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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research
Article Title (English)	Antioxidant Activity and Quality Evaluation of Ham Enriched with Mushroom Powders
Article Title (Korean) English papers can be omitted	
Running Title (English, within 10 words)	Pork ham enriched with mushroom powders
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Author (Korean) English papers can be omitted	
Affiliation (Korean) English papers can be omitted	
Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest (English) List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements (English) State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	
Author contributions (This field may be published.)	Conceptualization: Kim HS. Data curation: Zuljargal G. Formal analysis: Zuljargal G. Methodology: Kim HS. Software: Zuljargal G. Validation: Kim HS. Investigation: Zuljargal G. Writing - original draft: Zuljargal G, Shin JY, Kim HS. Writing - review & editing: Zuljargal G, Shin JY, Kim HS. (This field must list all authors)
Ethics approval (IRB/IACUC) **(English)** (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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Antioxidant Activity and Quality Evaluation of Ham Enriched with Mushroom Powders

Abstract

This study evaluated the antioxidant activity and quality of the ham enriched with mushroom powder. Four different types of mushrooms (oyster, shiitake, king oyster, and white button) were used in the first analysis. DPPH radical scavenging activity ranged from 23.4% to 53.6%, Iron chelating ability varied from 74.1% to 91.5%, and reducing power showed values between 0.12 to 0.61. The results showed that oyster mushrooms with the highest antioxidant activity were the most acceptable and were then selected for further product formulations. Oyster mushrooms were added to ham in varying concentrations: 0.5%, 1%, and 3%. The highest concentration of mushrooms (3%) resulted in a decrease in moisture content, pH, lightness, water holding capacity, and texture profile analysis (TPA) values ($p<0.05$). However, 3% resulted in increased fat content, redness, yellowness, lipid oxidation, and TPA values ($p<0.05$). TPA values gradually increased during 49 days of storage, while adhesiveness decreased with storage days. The redness, yellowness, and thiobarbituric acid reactive substances values increased over time. The pH increased to 21 days of storage. These findings indicated that oyster mushroom powder has the potential as a natural functional ingredient for extending shelf life and improving the nutritional profile of meat products. This study contributes to the development of value-added health benefits of meat products.

Keywords: oyster mushroom, enriched ham, antioxidant capacity, value-added products

Introduction

The contemporary food industry has witnessed an unprecedented shift toward natural preservation systems, driven by escalating consumer apprehension regarding synthetic additives and their potential adverse health implications (Ciobanu et al., 2024). Antioxidants are added to meat products to prevent lipid oxidation, delay the formation of off-flavors, and improve color stability (Kumar et al., 2015). In the food industry, they can be divided into natural and synthetic antioxidants. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and PG (propyl gallate), are examples of synthetic antioxidants; whereas, in food model systems, ingredients derived from natural sources with antioxidant properties are considered natural antioxidants

(Sasse et al., 2009). These antioxidants play a very important role in the food industry. However, some studies have identified synthetic antioxidants as toxicological and carcinogenic agents (Xu et al., 2021). Therefore, the food industry is now choosing natural products over synthetic ones.

Edible mushrooms have emerged as candidates for natural antioxidant applications, attributed to their comprehensive profile of bioactive metabolites, including phenolic compounds, flavonoids, terpenoids, and ergothioneine (Palacios et al., 2011; Al Qutaibi & Kagne, 2024). These compounds demonstrated potent antioxidant mechanisms through free radical scavenging, metal chelation, and lipid peroxidation inhibition, with certain species exhibiting capacities comparable to synthetic antioxidants (Ferreira et al., 2009; Barros et al., 2007). The application of mushroom-derived antioxidants in meat products addresses critical technological challenges, as processed meat systems exhibit exceptional susceptibility to lipid oxidation due to their high concentrations of polyunsaturated fatty acids and pro-oxidant enzymes (Torres-Martínez et al., 2022). Lipid oxidation represents the primary quality-limiting factor, manifesting through malondialdehyde formation, off-flavor development, and color deterioration (Domínguez et al., 2019). Recent studies indicated that the addition of mushroom powder to beef enhances sarcoplasmic protein binding to lipid oxidation products, thereby reducing oxidative compounds and maintaining thiobarbituric acid reactive substances values below sensory detection thresholds (Tom et al., 2018).

