Meat Science 166 (2020) 108151

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Utility of winter mushroom treated by atmospheric non-thermal plasma as an alternative for synthetic nitrite and phosphate in ground ham

Kyung Jo[a], Seonmin Lee[a], Cheorun Jo[b], Hee Joon Jeon[c], Jun Ho Choe[c], Yun-Sang Choi[d], Samooel Jung[a,*]

[a] Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Republic of Korea
[b] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea
[c] Meat Processing Division, Lotte R&D Center, Seoul 07209, Republic of Korea
[d] Researcher Group of Food Processing, Korea Food Research Institute, Wanju 55365, Republic of Korea

ARTICLE INFO

Keywords:
Atmospheric non-thermal plasma
Nitrite
Phosphate
Winter mushroom
Ground ham

ABSTRACT

The utility of plasma-treated winter mushroom powder (PWMP) as an alternative to synthetic nitrite and phosphate in ground ham was evaluated. Treatment of atmospheric non-thermal plasma generated nitrite in winter mushroom homogenate, and PWMP contained 4.87 g/kg of nitrite. Canned ground ham samples without nitrite and phosphate (NC), with sodium nitrite and sodium pyrophosphate (PC), and with PWMP (PWM) were manufactured, sterilized ($F_0 > 4$), and stored under accelerated conditions (40 °C) for 30 days. The nitrosylhemochrome content and color of PWM were similar to those of PC, without differences in the thiobarbituric acid reactive substances (TBARS) value. The proportion of jelly and melted fat exuded from ground hams in PWM exceeded that in PC but was lower than that in NC ($P < .05$). Therefore, PWMP can successfully replace synthetic nitrite, but it is an insufficient substitute for phosphate in ground ham.

1. Introduction

Various synthetic additives such as sodium nitrite, phosphate, and L-ascorbic acid have been used in the manufacture of meat products to improve their quality. However, many modern consumers prefer foods that use natural ingredients rather than foods with synthetic additives. Therefore, it has become preferable to replace synthetic additives with natural ingredients (Devcich, Pedersen, & Petrie, 2007; Jung et al., 2017a; Öztürk & Serdaroğlu, 2018).

Nitrite is an essential additive for cured meat products. A natural nitrite source is manufactured by converting nitrate to nitrite using a nitrate-reducing bacterium (Sebranek, 2009). However, a limitation of natural nitrite sources is the requirement of sufficient levels of nitrate, the precursor to nitrite, so that even if it has good functionality, it can't be used unless it contains enough nitrate (Jung et al., 2017a; Jung et al., 2017b). Therefore, a new approach to developing a natural nitrite source is required. Phosphate is another important additive in the manufacture of meat products, which serves to stabilize the emulsion of meat batter and to improve the water holding capacity through increasing the pH (Öztürk & Serdaroğlu, 2018). Several studies have been carried out to reduce the amount of phosphate using new processes or natural sources for satisfying the needs of consumers (Jo, Lee, & Jung, 2018; Öztürk & Serdaroğlu, 2018).

Plasma is considered the fourth state of matter and includes various species such as cations, anions, electron, and free radicals (Lee et al., 2017). Recently, the possibility of using atmospheric non-thermal plasma (ANP) as a new curing method has been suggested. The reactive nitrogen species in plasma can generate nitrite through a reaction with water molecules. Therefore, it can be utilized as a curing method for meat products (Jo, Lee, Lee, Lim, Choi, Jo, & Jung, 2020; Lee et al., 2017; Lee et al., 2018a; Oehmigen et al., 2010). Jung et al. (2015) have reported that the properties used to define the quality of emulsion type sausage manufactured using atmospheric-pressure plasma-treated water were similar to sausage prepared with synthetic nitrite. In previous studies, the nitrite content in a plant extract was found to increase with plasma treatment (Jung et al., 2017a), and the meat batter cured by the plasma-treated extract of a given plant showed characteristics of cured meat products (Lee, Jo, & Jung, 2018b). Therefore, natural nitrite sources can be generated by plasma treatment regardless of the nitrate content in the natural plant.

Mushrooms are rich in nutrients such as vitamins, proteins, minerals, and dietary fiber; they also contain high levels of antioxidants

* Corresponding author.
E-mail address: samooel@cnu.ac.kr (S. Jung).

https://doi.org/10.1016/j.meatsci.2020.108151
Received 17 December 2019; Received in revised form 5 April 2020; Accepted 10 April 2020
Available online 11 April 2020

such as phenolic compounds and ergothioneine (Kalaras, Richie, Calcagnotto, & Beelman, 2017). Mushrooms are commonly used as flavor enhancers, due to their unique flavor, and are recognized as a healthy food item because of their role in decreasing the incidence of hypertension, cancer and chronic diseases (Jo et al., 2018a). Dietary fiber, which is present in significant quantities in mushrooms, has properties that improve water and oil holding capacity, emulsification, and gel formation in food products (Mehta, Ahlawat, Sharma, & Dabur, 2015). Furthermore, a specific type of mushroom, called winter mushroom (*Flammulina velutipes*), has a high natural pH due to a large amount of basic amino acids, which further enhances the water holding capacity of meat products (Ito, Ueno, & Kikuzaki, 2017). Previous studies have shown that winter mushroom powder can effectively replace phosphate in pork sausage and low-salt chicken sausage (Cheo, Lee, Jo, Jo, Song, & Jung, 2018; Jo et al., 2018a). For this reason, we hypothesized that the use of plasma-treated winter mushroom could replace not only sodium nitrite but also phosphate in meat products.

Therefore, the aim of this study was to evaluate the effect of plasma-treated winter mushroom powder (PWMP) as an alternative to nitrite and phosphate in the manufacture of ground ham.

2. Materials and methods

2.1. Preparation of winter mushroom powder with plasma treatment

2.1.1. Plasma treatment of winter mushroom

Winter mushrooms were purchased from a local market (Daejeon, Korea). After the removal of the inedible part, winter mushrooms were washed using tap water and then ground using the food blender at speed level 2 for 2 min (FPM250, De'Longhi-Kenwood Appliances Co., Italy).

