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Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages

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ABSTRACT

This research evaluated the utilization of winter mushrooms as a replacement for phosphate in emulsion-type sausages. Winter mushroom powder (WMP) was added to the sausages at 0, 0.5, 1.0, 1.5, and 2.0% (w/w), and phosphate was added at 0.3% as a positive control. The WMP additions above 1.0% increased the pH of meat batter and efficiently inhibited the exudation of fat from the sausages ($p < 0.05$). Lipid oxidation of sausages was inhibited by the addition of WMP ($p < 0.05$). On the other hand, the addition of phosphate and WMP provided different instrumental texture properties. However, no adverse effects were observed with respect to the color and sensory properties of the sausages containing WMP, except for that containing 2.0% WMP. Therefore, this research indicates that WMP can effectively replace phosphate in meat products, and that the most effective addition level may be 1.0% WMP.

1. Introduction

Meat products have significant roles in the human diet because they contain various nutrients such as proteins, lipids, vitamins, and minerals. Various ingredients and additives are used to improve the quality and shelf life of meat products. Phosphates are a widely used additive for manufacturing meat products (Sebranek, 2009). In meat processing, one of the most beneficial functions of phosphates, especially alkaline phosphates, is the improvement of water holding capacity by raising the pH of meat batter. This results in improved cooking yield, texture, and eating quality, including improved tenderness and juiciness (Aberle, Forrest, Gerrard, & Mills, 2001; Sebranek, 2009). Other beneficial effects include (1) stabilization of emulsions and the texture of meat products by increasing the extraction of salt-soluble proteins based on increasing ion strengths and charges; and (2) reduction of lipid oxidation via their metal chelating activity, which subsequently inhibits off-flavor development (Aberle et al., 2001; Sebranek, 2009). However, phosphates are a chemical synthetic analogue. Recent research has revealed that consumers tend to choose natural sources of functional ingredients rather than chemical synthetic additives, and that they will pay significant premiums of 200% or more for natural ingredients (Carocho, Morales, & Ferreira, 2015; Sebranek & Bacus, 2007). Moreover, the meat processing industry has already taken steps to find suitable alternatives to synthetic additives, such as nitrites and ascorbic acids, to meet consumer demands (Jo, Lee, Lim, Hwang, & Jung, 2018; Jung et al., 2017). Thus, there is a requirement to find natural ingredients for the replacement of phosphates (E450).

Mushrooms, which originate from the natural environment, have been widely cultivated and consumed by humans since ancient times as a part of normal diet and as a delicacy because of their unique taste and flavor (Mattila, Suonpaa, & Piironen, 2000). Winter mushrooms (*Flammulina velutipes*) are widely distributed and are among the most popularly consumed mushrooms in South Korea, China, and Japan (Dong et al., 2017). Winter mushrooms have high levels of nutrients (protein, polysaccharides, fiber, and vitamins) and several biological benefits, such as antioxidant, antitumor, and antiinflammation properties, regardless of the extraction methods or active components (Dong et al., 2017; Kim & Kim, 2010; Leung, Fung, & Choy, 1997; Oh & Lee, 2010; Zhang et al., 2013). Moreover, some studies have shown that mushrooms can increase the pH of meat (Bao, Ushio, & Ohshima, 2008) and other food products (Ko & Kim, 2007) when used as a food additive for inhibiting the discoloration of meat via their antioxidant effects and improving their sensory and physicochemical properties. Considering both the antioxidant activities of winter mushrooms and the increase in pH of the meat products, we hypothesized that they could replace phosphates in meat products. Therefore, the aim of this study was to

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evaluate the potential use of winter mushrooms as an alternative to phosphates in emulsion-type sausages by testing various quality parameters including the pH of the meat batter, lipid oxidation, texture, and the sensory properties.

2. Materials and methods

2.1. Preparation of winter mushroom powder

Winter mushrooms (*Flammulina velutipes*) were purchased from a local market and washed using tap water. Winter mushrooms were lyophilized (Ilshin Co., Seoul, Korea) and pulverized using a bowl cutter (C4VV, Sirman, Curtarolo, Italy). Total phenolic content of WMP was estimated by the Folin-Ciocalteu method (Subramanian, Padmanaban, & Sarma, 1965).

2.2. Manufacture of emulsion-type sausages and sample collections

Winter mushrooms (*Flammulina velutipes*) were purchased from a local market and washed using tap water. Winter mushrooms were lyophilized (Ilshin Co., Seoul, Korea) and pulverized using a bowl cutter (C4VV, Sirman, Curtarolo, Italy).