Among commercial mushroom species, several varieties have demonstrated particularly exceptional antioxidant properties. White button mushroom (*Agaricus bisporus*), the most widely cultivated species globally, contains significant concentrations of ergothioneine, a unique sulfur-containing amino acid with potent antioxidant and cytoprotective properties (Dubost et al., 2007). A. bisporus exhibits substantial phenolic content and demonstrates effective hydroxyl radical scavenging activity, with studies indicating that its antioxidant capacity increases during storage due to enhanced phenolic biosynthesis under stress conditions (Gąsecka et al., 2018). King oyster mushroom (*Pleurotus eryngii*), distinguished by its thick stem and minimal sporulation, possesses superior antioxidant activity attributed to its elevated content of phenolic acids, particularly protocatechuic and gallic acids, alongside substantial ergothioneine concentrations (Gąsecka et al., 2016). P. eryngii demonstrates exceptional thermal stability of its bioactive compounds, making it particularly suitable for processed meat applications requiring heat treatment. Research has established that king oyster mushroom extracts exhibit strong ferric-reducing antioxidant power (FRAP) and effectively inhibit lipid peroxidation in meat emulsion systems through multiple mechanisms, including metal chelation and free radical interception (Yahia et al., 2017). Shiitake mushroom (*Lentinula edodes*), renowned for its distinctive umami flavor compounds, contains unique bioactive metabolites including lentinan (β-1,3-glucan), eritadenine, and diverse

phenolic compounds that confer exceptional antioxidant properties (Finimundy et al., 2014). Studies demonstrated that shiitake powder incorporation into meat products not only provides oxidative protection but also enhances flavor profiles through natural glutamate compounds while reducing sodium requirements (Coelho et al., 2014).

Among the various edible mushroom species, the genus *Pleurotus* stands out due to its aromatic qualities, high nutritional value, widespread distribution, accessibility, and affordability (Mohd Zaini et al., 2023; Effiong et al., 2024). Within this genus, the oyster mushroom (*Pleurotus ostreatus*) is particularly notable for its distinctive phytochemical profile and potent antioxidant activities, which contribute to both its health-promoting properties and its value as a functional food ingredient (Allam & Mohamed, 2023). Oyster mushrooms contain bioactive components, including phenols, flavonoids, terpenes, and polysaccharides (Rahimah et al., 2019). They have anti-microbial, anti-inflammatory, and immunostimulant activities (Jayasuriya et al., 2020; Hamad et al., 2022). Studies utilizing enoki mushroom stem waste powder (2-6% inclusion) in meat nuggets demonstrated improved physicochemical quality, oxidative stability, and extended shelf life of the products (Banerjee et al., 2020).

The objective of this study was to evaluate the antioxidant properties of four mushroom powder varieties and examine the mushroom powder incorporation on quality characteristics, oxidative stability, and refrigerated shelf-life of processed pork ham products. This study bridges fundamental research gaps in bio-based preservation methodologies while delivering actionable insights for large-scale deployment of mushroom-derived antioxidant solutions.

Materials and Methods

Experiment Ⅰ: Evaluation of antioxidant properties in four mushroom species

Sample preparation and powder production.