The homogenate of winter mushroom was treated by ANP using a remote infusion system, as shown in Fig. S1 of the supplementary data. The plasma discharge source used was a cylindrical dielectric-barrier discharge (DBD; Plasmapp Co., Ltd., Daejeon, Korea). Ambient air was used as the process gas for the plasma discharge, and the plasma was discharged at 1.5 kW and 60 kHz of power consumption. The plasma generated in the plasma discharge source was delivered to the food mixer containing the homogenate of winter mushroom through a polytetrafluoroethylene tube (5 mm internal diameter). A cooling path was formed inside the plasma discharge source, and 15 °C water was circulated to cool the plasma discharge source during plasma generation. The ANP was infused until the pH of the winter mushroom homogenate reached 6. Plasma-treated winter mushroom homogenate was then lyophilized (Bondiro, Ilshin Co., Seoul, Korea).

2.1.2. Properties of plasma-treated winter mushroom powder (PWMP)

Three samples of PWMP were prepared independently on different days. The nitrite content of PWMP was analyzed according to the AOAC method 973.31 (AOAC, 1990).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total phenolic content, and ergothioneine content of winter mushroom powder were measured for evaluation of antioxidant activity. Briefly, 0.5 g of winter mushroom powder and 70% methanol were mixed with the final volume adjusted to 50 mL for measuring DPPH radical scavenging activity and total phenolic content. DPPH radical scavenging activity was measured according to the method described by Jung et al. (2017a).

Total phenolic content was analyzed by the Folin Ciocalteu method (Subramanian, Padmanaban, & Sarma, 1965). Ergothioneine was extracted from winter mushroom powder by modifying the method of Kalaras et al. (2017). Ergothioneine content was analyzed by high-performance liquid chromatography (HPLC; 1200 series, Agilent Technologies, USA). A Luna HILIC column (150 × 4.6 mm, 3 μm particles) was used for analysis and the mobile phase employed included acetonitrile (A) and 20 mM ammonium acetate (pH adjusted to 6.0 with acetic acid) (B). The flow rate of the mobile phase was 1 mL/min, with an isocratic elution profile of A:B = 85:15 for 20 min. The column temperature was maintained at 40 °C, and the UV wavelength used to monitor the signals was 254 nm.

2.2. Quality properties of ground ham

2.2.1. Manufacture of canned ground ham

The ground hind-leg pork and back fat for canned ground ham were purchased at a local market (Daejeon, Korea). The meat batters were manufactured depending on the formula for each of the three treatments (Table S1). The three treatments were classified: 1) negative control (NC), ground ham manufactured without sodium pyrophosphate and nitrite source; 2) positive control (PC) ground ham manufactured with sodium nitrite (48 mg of nitrite/kg) and sodium pyrophosphate (3 g/kg); and 3) PWM, ground ham manufactured with 1% PWMP (10 g/kg).

PWMP samples prepared independently were mixed and added to the meat batter. The three meat batters from each treatment were prepared in three independent manufacturing processes (batches) following a random order and stored in a refrigerator at 4 °C for 12 h prior to the manufacture of canned ground hams. Meat batters for all three treatments were manufactured in each batch, and three independent batches were prepared on independent days. The meat batter (200 g) was packed into a steel can and then sealed using an automatic closing machine (DWC-160, Duckwoo Machinery Co., Korea). Cans were placed inside the retort machine (GHPR-300, Hyupjin Co., Korea), and heated for 45 min for sterilization ($F_0 > 4$). After the retort process, the cans were cooled using tap water for 1 h, followed by drying and storage of the cans. Ten samples of canned ground ham were prepared using each treatment/batch method, and three samples of each treatment/batch were randomly selected for used in the quality analysis. For accelerated storage test, three samples of canned ground ham for each treatment/batch were stored at 40 °C for 30 days.

2.2.2. Residual nitrite contents of ground ham

The residual nitrite content of ground ham was measured according to AOAC method 973.31 (AOAC, Association of Official Analytical Chemists, 1990).

2.2.3. Nitrosyl-hemochrome content of ground ham

To determine the nitrosyl-hemochrome content of ground ham, nitroso pigments and total pigments were measured according to the method described by Lee et al. (2018a). Nitrosyl hemochrome content was expressed as the ratio of nitroso pigments to the total pigments.

2.2.4. Color of ground ham

CIE lightness (L*), redness (a*), and yellowness (b*) of ground ham were measured using a spectrophotometer (CM-3500d, Konica Minolta Inc., Tokyo, Japan) with illuminant D_{65}, 10° standard observer, and 30 mm diameter quartz plate. The measurements were performed perpendicular to the surface of the ground ham at two different locations per each sample. The results were analyzed using SpectraMagic software (SpectramagicTM NX, Konica Minolta Inc., Tokyo, Japan).

The color difference (ΔE) which represents the degree of color change during storage was calculated using following equation. $\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$.

Where L_1^*, a_1^*, and b_1^* were the value of ground ham color at 0 days and L_2^*, a_2^*, and b_2^* were the value of ground ham color at 30 days of storage.

2.2.5. Lipid and protein oxidation

Lipid oxidation of ground ham was evaluated using the 2-thiobarbituric acid reactive substances (TBARS) value according to the method described by Jung, Nam, and Jo (2016).

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with 2,4-dinitrophenylhydrazine (DNPH) according to the method described by Jo et al. (2018a).

2.2.6. pH of the meat batter

To measure meat batter pH, 1 g of meat batter was homogenized with 9 mL distilled water (T25 basic, IKA GmbH & Co. KG, Germany). The homogenates were centrifuged at 2090 ×*g* for 15 min (1580R, LaboGene, Lynge, Denmark) and filtered using Whatman N. 4 filter paper (Whatman, Maidstone, England). The pH of filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Intl Inc., Schwerzenbach, Switzerland). The pH meter was calibrated at pH 9.21, 7.00, and 4.01 buffer solutions (pH buffer solution, Mettler-Toledo Intl Inc., Schwerzenbach, Switzerland) before the measurement of sample pH.

2.2.7. Proportion of jelly and melted fat exuded from ground ham

The ground ham was taken out of the can and separated from the exuded jelly and melted fat. The ground ham was weighed, and the results were expressed as a percentage of the weight of the meat batter packed in the can.