Hind-leg pork and fat (after 24–36 h postmortem) were purchased from a local market (Daejeon, South Korea). Excessive visible fat and connective tissue were removed from the pork, and then ground by using a meat grinder (M-12S; Hankook Fugee Industries Co., Ltd., Hwaseong, Korea) with a 6-mm plate. Ground pork (1.6 kg) was mixed with back fat (0.4 kg), ice (0.4 kg), sodium chloride (1.5%), L-ascorbic acid (0.02%), and sodium nitrite (0.01%) in a silent cutter (12VV, Sirman, Curtarolo, Italy). Sodium pyrophosphate or different level of winter mushroom powder (WMP) were added depending on the formula for each of the six treatments: 1) Phosphate: sausages manufactured with 0.3% sodium pyrophosphate, 2) WMP 0: sausages manufactured without sodium pyrophosphate or WMP, 3) WMP 0.5: sausages manufactured with 0.5% WMP, 4) WMP 1.0: sausages manufactured with 1.0% WMP, 5) WMP 1.5: sausages manufactured with 1.5% WMP, and 6) WMP 2.0: sausages manufactured with 2.0% WMP (Table 1). The meat batters of 6 treatments were manufactured in each batch and 3 independent batches were prepared at different times on the same day. Eighteen meat batters (6 treatment × 3 batches) were stored in a refrigerator at 4 °C for 12 h prior to manufacturing the sausages. The meat batter (200 g) was packed into a steel can (95 mm × 50 mm × 50 mm) and then sealed using an automatic closing machine (DWC-160, Duckwoo Machinery Co., Korea). The cans were heated for 1 h in a water bath at 85 °C. After the heating process, the cans were cooled in tap water for 30 min. The cans were then dried and placed in a refrigerator at 4 °C. Ten sausages (10 cans) were manufactured from each treatment/batch. Three sausages from each treatment/batch were randomly collected and total nine sausages (3 sausages × 3 batches) of each treatment were used for quality analysis. The remaining sausages were used for sensory analysis. After storing the sausages for one day in a refrigerator at 4 °C, the proportion of jelly and melted fat, instrumental color, and texture properties of the sausages were measured with three replicates per treatment. Samples were collected in test tubes for lipid oxidation and stored at −70 °C until analysis.

Table 1
Formulations (%) for manufacturing emulsion-type sausages.

	Phosphate	Winter mushroom powder (%, w/w)				
		0	0.5	1.0	1.5	2.0
Ingredients						
Pork hind leg meat	80.0	80.0	80.0	80.0	80.0	80.0
Pork fat	20.0	20.0	20.0	20.0	20.0	20.0
Total	100	100	100	100	100	100
Ice	20	20	20	20	20	20
Additives						
Sodium chloride	1.5	1.5	1.5	1.5	1.5	1.5
L-ascorbic acid	0.02	0.02	0.02	0.02	0.02	0.02
Sodium nitrite	0.01	0.01	0.01	0.01	0.01	0.01
SPP[a]	0.3	–	–	–	–	–
WMP[b]	–	–	0.5	1.0	1.5	2.0

[a] Sodium pyrophosphate.
[b] Winter mushroom powder.

2.3. pH of the meat batter.

Three samples were collected from each meat batter for pH measurements, and the pH was measured before the manufacture of sausages. A meat batter sample (1 g) was homogenized with 9 mL of distilled water using a homogenizer (T25 basic, IKA GmbH & Co. KG, Germany). The homogenates were filtered through Whatman No. 4 filter paper (Whatman, Maidstone, England) after centrifugation at 2090 ×*g* for 15 min (Union 32R, Hanil Co., Ltd., Incheon, Korea). The pH of the filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Intl Inc., Schwerzenbach, Switzerland).

2.3. Proportion of jelly and melted fat exuded from sausages

The canned sausages were opened, and the sausages were removed from the can and placed on a cutting board. The sausages were weighed after removing the jelly and melted fat that exuded from the sausages. The results were expressed as a percentage in relation to the net weight of the packed meat batter.

2.4. Lipid oxidation

Lipid oxidation of sausages was estimated by detecting malondialdehyde (MDA). This procedure was conducted according to the method described by Jung, Nam, and Jo (2016). For this analysis, MDA was extracted from the samples with acetonitrile as follows. A 3 g sample was homogenized with 6 mL of distilled deionized water and 50 μL of 7.2% 2,6-Di-tert-butyl-4-methylphenol in ethanol using a homogenizer (T-25 Basic) at 1130 ×*g* for 1 min. Next, 500 μL of the homogenate was transferred into an Eppendorf tube, and 100 μL of a 6 M NaOH solution (final concentration: 1 M) was added for alkaline hydrolysis of the protein-bound MDA. The tubes were incubated in a water bath at 60 °C for 45 min. After cooling in ice for 5 min, 1 mL of acetonitrile was added to the tube, and the mixture was vigorously vortexed. The tube was centrifuged at 13,000 ×*g* for 10 min (HM-150IV, Hanil Co., Ltd., Incheon, Korea). The upper clear phase of the supernatant contained the MDA extract. As an MDA standard, a 1,1,3,3-tetraethoxypropane solution (3.2 mM) was diluted with distilled deionized water to 0.1, 0.2, 0.4, 0.8, or 1.6 μM. Subsequently, 1 mL of the MDA extract, standard, or distilled deionized water (blank) was passed through a 0.2 μm polyvinylidene fluoride syringe filter (Whatman), and the filtrate was collected into a vial. The MDA concentration was then analyzed by HPLC (ACME 9000, Young Lin Instruments Co., Ltd., Daejeon, Korea) using an Atlantis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) with a mobile phase consisting of 30 mM K_2HPO_4 (pH adjusted to 6.2 with H_3PO_4). The isocratic flow rate of the mobile phase was 1.2 mL/min, and the injection volume was 50 μL. The column temperature was maintained at 35 °C and the UV/VIS detector was set to a wavelength of 254 nm. The concentration of MDA in each sample was expressed as mg of MDA/kg of sausage.

2.5. Instrumental color measurements

The color of each sausage was measured using a colorimeter (CM-3500d, Minolta, Japan). Measurements were taken perpendicular to the surface of the sausage with a 30 mm diameter illumination area at two different locations per sample. The results were analyzed using the SpectraMagic software (Minolta, Japan) and were expressed CIE