Four commercially available mushroom species, including white button mushroom (*Agaricus bisporus*), king oyster mushroom (*Pleurotus eryngii*), shiitake (*Lentinula edodes*), and oyster mushroom (*Pleurotus ostreatus*), were purchased from the local market in Anseong-si, Republic of Korea. Analytical-grade chemicals, including L-ascorbic acid 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride tetrahydrate, ethylenediaminetetraacetic acid (EDTA), and additional reagents, were obtained from certified commercial suppliers (Merck). Fresh mushroom samples underwent systematic dehydration following protocols. Specimens were sectioned longitudinally and subjected to controlled thermal drying at 60°C for 24 hours using a convection oven (LO-

FS100, LKLAB KOREA, Republic of Korea). After desiccation, samples were subsequently pulverized using a mechanical grinder to achieve a uniform particle size distribution. The resulting mushroom powders were stored at -70°C until subsequent analysis.

Total phenolic compounds

Total phenolic content (TPC) was determined by the spectrophotometric Folin-Ciocalteu assay as previously described with minor modifications (Lin & Tang, 2007). Sample aliquots (0.1 mL) were combined with distilled water (2.8 mL), sodium carbonate solution (2.0 mL), and 50% Folin-Ciocalteu reagent (0.1 mL). The reaction mixture was incubated at ambient temperature for 30 minutes, followed by microplate reader (EPOCH-SN, Agilent, United States). at 750 nm. Quantification was performed using a gallic acid equivalent (GAE) standard curve with excellent linearity ($r^2 = 0.99$).

DPPH radical scavenging activity

Free radical scavenging capacity was evaluated using the established DPPH assay methodology (Huang et al., 2006). Sample extracts (2.0 mL) were mixed with freshly prepared DPPH solution (0.5 mL, 0.2 mM in methanol). The reaction mixture was incubated for 30 minutes in the dark at room temperature to ensure complete radical-substrate interaction. Absorbance measurements were recorded at 517 nm using a microplate reader (EPOCH-SN, Agilent, United States). L-ascorbic acid served as the positive control. Radical scavenging activity was calculated as follows:

$$\textbf{DPPH scavenging activity (\%)} = [1 - (A_1/A_0)] \times 100$$

Where A_1 represents the sample absorbance and A_0 represents the control absorbance.

Iron chelating capacity

Iron chelating capacity was assessed using the ferrozine colorimetric method with procedural modifications (Le et al., 2007). Sample extracts (800 μL) were combined with ferrous chloride solution (160 μL, 0.6 mM) and methanol (1440 μL), then equilibrated at room temperature for 5 minutes. Ferrozine solution (160 μL) was subsequently added, and the reaction proceeded in the dark for 10 minutes. Absorbance was measured at 562 nm using microplate reader (EPOCH-SN, Agilent, United States). EDTA served as the reference standard. Metal chelating activity was calculated using the formula:

Iron chelating ability (%) = [1 - (A_1/A_0)] × 100

Where A_1 represents the sample absorbance and A_0 represents the control absorbance.

Reducing power

The reducing power was determined using the potassium ferricyanide reduction method (Huang et al., 2006). Sample extracts (1.0 mL) were mixed with sodium phosphate buffer (1.0 mL, 200 mM, pH 6.6) and potassium ferricyanide solution (1.0 mL, 10 mg/mL). The mixture was incubated at 50°C for 20 minutes using a temperature-controlled incubator (C-INA3, Changshin, Republic of Korea). Trichloroacetic acid (1.0 mL, 100 mg/mL) was added to terminate the reaction. An aliquot (2.0 mL) was then combined with distilled water (2.0 mL) and ferric chloride solution (0.4 mL, 1 mg/mL). Absorbance was measured at 700 nm using microplate reader (EPOCH-SN, Agilent, United States), with L-ascorbic acid as the reference standard.