2.2.8. Texture properties of ground ham

Texture properties were analyzed by a two-bite system using a Texture Analyzer (Model A-XT2, Stable Micro Systems Ltd., UK) with a 70 mm diameter compression probe. The ground ham was cut to a standard size (2 × 2 × 1.5 cm) and underwent two cycles of 70% compression at a test speed of 2 mm/s. Texture properties of ground ham were expressed as hardness, springiness, cohesiveness, gumminess, and chewiness.

2.3. Statistical analysis

This study was repeated three times independently. Properties of winter mushroom powder were statistically analyzed by *t*-test. The quality of the canned ground hams was statistically analyzed using the general linear model (GLM) under a randomized complete block design (with a batch corresponding to a block). The main effect of the statistical model was set as a nitrite source and addition of phosphate. The interaction effects between treatment and storage time were included in the model when it was significant. The results were expressed as least-square mean and standard error of the least-square mean, and specific comparisons were performed by Tukey's multiple-range test when the main effect was significant ($P < .05$). SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

3. Results and discussion

3.1. Properties of PWMP

Nitric oxide and nitric dioxide represent the reactive nitrogen species in plasma and are the precursors for nitrite generated upon reaction with water molecules (Jo et al., 2019; Oehmigen et al., 2010). However, in previous studies, a temporary increase and continuous decrease in nitrite content in the plasma-treated liquid was observed with an increase in the plasma treatment time (Jung et al., 2015; Oehmigen et al., 2010). The reaction of nitrite precursors in plasma, such as nitric oxide and nitric dioxide, with water molecules generates nitrous acid, which has a pKa value of 2.8–3.3 (Da Silva, Kennedy, & Dlugogorski, 2006).

The nitrous acid forms nitrite via deprotonation under alkaline condition, which leads to the acidification of plasma-treated liquids, and conversely, it is disproportionated into nitric acid and nitric oxide in acidic condition (Jung et al., 2015; Oehmigen et al., 2010). Previous studies have found that the protonation of nitrite was accelerated at pH values below 6 (Braida & Ong, 2000). Therefore, pH is an important factor for the continuous generation of nitrite by plasma treatment, and synthetic additives, such as sodium hydroxide or phosphate, were added to increase the amount of nitrite formed by creating more alkaline conditions when nitrite is generated in water or plant extracts using ANP treatment (Jung et al., 2015; Jung et al., 2017a). However, the use of synthetic additives in the development of a new natural nitrite source, intended to substitute for synthetic nitrite sources in meat products, may not be appropriate.

Table 1
Properties of winter mushroom powder treated by atmospheric non-thermal plasma.

	Control[1]	PWMP[2]	SEM[3] a-b
pH	6.81[a]	6.03[b]	0.109
Nitrite content (g/kg)	–	4.87 ± 0.09	–
Antioxidative activity			
Total phenolic content (g/kg)	4.53	4.50	0.027
Ergothioneine content (g/kg)	2.12[a]	1.20[b]	0.092
DPPH radical scavenging activity (%)	79.8	78.9	0.79

[1] Lyophilized winter mushroom powder without treatment by atmospheric pressure plasma.
[2] Lyophilized winter mushroom powder treated by atmospheric non-thermal plasma.
[3] Standard error of the mean ($n = 6$).
[a-b] Different letters within the same column represent significant differences ($P < .05$).

Plasma generated in the cylindrical dielectric-barrier discharge source was remotely infused into a food mixer containing the winter mushroom homogenate, without alkaline substances, in the present study. The intrinsic pH of the winter mushroom homogenate was 6.8, and the plasma was continuously infused into the winter mushroom homogenate until its pH decreased to 6.03 (Table 1). After lyophilization of the plasma-treated winter mushroom homogenate, the obtained PWMP contained 4.87 g/kg of nitrite (Table 1). Jung et al. (2017a) reported that the lyophilized powder of plasma-treated red perilla extract, with the addition of sodium hydroxide to adjust the pH to 9 prior to plasma treatment, contained 3.74 g/kg nitrite. Lee, Lee, Jo, and Jung (2018) found that the optimal nitrite levels of lyophilized garlic and onion powder after plasma treatment were 1.95 and 1.80 g/kg, respectively, when the homogenates of garlic and onion were treated by ANP without the addition of alkaline substances. Therefore, it was considered that a significant and comparable level of nitrite content could be generated in winter mushroom homogenate, and this might be due to its high intrinsic pH and buffering capacity (Ito et al., 2017). The intrinsic pH of winter mushroom homogenate was 6.8 while those of garlic and onion homogenates were 6.2 and 5.8, respectively (Lee et al., 2018c).

The plasma treatment had no influence on the total phenolic content of PWMP (Table 1). This result was similar with that of a study by Jung et al. (2017a). They found no change in the total phenolic content of red perilla after plasma treatment (Jung et al., 2017a).

However, the ergothioneine content of mushroom powder significantly decreased upon plasma treatment ($P < .05$). Plasma treatment generates reactive oxygen and nitrogen species, which can induce oxidation. A previous study using the same plasma system, found that lipid and protein oxidation increased in meat batter with plasma treatment (Jo, Lee, Lim, Hwang, & Jung, 2018). Ergothioneine is a naturally occurring, sulfur containing amino acid that is synthesized by the incorporation of the sulfhydryl group into the intermediate precursor produced from L-histidine (Servillo et al., 2015). Oxidation of ergothioneine in the presence of oxidants, such as peroxynitrite and hydrogen peroxide, forms ergothioneine disulfide. Ergothioneine disulfide is stable when dry or in strongly acidic conditions. It is unstable at physiological pH, and it slowly decomposes hydrolytically upon an increase in pH to partially reform ergothioneine (Servillo et al., 2015). Therefore, ergothioneine in winter mushroom homogenates may be oxidized to the disulfide variant by reactive species produced through

plasma treatment. In addition, ergothioneine disulfide could be relatively stable in winter mushroom homogenates because the pH is decreased to 6.03 upon plasma treatment.

