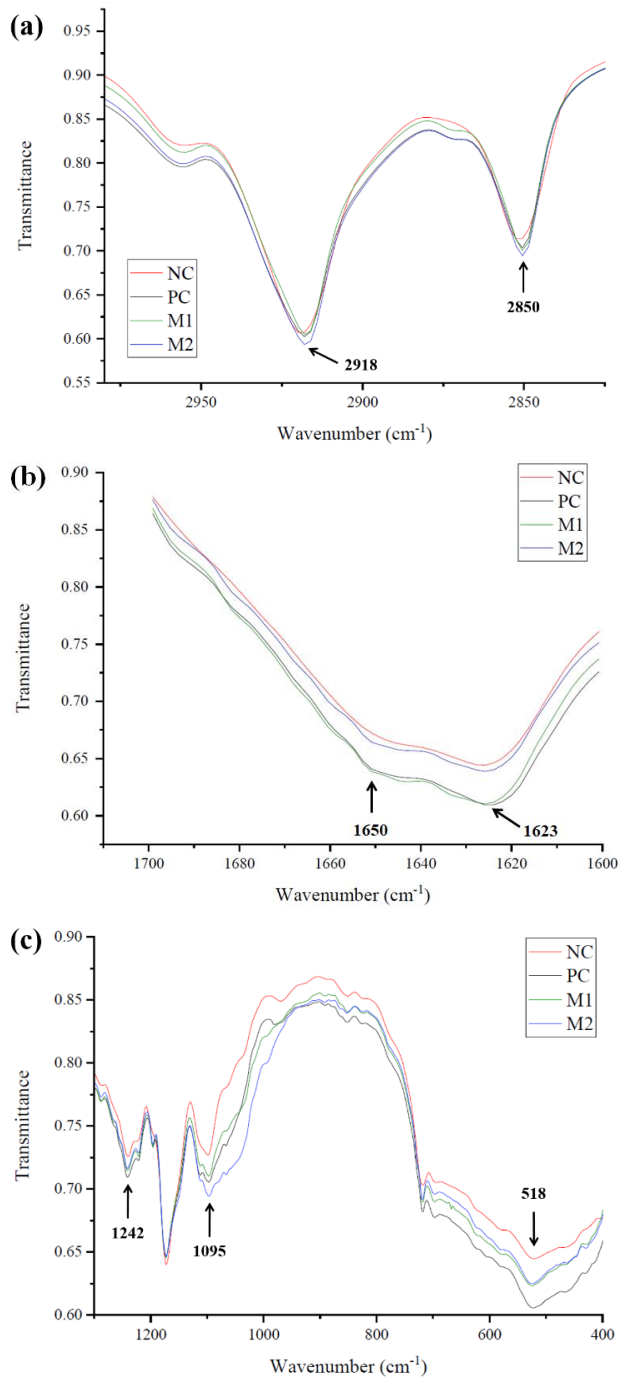

Figure 5. FTIR spectral region in 300-2800 cm^{-1} (a), 1700-1600 cm^{-1} (b), and 1300-400 cm^{-1} (c) of lyophilized sausages with different concentrations of oyster mushroom powder. NC, Negative control; PC, Positive control; M1, 1% OMP; M2, 2% OMP.

2.4. Conclusion

The effect of OMP on sausage as a phosphate replacement is summarized in Fig. 6. The addition of OMP improved the water and fat binding properties as well as overall physicochemical traits (moisture content, pH, and storage stability) of phosphate-free emulsion-type sausage. The stabilized emulsion structure by adding OMP could be caused by a complicated interaction between polysaccharides and proteins, which was different from those of phosphate. Namely, the formation of the interface of fat globules by proteins and the increasing viscosity and the formation of stable emulsion networks by polysaccharides were caused by OMP. These specific structural changes contributed to improving water- and fat-binding properties.

Considering the improved quality of sausages by the stabilized emulsion structure and long-term storage stability, OMP may be used as a phosphate substitute; in particular, 2% of the addition level could be most effective in replacing phosphate. However, considering the changed textural traits by adding OMP, it seems preferable to apply for meat products with a relatively smooth texture.

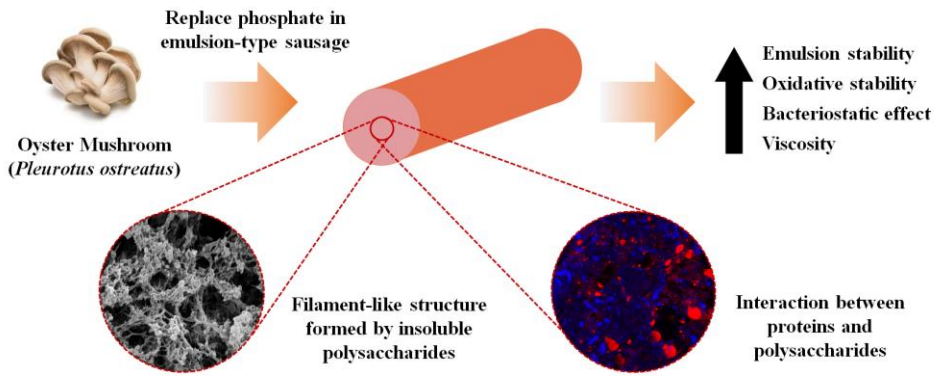

Figure 6. Overview of the quality changes of sausage containing oyster mushroom powder.

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Summary in Korean

유화형 소시지에서 느타리버섯 분말의 인산염 대체 효과

정두연
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본 연구는 유화형 소시지의 유화 안정성을 향상시킬 수 있는 인산염 대체재로써 느타리버섯 분말의 잠재성을 확인하기 위해 수행되었다. 분석을 위해 인산염 무첨가 소시지, 0.2% 인산염 첨가 소시지, 그리고 각각 1%와 2% 느타리버섯 분말을 첨가한 소시지를 제조하였다. 느타리버섯 분말의 첨가는 소시지의 물리화학적 특성을 향상시켰다. 또한, 28일의 냉장 저장 동안 지질 산화를 효과적으로 억제하였으며, 호기성 미생물의 성장을 지연시켰다. 1%와 2%의 느타리버섯 분말을 소시지에 첨가하였을 때 인산염을 첨가한 경우와 유사한 수준으로 유화 안정성이 개선되었다. 또한, 느타리버섯 분말 2%를 첨가하였을 때 소시지의 보수력과 육반죽의 겉보기 점도가 가장 높고, 조리 손실은 가장 낮게 나타났다. 한편, 느타리버섯 분말이 혼입된 소시지의 조직감은 인산염 첨가 또는 무첨가 소시지와 비교하여 다른 특성

을 나타냈다. 느타리버섯 분말의 첨가된 소시지에서 다당류로부터 유래한 필라멘트 유사 성분에 의해 지방구들이 무작위로 포획된 유화물 구조가 나타났으며, 이러한 특이적 구조가 소시지의 조직감 특성을 변화시킨 것으로 사료된다. 결과적으로, 느타리버섯 분말에 존재하는 다당류가 유화물 구조를 안정화 하는데 주요하게 기여한 것으로 판단되며, 그 기작은 수용성 다당류에 의한 점도 향상 및 불용성 다당류에 의한 입체 장애 효과이다. 결론적으로, 2% 수준의 느타리버섯 분말의 첨가가 육제품에 첨가되는 인산염을 대체하는데 가장 효과적일 것으로 사료된다.