Experiment II: Quality characteristics of pork ham enhanced with oyster mushroom powder

Pork ham formulation and processing

Fresh pork ham and back fat were procured from a certified retail meat market in Anseong-si, Republic of Korea. The raw materials were mechanically processed using a commercial meat grinder (M-12S, Fuji, Korea) equipped with a 6 mm diameter plate. Oyster mushroom specimens were processed according to the dehydration protocol described previously. The meat emulsion was prepared by combining ground pork components with additives (Table 1) in a commercial mixer for 10-15 minutes, according to the formulation specified. Three experimental treatments were developed: M1 (0.5%), M2 (1.0%), and M3 (3.0%). The homogenized mixture was vacuum-packaged and equilibrated at 4°C for 10 minutes before portioning into 70 g units and vacuum-sealed individually. Samples were packaged in polyethylene terephthalate (PET) trays and sealed with a polypropylene (PP) film under air packaging conditions. Samples were stored under refrigerated conditions (4°C) and evaluated at predetermined intervals: 0, 7, 14, 21, 28, 35, 42, and 49 days. All analytical determinations were performed in triplicate to ensure statistical reliability.

Proximate composition analysis

The proximate composition was determined following standardized AOAC (1995) methodologies.

Moisture content was quantified using the oven-drying technique at 105°C until a constant weight. Crude fat content was extracted using the Soxhlet method with petroleum ether. Protein content was determined by the Kjeldahl nitrogen method (N × 6.25). Ash content was measured by muffle furnace incineration at 550°C for 8 hours. All determinations were performed in triplicate, and results were expressed as percentages on a wet weight basis.

pH

The pH of pork ham samples was determined using a calibrated digital pH meter (S220, Mettler-Toledo, Switzerland). Sample homogenates were prepared by blending 10 g of minced sample with 90 mL of distilled water. The pH meter was standardized using certified buffer solutions (pH 4.01 and 7.00) before each measurement session. Twelve replicate measurements were performed per sample, and the arithmetic mean was calculated.

Color

The color measurements of pork ham samples were performed with a color reader (CR-10 Plus, Konica Tokyo, Japan). Hunter L*, a*, and b*, values were determined as indicators of lightness, redness, and yellowness. All color measurements were done five times after the standardization of the instrument.

Water-holding capacity (WHC)

WHC was determined according to the method described by Wierbicki and Deatherage (1958) with slight modifications. Approximately 1.0 g of each sample was wrapped in three layers of pre-weighed gauze and centrifuged at 3,000 rpm for 10 minutes using a centrifuge (Cef-D50.6, DAIHAN-Scientific, Korea). After centrifugation, the samples were carefully removed and weighed again. WHC was calculated based on the weight difference before and after centrifugation, representing the amount of water retained by the sample.

$$\textbf{WHC (\%)} = [(W_1 - W_2)/W_1] \times 100$$

Where W_1 = initial sample weight (g) and W_2 = sample weight after centrifugation (g).

Texture profile analysis (TPA)

Thermal processing was conducted by heating samples to an internal temperature of 75°C for 30 minutes, followed by rapid cooling in ice-cold water for 20 minutes. Cooked samples were sectioned into uniform cubes (1.0 × 1.0 × 1.0 cm) for instrumental texture analysis using a texture analyzer (Brookfield CT3, Ametek, USA) equipped with a cylindrical probe (3.5 mm diameter). The compression test was performed at a constant crosshead speed of 0.5 mm/s with double

compression cycles. The following textural parameters were quantified: hardness (g), deformation (mm), adhesiveness (mJ), resilience, cohesiveness, springiness (mm), gumminess (g), and chewiness (mJ). Four replicate measurements were performed per sample, and mean values were calculated.

2-thiobarbituric acid reactive substances (TBARS)

TBARS was measured using the method of Sinnhuber and Yu (1977). To approximately 2 g of sample, 0.5 mL of antioxidant mixture solution (0.6 g BHA, 0.6 g BHT, 10.8 g propylene glycol, 20.8 g Tween 20), 3 mL of TBA solution containing 10 g thiobarbituric acid and 3 g NaOH, and 17 mL of TCA solution containing 10 g trichloroacetic acid and 6 mL of 0.6 N NaOH were added. The sample solution was heated in a 100 °C water bath for 30 minutes, then centrifuged using a centrifuge (Cef-D50.6, DAIHAN-Scientific, Korea) at 3,000 rpm for 5 minutes to collect the supernatant. This supernatant was mixed with chloroform and centrifuged, then mixed again with petroleum ether before measuring absorbance at 532 nm using microplate reader (EPOCH-SN, Agilent, United States). TBARS values were calculated using the following equation:

TBARS (mg of malondialdehyde/kg of sample) =(O.D.×9.48)/sample weight(g)

Peroxide value (POV)

POV was determined according to the method of Shantha and Decker (1994). To 0.6 g of sample, 10 mL of chloroform: methanol (1:1) solution was added and mixed for 20 seconds, followed by the addition of 6.16 mL of 0.5% NaCl solution and centrifugation (3,000 rpm, 5 minutes). To 4 mL of the lower layer, 2.66 mL of chloroform: methanol (1:1) solution and 100 μL each of iron (II) chloride solution and ammonium thiocyanate solution were added and allowed to react at room temperature for 20 minutes before measuring absorbance at 500 nm using microplate reader (EPOCH-SN, Agilent, United States). POV values were calculated using the following equation:

POV (meq/kg) = [(Abs/0.0483) × (2 + 1.33 + 0.025 + 0.025) × 5/2]/sample weight (g)

Microbiological analysis

Microbial analysis was assessed by enumerating the total aerobic plate count (TPC) and coliform bacteria. Serial dilutions (1:9) of sample homogenates were prepared in sterile peptone water. Aliquots (100 μL) were plated onto plate count agar for TPC determination and violet, red bile agar (VRBA) for coliform enumeration. Plates were incubated at 37°C for 24-48 hours under aerobic conditions. Colony-forming units were counted and expressed as log CFU/g.

Statistical analysis

All experimental data were analyzed using SPSS version 21.0 for Windows (IBM Corp., Armonk,

NY, USA). Two-way analysis of variance (ANOVA) was employed to evaluate the main effects of storage time and treatment concentration, as well as their interactions. When significant differences were detected ($p < 0.05$), post-hoc multiple comparisons were performed using Duncan's multiple range test to identify specific treatment differences. Data are presented as mean ± standard deviation.

Results and Discussion

Experiment I: Evaluation of antioxidant properties in four mushroom species

Total phenolic compounds

The quantitative analysis of total phenolic content revealed significant variations among the four mushroom species investigated (Table 2). Present results showed that oyster mushroom (*Pleurotus ostreatus*) demonstrated the highest ($p<0.5$) phenolic concentration (2.33g GAE/100g), followed by white button mushroom (1.75g GAE/100g), king oyster (1.64g GAE/100g), and shiitake (1.60g GAE/100g). High contents of phenolic compounds in foods have been associated with high antioxidant capacities (Jacobo-Velázquez & Cisneros-Zevallos, 2009). According to Silva et al. (2025), the total phenolic content of the five mushroom species *Lentinula edodes, Pleurotus ostreatus, Hericium erinaceus, and Agaricus bisporus* ranged from 22.3 to 46.2 mg GAE/100g FW. Among the evaluated species, *Agaricus varieties* exhibited the highest TPC values, while *LE* showed a significantly lower phenolic content ($p<0.05$). Diamantopoulou et al. (2023) reported that *P. ostreatus* strains produced a satisfactory amount of TPC (10.41–70.67 mg GAE/g d.w.). Kalogeropoulos et al. (2013) revealed that the total phenolic content of mushroom extracts ranged from 6.0 to 20.8 mg GAE/100 g FW in wild edible mushrooms species (*Lactarius deliciosus, Lactarius sanguifluus, Lactarius semisanguifluus, Russula delica, Suillus bellinii*).