Ergothioneine content was significantly decreased by plasma treatment, but there was no significant difference in DPPH radical scavenging activity. Ergothioneine contains a thiol group and has antioxidant activity through electron transfer from the thiol group (Bao, Ushio, & Ohshima, 2009). Ergothioneine disulfide decomposes hydrolytically into ergothioneine, hercynine, and sulfurous acid at physiological pH (Servillo, D'Onofrio, & Balestrieri, 2017). Ergothioneine of the winter mushroom homogenate was oxidized by the plasma treatment to form ergothioneine disulfide, some of which remained in disulfide form due to low pH, but some could have been hydrolyzed. Sulfurous acid produced as a result of the hydrolysis of ergothioneine disulfide is a strong reducing agent (Liu, Nam, & Lee, 2014). Moreover, a previous study has reported that six electrons can be irreversibly transferred to oxidants while ergothioneine forms hercynine and sulfurous acid during oxidation (Servillo, D'Onofrio, & Balestrieri, 2017). For this reason, the ergothioneine content was reduced by plasma treatment, but there was no significant difference in the antioxidant activity of the winter mushroom.

3.2. Residual nitrite, nitrosyl-hemochrome content and color of ground ham

The residual nitrite content of meat products is affected by various factors such as initial nitrite level, meat pH, heating temperature, and the presence of a reducing agent in the meat (Honikel, 2008; Lee et al., 2018b). The residual nitrite content at 0 days of PC and PWM was 3.2 mg/kg and 1.7 mg/kg respectively, which shows a rapid decrease from the 48 mg/kg level initially present during the manufacture of meat batter (Table 2). The large loss of nitrite at 0 days may be due to the use of high heating temperature. In this study, the ground ham was heated to 120 °C for sterilization. Loss of nitrite is greater at higher temperatures (Honikel, 2008).

The loss of nitrite in PWM was significantly higher than PC at 0 days because the pH of the meat batter was lower in PWM (Table 2). Nitrite is transformed into nitric oxide at pH 6.0 or less, and a decrease in pH of 0.2–0.3 units can double the rate of the production of nitric oxide (Sebranek, 2009). Li, Shao, Zhu, Zhou, and Xu (2013) reported that compounds found in plant extract, especially flavonoids and polyphenols, can react with nitrite, which may reduce residual nitrite content. Therefore, the low residual nitrite content in PWM may be due to phenolic compounds and ergothioneine in the winter mushroom as well as the pH of the meat batter. The residual nitrite content was also found to decrease with increasing storage time in both PC and PWM samples. Nitrite content gradually decreases with storage time due to the conversion to nitric oxide or nitrate, and reaction with meat substances such as proteins (Honikel, 2008).

The nitrosyl-hemochrome content of ground ham was no different for PC and PWM after 0 and 30 days of storage (Table 2). Nitrosyl-hemochrome content was significantly increased in NC and PWM with storage time. This result might be due to the instability of nitrosyl-hemochrome in NC and PWM. The stability of nitrosyl-hemochrome is affected by the pH and redox stability of meat products (Yu, Jiao, Ma, & Sun, 2016). Nitrosyl-hemochrome is unstable in weakly acidic pH conditions and undergoes changes in absorbance (Yu et al., 2016). In addition, the absorbance of myoglobin is directly dependent on the structure of the heme group, and therefore, changes in the structure of nitrosyl-hemochrome in myoglobin can affect the absorbance of pigments in meat products (Anderson & Robertson, 1995). The pH of NC and PWM were lower than that of PC in the present study, and the oxidation stability of NC might be lower than that of PWM and PC, because of the absent of antioxidants, such as nitrite and phosphate in NC. Therefore, nitrosyl-hemochrome in NC and PWM samples might be less stable than that of PC.

In NC, the a* value was significantly lower, and the b* value was higher than those of PC and PWM. This is due to the addition of nitrite, which triggers the formation of nitrosyl-hemochrome and the development of a pink color by increasing the redness and decreasing yellowness in cured meats as compared to non-cured meat products (Jung et al., 2015). Although there was no significant difference in nitrosyl-hemochrome content between PC and PWM, the a* value of PWM was significantly higher than that of PC at 0 day. There are three main forms of myoglobin present in meat, namely, metmyoglobin, oxymyoglobin, and deoxymyoglobin, and their chemical form affects the color of cooked meat (King & Whyte, 2006). Metmyoglobin has the lowest heat resistance and denatures upon heating to ferrihemochrome, which is brown globin hemichromogen. On the other hand, oxymyoglobin and deoxymyoglobin are more heat resistance than metmyoglobin and denatured to ferrohemochrome, which is red globin hemochromogen (King & Whyte, 2006). Previous studies have reported that the presence of antioxidants in meat promotes the formation of oxymyoglobin and deoxymyoglobin (Suman, Nair, Joseph, & Hunt, 2016). Bao et al. (2009) reported that the use of winter mushroom extract to prevent discoloration during storage of beef and fish inhibits the formation of metmyoglobin due to the reducing power of ergothioneine. Therefore, antioxidants in winter mushroom could have inhibited the oxidation of myoglobin and increased the oxymyoglobin content, which would increase the amount of redness after cooking. The b* value was significantly higher in PWM than PC, at all storage periods evaluated. In a previous study, the addition of winter mushroom powder to chicken sausage increased the yellowness of products (Jo et al., 2018a). In this study, the winter mushroom powder was browned after plasma treatment, which may increase the yellowness of the ground ham (data not shown). The L* and a* values were significantly increased in NC and the a* value was significantly increased in PC with increased storage time.

Table 2
Residual nitrite content, nitrosyl-hemochrome content, and color of ground ham during storage under accelerated conditions (40 °C).