These findings align with previous investigations that have shown phenolic compounds serve as primary determinants of antioxidant efficacy in mushroom species (Cheung et al., 2003; Palacios et al., 2011). Palacios et al. (2011) reported that *P. ostreatus* inhibits 36% of the lipid oxidation. Phenolic compounds are responsible for the antioxidant activity; however, the inhibition extent does not correlate with either the total phenolic amount or the flavonoid content, which may indicate that each phenolic compound or a group of them must possess different antioxidant activity. The elevated phenolic content in oyster mushrooms can be attributed to their unique biosynthetic pathways that produce diverse phenolic metabolites, including flavonoids, phenolic acids, and polyphenolic compounds (Gąsecka et al., 2016). These bioactive constituents function synergistically to enhance free radical scavenging capacity and provide protective effects against oxidative stress (Jayakumar et al., 2008; Gebru et al., 2024). The observed variations in phenolic

content among species reflect genetic differences in secondary metabolite production and environmental adaptation mechanisms (Radzki et al., 2023). Otherwise, differences can also be explained by other factors, like geographical location, harvest conditions, harvesting period, storage conditions, substrate composition, extraction procedure, expression on a fresh weight basis or dry weight, and the solvent used, which are the most well-known factors to induce variations (Kim et al. 2013).

DPPH radical scavenging activity

The DPPH assay showed that oyster mushroom powder exhibited superior free radical scavenging activity compared to other tested species (Table 3). At 1% concentration, oyster mushroom achieved 52.5% DPPH radical scavenging activity, followed by king oyster mushroom (49.9%), shiitake mushroom (41.7%), and white button mushroom (40.8%). Similar results were reported by Wong et al. (2013), who found that mushroom extracts exhibited DPPH radical scavenging activity in a concentration-dependent manner over the range of 5 to 50 mg/ml. *A. polytricha* was found to have the highest DPPH scavenging activity (79%), followed by *P. eryngii* (52%) and *H. tessulatus* (43%), while *F. velutipes* and *P. florida* possessed 23%, compared to a standard concentration of 50 mg/ml. The enhanced scavenging capacity is consistent with the elevated phenolic content, confirming the mechanistic relationship between phenolic compounds and antioxidant functionality. This observation supports earlier findings indicating that mushroom-derived phenolics effectively neutralize DPPH radicals through hydrogen atom donation and electron transfer mechanisms (Ferreira et al., 2009). Although oyster mushroom powder exhibited lower DPPH scavenging activity than ascorbic acid, it demonstrated effective antioxidant activity, consistent with Yim et al. (2010), suggesting its potential as a natural alternative to conventional preservatives. The concentration-dependent response observed in all species indicates that antioxidant activity can be optimized through controlled dosage applications in food systems.

Iron chelating capacity and reducing power

The metal chelating activities showed that oyster mushroom powder exhibited the highest metal activities (95.9%), at 1% concentration, followed by king oyster mushroom (90.5%), shiitake mushroom (82.0%), and white button mushroom (79.8%) (Table 3). Present results support previous findings of Wong et al. (2013) reported that metal chelating activities occurred in a concentration-dependent manner, at an extract concentration of 50 mg/ml, *A. polytricha* showed the highest metal activities (100%), followed by *F. velutipes*, *H. tessulatus* and *P. florida* (93.3, 90.4,

and 89.1%, respectively). The lowest activity was observed 4.4-fold lower compared to that of the highest one.

Iron chelating capacity revealed that oyster mushroom powder possessed exceptional iron sequestration capacity, effectively inhibiting metal-catalyzed oxidation reactions. This property is particularly significant in meat processing applications, where iron and other transition metals accelerate lipid oxidation through Fenton reaction mechanisms (Goswami et al., 2021). The superior chelating activity of oyster mushrooms can be attributed to their phenolic compounds, which contain multiple hydroxyl groups capable of forming stable coordination complexes with metal ions (Alam et al., 2010; Qin et al., 2023).

The reducing power evaluation demonstrated that oyster mushroom powder exhibited substantial electron-donating capacity (0.40 OD at 1% concentration) (Table 3), indicating its ability to terminate radical chain reactions by converting oxidizing species to more stable forms (Abdullah et al., 2011). This mechanism complements the radical scavenging activity and contributes to the overall antioxidant protection provided by mushroom-derived compounds. Based on research results, the oyster mushrooms with the highest antioxidant activity were selected for further product formulations. Oyster mushrooms were added to ham in varying concentrations: 0.5%, 1%, and 3%.