	Treatment[1] 2, 3, a-c, X-Y	0 day	30 day	SEM[3]
Residual nitrite content (mg/kg)	NC	0.0^{cY}	0.1^{cX}	0.01
	PC	3.2^{aX}	1.0^{bY}	0.11
	PWM	1.7^{bX}	1.2^{aY}	0.05
	SEM[2]	0.09	0.04	
Nitrosyl-hemochrome content (%)	NC	4.0^{bY}	12.0^{bX}	0.80
	PC	34.4^{a}	36.4^{a}	1.48
	PWM	34.0^{aY}	39.5^{aX}	1.11
	SEM[2]	1.10	1.29	
Color	L* value			
	NC	67.2^{bY}	69.5^{aX}	0.27
	PC	68.7^{a}	69.1^{a}	0.23
	PWM	66.6^{bY}	67.9^{bX}	0.22
	SEM[2]	0.20	0.22	
	a* value			
	NC	3.8^{cY}	5.9^{bX}	0.12
	PC	7.9^{bY}	8.8^{aX}	0.15
	PWM	8.8^{a}	8.8^{a}	0.12
	SEM[2]	0.12	0.07	
	b* value			
	NC	15.4^{a}	15.1^{a}	0.14
	PC	10.9^{c}	11.0^{c}	0.15
	PWM	13.0^{bX}	12.6^{bY}	0.17
	SEM[2]	0.15	0.13	

[1] Negative control (NC): ground ham manufactured without sodium pyrophosphate and nitrite source; Positive control (PC): ground ham manufactured with sodium nitrite and sodium pyrophosphate; PWM, ground ham manufactured with 1% PWMP.

[2, 3] Standard error of the least square mean ($n = 9$)[2], ($n = 6$)[3].

[a-c] Different small letters in the same column indicate significant differences between means ($P < .05$).

[X-Y] Different capital letters in the same row indicate significant differences between means ($P < .05$).

The b* value was a significant difference only in PWM over the storage time.

Mancini, Kropf, Hunt, and Johnson (2005) reported an increase in the redness during the storage of cooked pork chops. Specifically, they report that pH affects the return of redness in pork, and that the return of redness significantly increases in high pH pork with storage time (Mancini et al., 2005). Therefore, the a* value of PC was lower than PWM on day 0, but the redness returned due to the high pH of PC, and the a* value showed no significant difference between the PC and PWM at the final storage time evaluated.

The ΔE value, which represents the degree of color change during storage, was the highest at 3.66 in NC ($P < .05$), and there was no significant difference between the values for PC and PWM (1.46 and 1.26, respectively; data not shown). Endogenous factors such as pH and oxidation stability can affect the stability of myoglobin, and consequently, affect the color of meat products (King & Whyte, 2006). In this study, NC had the lowest meat batter pH and lower oxidation stability than other treatments, due to the absence of nitrite and phosphate. As a result, the largest color change was observed in NC. Although the pH of PWM was lower than PC, the high oxidation stability, due to antioxidants in winter mushroom, inhibited myoglobin denaturation, resulting in less color change during storage periods. Therefore, the use of PWMP can serve as a substitute for synthetic nitrite for color development and color stability of cured meat products.

3.3. Lipid and protein oxidation of ground ham

NC showed the highest TBARS values at all storage periods (Table 3, $p < .05$). This result is due to the absence of nitrite and phosphate in NC. Nitrite is a well-known antioxidant in cured meat products. Nitric oxide (NO) derived from nitrite combines lipid peroxyl radicals to form non-radical products and consequently terminate the radical chain reaction (Berardo et al., 2016). In addition, nitrite reduces the amount of free iron in meat products through stabilizing myoglobin (Berardo et al., 2016). Phosphate is also an effective antioxidant due to its chelation effect on transition metal ions. Free transition metal ions catalyze lipid oxidation and, specifically, free iron derived from the denaturation of myoglobin during cooking and storage, is a major catalyst for oxidation in meat and meat products (Estévez, 2011).

Despite the absence of sodium nitrite and phosphate in PWM, there was no significant difference in TBARS value between PWM and PC at all storage periods. This was because of the effect of nitrite generated by plasma treatment in winter mushroom and the presence of antioxidants in winter mushroom powder. Winter mushrooms are known to be rich in antioxidants such as phenolic compounds and ergothioneine (Kalaras et al., 2017). Phenolic compounds from natural plants have antioxidant activity due to their free radical scavenging, electron donation, and metal chelating properties (Li et al., 2013). Ergothioneine contained in winter mushroom is an effective antioxidant, which acts as a strong scavenger of hydroxyl radicals as well as metal ion chelator (Bao et al., 2009). Bao et al. (2009) reported that the addition of winter mushroom extract to bigeye tuna meat significantly enhanced lipid oxidation stability. Choe et al. (2018) found that the inhibition of lipid oxidation in pork sausages upon addition of more than 1% of winter mushroom powder was greater than the addition of phosphate (0.3%). Jo et al. (2018a) also reported a significant antioxidant effect of winter mushroom powder in chicken sausages. The TBARS value of ground ham showed no significant differences during storage in all treatments. Oxygen is an important factor in the oxidation of meat products. It reacts with lipid radicals in the propagation phase of the lipid oxidation chain reaction to produce peroxide Bao et al., 2009). In this study, the meat batter was packed in a steel can without oxygen, thus, inhibition of oxidation may have achieved due to our storage condition.

Measurements of the carbonyl content, for the evaluation of the degree of protein oxidation, revealed higher levels in NC than PC and PWM, but there were no significant differences among the treatments over the storage period (Table 3, $p > .05$). Nitrite and phosphate can inhibit lipid oxidation and protein oxidation, which both occur through a free radical chain reaction. However, (Vossen and De Smet, 2015) have reported that the addition of sodium nitrite has no antioxidant effect on protein oxidation in isolated myofibrillar protein isolates and pork patties. Berardo et al. (2016) have reported that nitrite can inhibit lipid oxidation in cured meat products, but increased the formation of protein carbonyl, when added with ascorbic acid. Protein carbonyl can be formed through metal-catalyzed oxidation, non-enzymatic glycation by reducing sugar, and covalent binding with non-protein carbonyl compounds such as malondialdehyde (Estévez, 2011). Ascorbic acid is reduced to dehydroascorbic acid by nitrite in cured meat products, and it can cause glycation of proteins (Berardo et al., 2016). For this reason, despite the addition of nitrite and phosphate, which could act as antioxidants in meat products, there was no observed difference in carbonyl content between PC and NC. Similarly, despite the absence of phosphate in PWM, it has no significant difference compared to PC, possibly due to the antioxidant activity of phenolic compounds and ergothioneine present in winter mushroom. Natural antioxidants, such as phenolics, have been reported to inhibit protein carbonylation in meat products (Estévez, 2011). The carbonyl content of ground ham showed no significant difference during storage in all treatments. This might be due to the inhibition of oxidation through the removal of oxygen in the steel can used for storage.