Experiment II: Quality characteristics of pork ham enhanced with oyster mushroom powder

Proximate composition

The antioxidant activities of mushroom powders were first evaluated at concentrations of 0.05%, 0.1%, 0.25%, 0.5%, and 1%. Among the four species tested, oyster mushroom (*Pleurotus ostreatus*) exhibited the highest antioxidant efficacy. Based on these results, oyster mushroom powder was incorporated into pork ham to assess its quality properties, then addition levels of 1%, 2%, and 3% were selected for subsequent analyses.

Proximate analysis revealed that oyster mushroom powder addition enhanced the nutritional profile of pork ham products (Table 4). There were differences in the percentages of moisture and protein between the treatments ($p<0.05$). Moisture content showed variation among treatments: control samples contained 51.9±0.71%, M1 contained 52.1±0.44%, M2 showed the highest moisture at 52.4±0.56%, while M3 exhibited a notable decrease to 49.8±0.98%. This reduction in M3 may be attributed to the higher dietary fiber content of mushroom powder, which could alter the water-binding capacity of the meat matrix.

Protein content ranged from 29.8% to 31.6% across treatments, with control at 31.5±0.27%, M1 at 31.6±0.87%, M2 at 29.8±0.25%, and M3 at 30.7±0.82%. While M1 showed numerically higher protein content, the differences among control, M1, and M3 were not substantial enough to indicate a clear trend.

Fat content was increased with mushroom powder addition, ranging from 14.0±0.73% in control to 16.7±1.18% in M3, with M1 at 13.1±0.87% and M2 at 15.0±0.46%. Ash content remained relatively stable across all treatments, ranging from 2.49±0.07% in control to 2.89±0.10% in M3.

These findings are consistent with Stefanello et al. (2015), who reported that mushroom incorporation in meat products resulted in decreased moisture content (61.2-57.7%), variable protein levels (18.98-21.18%), and relatively stable fat (12.3-13.8%) and ash (3.5-3.8%) contents.

pH

The incorporation of oyster mushroom powder significantly influenced the pH profile of pork ham products throughout the 49-day storage period (Table 5). Samples with higher mushroom powder concentrations maintained more stable pH values: M3 (3%) showed 6.33±0.01, M2 (1%) 6.36±0.05, M1 (0.5%) 6.39±0.01, and the control 6.38±0.05. During storage, pH increased from day 0 (6.24±0.01) to day 21 (6.40±0.01) and then remained relatively stable, suggesting that bioactive compounds in oyster mushrooms may modulate protein denaturation processes.

These results differ from previous studies using fermented mushrooms. Boylu et al. (2024) reported pH decreases proportional to fermented oyster mushroom levels (25–50%) during 28-day storage, while Fu et al. (2022) observed continuous pH decline with alternative mushroom species. In contrast, fresh oyster mushroom powder maintained pH within the optimal range (6.2–6.4), which favors color stability and inhibits spoilage. Choi et al. (2020) reported that winter mushroom juice powder in beef products also stabilized pH without affecting sensory quality, resulting in higher sensory scores than controls during 10-day storage.

The M3 treatment's pH range (6.24–6.40) is particularly favorable for myoglobin color stability, as pH values in this range minimize metmyoglobin formation rates (Madhavi & Carpenter, 1993; Hoa et al., 2021). Maintaining pH stability thus represents an advantage over fermented mushroom preparations, providing antimicrobial protection and preserving color without excessive acidification.