Table 3
TBARS value and carbonyl content of ground ham during storage under accelerated conditions (40 °C).

	Treatment[1] 2, 3, a-b	0 day	30 day	SEM[3]
TBARS value (mg/kg)	NC	1.75[a]	1.63[a]	0.108
	PC	0.31[b]	0.33[b]	0.029
	PWM	0.38[b]	0.40[b]	0.028
	SEM[2]	0.041	0.088	
Carbonyl content (nmol/mg)	NC	1.21	1.17	0.063
	PC	0.92	0.98	0.079
	PWM	0.92	0.98	0.063
	SEM[2]	0.082	0.059	

[1] Negative control (NC): ground ham manufactured without sodium pyrophosphate and nitrite source; Positive control (PC): ground ham manufactured with sodium nitrite and sodium pyrophosphate; PWM, ground ham manufactured with 1% PWMP.

[2, 3] Standard error of the least square mean (n = 9)[2], (n = 6)[3].

[a-b] Different small letters in the same column indicate significant differences between means ($P < .05$).

3.4. pH of meat batter and proportion of jelly and melted fat exuded from ground ham

The pH was the lowest in PWM and NC, which contained no phosphate, and the highest in PC, containing 0.3% phosphate (Table 4, $p < .05$). Choe et al. (2018) found that the addition of 1% winter mushroom powder into the meat batter of pork sausage increased the pH to a level similar to that containing 0.3% phosphate. However, the meat batter pH of PWM was significantly lower than that of PC ($P < .05$). In the present study, the pH of winter mushroom homogenate was declined to 6 with the plasma treatment. Therefore, the effect of the addition of winter mushroom powder on the meat batter pH observed here was less than that in study by Choe et al. (2018).

The use of alkaline phosphate in meat products improves the water holding capacity by increasing the pH and ionic strength (Choe et al., 2018; Sun & Holley, 2011). Moreover, phosphate in meat batter improves the solubilization of actomyosin via the neutralization of Mg^{2+} and Ca^{2+}, which improves gel formation of comminuted meat products (Sebranek, 2009; Sun & Holley, 2011). Consequently, the addition of phosphate increases the water holding capacity and the solubility of

Table 4
pH of meat batter and proportion of jelly and melted fat exuded from ground ham during storage under accelerated conditions (40 °C)

Treatment[1] 2, 3, a-c	pH	Proportion of jelly and melted fat (%)		
		0 day	30 day	SEM[3]
NC	5.80[c]	27.3[a]	27.6[a]	0.34
PC	6.18[a]	10.6[c]	13.0[c]	1.12
PWM	5.90[b]	20.6[b]	22.3[b]	0.63
SEM[2]	0.003	0.99	0.50	

[1] Negative control (NC): ground ham manufactured without sodium pyrophosphate and nitrite source; Positive control (PC): ground ham manufactured with sodium nitrite and sodium pyrophosphate; PWM, ground ham manufactured with 1% PWMP.
[2, 3] Standard error of the least square mean (n = 9)[2], (n = 6)[3].
[a-c] Different small letters in the same column indicate significant differences between means ($P < .05$).

myofibrillar proteins and thereby improves the water and fat binding abilities in meat products (Sebranek, 2009).

The proportion of jelly and melted fat exuded from ground ham was the highest in NC and the lowest in PC (Table 4, $p < .05$). These results were due to the improved water and fat binding ability with the addition of alkaline phosphate in PC. PWM showed a lower degree of exudation of water and fat than did NC, even though phosphate was not added. This may be related to not only the pH of meat batter, but also the dietary fiber content in the winter mushroom. Jo et al. (2018a) reported that the dietary fiber composition of winter mushroom powder was 44.5%. The water and fat binding ability of dietary fibers in meat products have been reported (Mehta et al., 2015). Previous studies found that various dietary fibers such as wheat, oat, and rice bran added to meat products enhanced cooking yield by suppressing water and fat exudation from the meat products (Jung et al., 2018; Mehta et al., 2015). Choe et al. (2018) and Jo et al. (2018a) found that the cooking yield was increased with the addition of 1% of winter mushroom powder into emulsion pork sausages and chicken sausage, and it was found to be equal or better than that of sausages manufactured with 0.3% phosphate. However, PWM showed higher exudation of water and fat than PC. This was because the pH of PWMP was lower than that of winter mushroom powder without plasma treatment. Therefore, the phosphate substitution with PWMP in ground hams was insufficient. The proportion of jelly and melted fat tended to increase with the length of the storage period, but there was no significant difference between treatments.

3.5. Texture properties of ground ham

The hardness was significantly lower in PC, than that of NC and PWM, and there were no significant differences in springiness, cohesiveness, and chewiness among treatments at 0 days of storage (Table 5). Choe et al. (2018) and Jo et al. (2018a) has reported that the addition of winter mushroom powder to pork and chicken sausage resulted in low hardness, due to the dietary fiber contained in mushroom, which disrupted the protein gel formation. Nevertheless, the hardness of PWM was higher than PC, and showed no significant difference compared to NC at 0 days of storage. Fat and water in meat products leads to increased softness in meat products. The proportion of jelly and melted fat exuded from ground ham was high in NC and PWM, when compared to that of PC (Table 3). In addition, the texture properties of meat products are highly dependent on pH (Ni et al., 2014; Sun & Holley, 2011). At a pH near the pI of myofibrillar protein (pH 5.5–6.0), the α-helix content of myosin decreases and the β-sheet content increases (Sun & Holley, 2011). The increase of the β-sheet fraction, before heating, results in the formation of a protein gel with high hardness and low water holding capacity. Therefore, the hardness of ground ham

Table 5
Texture properties of ground ham during storage under accelerated conditions (40 °C).

	Treatment[1] 2, 3, a-b, X-Y	0 day	30 day	SEM[3]
Hardness (N)	NC	76.6[aX]	68.0[aY]	2.14
	PC	55.6[b]	51.5[b]	2.34
	PWM	70.1[aX]	60.1[abY]	2.70
	SEM[2]	2.71	2.67	
Springiness	NC	0.6[×]	0.5[bY]	0.03
	PC	0.7	0.7[a]	0.03
	PWM	0.6[×]	0.5[bY]	0.03
	SEM[2]	0.03	0.03	
Cohesiveness	NC	0.3[×]	0.2[bY]	0.01
	PC	0.3[×]	0.2[aY]	0.01
	PWM	0.2[×]	0.2[abY]	0.01
	SEM[2]	0.01	0.01	
Gumminess	NC	19.8[aX]	12.5[Y]	0.65
	PC	14.3[bX]	11.0[Y]	0.75
	PWM	16.5[abX]	12.1[Y]	0.93
	SEM[2]	0.93	0.71	
Chewiness	NC	12.0[×]	6.2[Y]	0.59
	PC	9.5	7.8	0.73
	PWM	10.8[×]	6.6[Y]	0.87
	SEM[2]	0.89	0.71	

[1] Negative control (NC): ground ham manufactured without sodium pyrophosphate and nitrite source; Positive control (PC): ground ham manufactured with sodium nitrite and sodium pyrophosphate; PWM, ground ham manufactured with 1% PWMP.
[2, 3] Standard error of the least square mean (n = 9)[2], (n = 6)[3].
[a-b] Different small letters in the same column indicate significant differences between means ($P < .05$).
[X-Y] Different capital letters in the same row indicate significant differences between means ($P < .05$).

was high due to the high loss of fat and water and low pH in NC and PWM compared to those of PC.

The hardness, cohesiveness, gumminess, and chewiness were decreased with storage time in NC and PWM. The texture properties of PWM were not different from those of PC after 30 days of storage. Changes in the texture properties of ground ham during storage may be due to the differences in pH of each treatment. The pH of meat changes the properties of proteins, such as protein unfolding, denaturation, and solubility, and these changes significantly affect protein gel formation (Ni et al., 2014). Changes in protein properties induced by low pH might affect not only gel formation but also gel structural stability during storage of NC and PWM, which might also affect the texture properties. However, the precise mechanism is unclear and further study is needed.

4. Conclusion

ANP treatment of winter mushroom homogenate generated nitrite, notably without any effect on the DPPH radical scavenging activity of winter mushroom. PWM showed similar curing properties as PC in terms of nitrosyl-hemochrome content and color. In addition, the TBARS value of PWM was not significantly different from that of PC after 30 days of storage under accelerated conditions (40 °C) despite the lack of phosphate. The proportion of jelly and melted fat exuded from the ground ham was significantly lower in PWM than in NC, and the lowest in PC. These results indicate that PWMP can be used as nitrite source; however, it cannot completely replace phosphate in cured meat products.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2020.108151.

Author contributions

Conceptualization: Samooel Jung, Kyung Jo. Formal analysis:

Kyung Jo, Seonmin Lee, Hee Joon Jeon, Jun Ho Choe. Writing-original draft: Kyung Jo. Writing-Review & Editing: Cheorun Jo, Yun-Sang Choi, Samooel Jung

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) and a grant funded by the Korean government (MSIT) (No. R1F1A1059255).

Author contributions

Conceptualization: Samooel Jung, Kyung Jo. Formal analysis: Kyung Jo, Seonmin Lee, Hee Joon Jeon, Jun Ho Choe. Writing-original draft: Kyung Jo. Writing-Review & Editing: Cheorun Jo, Yun-Sang Choi, Samooel Jung

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Anderson, A. B., & Robertson, C. R. (1995). Absorption spectra indicate conformational alteration of myoglobin adsorbed on polydimethylsiloxane. *Biophysical Journal, 68*(5), 2091–2097.

AOAC, Association of Official Analytical Chemists (1990). *Nitrites in cured meats official method 973.31 official methods of analysis* (15th ed.). Arlington, VA: AOAC.

Bao, H. N. D., Ushio, H., & Ohshima, T. (2009). Antioxidative activities of mushroom (Flammulina velutipes) extract added to bigeye tuna meat: Dose-dependent efficacy and comparison with other biological antioxidants. *Journal of Food Science, 74*(2), C162–C169.

Berardo, A., De Maere, H., Stavropoulou, D. A., Rysman, T., Leroy, F., & De Smet, S. (2016). Effect of sodium ascorbate and sodium nitrite on protein and lipid oxidation in dry fermented sausages. *Meat Science, 121*, 359–364.

Braida, W., & Ong, S. K. (2000). Decomposition of nitrite under various pH and aeration conditions. *Water, Air, and Soil Pollution, 118*(1–2), 13–26.

Choe, J., Lee, J., Jo, K., Jo, C., Song, M., & Jung, S. (2018). Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. *Meat Science, 143*, 114–118.

Da Silva, G., Kennedy, E. M., & Dlugogorski, B. Z. (2006). Ab initio procedure for aqueous-phase pKa calculation: The acidity of nitrous acid. *The Journal of Physical Chemistry A, 110*(39), 11371–11376.

Devcich, D. A., Pedersen, I. K., & Petrie, K. J. (2007). You eat what you are: Modern health worries and the acceptance of natural and synthetic additives in functional foods. *Appetite, 48*(3), 333–337.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*(3), 259–279.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science, 78*(1–2), 68–76.

Ito, H., Ueno, H., & Kikuzaki, H. (2017). Construction of a free-form amino acid database for vegetables and mushrooms. *Integrative Food Nutrition and Metabolism, 4*, 1–9.

Jo, K., Lee, J., & Jung, S. (2018). Quality characteristics of low-salt chicken sausage supplemented with a winter mushroom powder. *Korean Journal for Food Science of Animal Resources, 38*(4), 768.

Jo, K., Lee, J., Lee, S., Lim, Y., Choi, Y. S., Jo, C., & Jung, S. (2020). Curing of ground ham by remote infusion of atmospheric non-thermal plasma. *Food Chemistry, 309*, 125643.

Jo, K., Lee, J., Lim, Y., Hwang, J., & Jung, S. (2018). Curing of meat batter by indirect treatment of atmospheric pressure cold plasma. *Korean Journal of Agricultural Science, 45*(1), 94–104.

Jung, S., Kim, H. J., Park, S., Yong, H. I., Choe, J. H., Jeon, H. J., ... Jo, C. (2015). The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage. *Meat Science, 108*, 132–137.

Jung, J. T., Lee, J. K., Choi, Y. S., Lee, J. H., Choi, J. S., Choi, Y. I., & Chung, Y. K. (2018). Effect of rice bran and wheat fibers on microbiological and physicochemical properties of fermented sausages during ripening and storage. *Korean Journal for Food Science of Animal Resources, 38*(2), 302.

Jung, S., Lee, C. W., Lee, J., Yong, H. I., Yum, S. J., Jeong, H. G., & Jo, C. (2017a). Increase in nitrite content and functionality of ethanolic extracts of *Perilla frutescens* following treatment with atmospheric pressure plasma. *Food Chemistry, 237*, 191–197.

Jung, S., Lee, J., Lim, Y., Choe, W., Yong, H. I., & Jo, C. (2017b). Direct infusion of nitrite into meat batter by atmospheric pressure plasma treatment. *Innovative Food Science & Emerging Technologies, 39*, 113–118.

Jung, S., Nam, C. K., & Jo, C. (2016). Detection of malondialdehyde in processed meat products without interference from the ingredients. *Food Chemistry, 209*, 90–94.

Kalaras, M. D., Richie, J. P., Calcagnotto, A., & Beelman, R. B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. *Food Chemistry, 233*, 429–433.

King, N. J., & Whyte, R. (2006). Does it look cooked? A review of factors that influence cooked meat color. *Journal of Food Science, 71*(4), R31–R40.

Lee, J., Jo, K., & Jung, S. (Jo, & Jung, 2018b). The curing of meat batter by the plasma treated juice of red perilla. *Korean Journal of Agricultural Science, 45*(3), 475–484.

Lee, S., Lee, J., Jo, K., & Jung, S. (2018, May). Nitrite generation in ground garlic and onion by the treatment of atmospheric cold plasma. *Poster session presentation at the 50th Korean Society for Food Science of animal resources international symposium and annual meeting*. Jeju: Korea.

Lee, J., Jo, K., Lim, Y., Jeon, H. J., Choe, J. H., Jo, C., & Jung, S. (2018a). The use of atmospheric pressure plasma as a curing process for canned ground ham. *Food Chemistry, 240*, 430–436.

Lee, J., Lee, C. W., Yong, H. I., Lee, H. J., Jo, C., & Jung, S. (2017). Use of atmospheric pressure cold plasma for meat industry. *Korean Journal for Food Science of Animal Resources, 37*(4), 477.

Li, L., Shao, J., Zhu, X., Zhou, G., & Xu, X. (2013). Effect of plant polyphenols and ascorbic acid on lipid oxidation, residual nitrite and N-nitrosamines formation in dry-cured sausage. *International Journal of Food Science & Technology, 48*(6), 1157–1164.

Liu, Y., Nam, S. H., & Lee, M. (2014). Stripping of Fe (III) from the loaded mixture of D2EHPA and TBP with sulfuric acid containing reducing agents. *Bulletin of the Korean Chemical Society, 35*(7), 2109–2113.

Mancini, R. A., Kropf, D. H., Hunt, M. C., & Johnson, D. E. (2005). Effects of endpoint temperature, pH, and storage time on cooked internal color reversion of pork longissimus chops. *Journal of Muscle Foods, 16*(1), 16–26.

Mehta, N., Ahlawat, S. S., Sharma, D. P., & Dabur, R. S. (2015). Novel trends in development of dietary fiber rich meat products—A critical review. *Journal of Food Science and Technology, 52*(2), 633–647.

Ni, N., Wang, Z., He, F., Wang, L., Pan, H., Li, X., ... Zhang, D. (2014). Gel properties and molecular forces of lamb myofibrillar protein during heat induction at different pH values. *Process Biochemistry, 49*(4), 631–636.

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K. D., & Von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers, 7*(3–4), 250–257.

Öztürk, B., & Serdaroğlu, M. (2018). Effects of Jerusalem artichoke powder and sodium carbonate as phosphate replacers on the quality characteristics of emulsified chicken meatballs. *Korean Journal for Food Science of Animal Resources, 38*(1), 26.

Sebranek, J. G. (2009). *Ingredients in meat products: Properties, functionality and applications.* New York: Springer Science & Business Media (Chapter 1).

Servillo, L., Castaldo, D., Casale, R., D'Onofrio, N., Giovane, A., Cautela, D., & Balestrieri, M. L. (2015). An uncommon redox behavior sheds light on the cellular antioxidant properties of ergothioneine. *Free Radical Biology and Medicine, 79*, 228–236.

Servillo, L., D'Onofrio, N., & Balestrieri, M. L. (2017). Ergothioneine antioxidant function: From chemistry to cardiovascular therapeutic potential. *Journal of Cardiovascular Pharmacology, 69*(4), 183–191.

Subramanian, K. N., Padmanaban, G., & Sarma, P. S. (1965). Folin-Ciocalteu reagent for the estimation of siderochromes. *Analytical Biochemistry, 12*(1), 106–112.

Suman, S. P., Nair, M. N., Joseph, P., & Hunt, M. C. (2016). Factors influencing internal color of cooked meats. *Meat Science, 120*, 133–144.

Sun, X. D., & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. *Comprehensive Reviews in Food Science and Food Safety, 10*(1), 33–51.

Vossen, E., & De Smet, S. (2015). Protein oxidation and protein nitration influenced by sodium nitrite in two different meat model systems. *Journal of Agricultural and Food Chemistry, 63*(9), 2550–2556.

Yu, C., Jiao, J., Ma, L., & Sun, W. (2016). Effect of pH on the stability and molecular structure of nitrosyl hemochromogen. *Food Chemistry, 196*, 503–508.