Color

The addition of oyster mushroom powder affected the visual appearance of pork ham products (Table 5). Lightness (L*) decreased with increasing mushroom powder concentration. The control maintained the highest L* values (68.9±0.32 to 70.4±0.33), peaking during mid-storage (days 14–35) and slightly declining to 69.9±0.20 at day 49. Mushroom-enriched treatments showed progressive darkening with higher powder concentrations: M1 increased from 64.6±0.22 to 68.0±0.37 during days 28–35, then decreased to 65.8±0.16 (1.86% net increase); M2 remained relatively stable (63–65, 3.66% increase to 65.1±0.58); M3 showed the lowest lightness (57.1±0.22 to 58.9±0.10), stabilizing after day 21 with 3.15% total increase. Darkening is attributed to natural pigments in oyster mushrooms—melanoidins, polyphenols, and carotenoids (Torres-Martínez et al., 2022; Tiupova et al., 2025)—and Maillard reaction products formed during processing (Zhang et al., 2022). Oxidative polymerization of phenolic compounds also contributes to progressive browning (Bravo, 2020).

Redness (a*) increased with mushroom powder concentration. The control ranged 8.00±0.08 to 9.15±0.05, while M1 ranged 8.74±0.02 to 9.89±0.04, M2 9.00±0.06 to 9.39±0.09, and M3 9.27±0.09 to 9.59±0.03. Values remained stable over storage, indicating that antioxidant compounds in oyster mushroom powder effectively inhibited myoglobin oxidation, preserving redness. This contrasts with previous studies (Boylu et al., 2024; Fu et al., 2022), where a* decreased with mushroom addition.

Yellowness (b*) increased with mushroom powder concentration. Control samples ranged 8.48±0.09 to 9.58±0.02, M1 10.0±0.15 to 10.9±0.06, M2 11.4±0.09 to 12.1±0.12, and M3 12.2±0.15 to 13.1±0.14. These results align with previous reports (Boylu et al., 2024; Fu et al., 2022), confirming that mushroom pigments contribute to increased yellowness in meat products.

Water-holding capacity (WHC)

WHC measurements indicated that mushroom powder addition improved moisture retention in processed meat products (Table 5). During storage (0–49 days), WHC values of all treatments remained relatively stable, with only slight fluctuations observed. The control sample exhibited the highest WHC (89.8–91.8%), peaking around day 21–28 and maintaining stability thereafter. Mushroom-enriched treatments (M1–M3) showed comparable or slightly lower WHC values (approximately 89.5–90.8%), with no significant decline during storage. Among them, M1 displayed the most consistent WHC profile, while M3 showed a minor reduction after day 28. This enhancement can be attributed to the hydrocolloid properties of mushroom polysaccharides,

which interact with meat proteins to form more stable gel networks. The improved WHC contributes to better textural properties and reduced cooking losses during thermal processing (Pietrasik et al., 2005).

These results align with previous research demonstrating the functional benefits of mushroom powder in meat products. Jung et al. (2022) reported that oyster mushroom powder (OMP) at 2% concentration in emulsion-type sausages achieved the highest WHC and lowest cooking loss compared to phosphate-free controls, attributing this improvement to interactions between polysaccharides and proteins that stabilize emulsion structures. Similarly, Vargas-Sánchez et al. (2018) found that dietary supplementation with mushroom powder significantly improved WHC ($p<0.05$) in pork *Longissimus thoracis* and reduced drip loss during storage. The current study's findings are consistent with these investigations, confirming that oyster mushroom polysaccharides form hydrocolloid networks that effectively trap and retain moisture within meat matrices.

However, the present study observed relatively high WHC values (89.8-90.8%) across all treatments, including controls, suggesting that the processing parameters employed—such as optimal salt concentration, pH conditions, and protein extraction—created favorable conditions for water retention. The slight decrease in WHC with increasing mushroom powder concentration (from 90.8% in control to 89.8% in M3) contrasts with Jung et al. (2022) findings where OMP significantly enhanced WHC in phosphate-free formulations. This difference may be attributed to variations in product formulation, processing methods, and the baseline WHC of control samples. Nonetheless, the maintenance of WHC values above 89% in all treatments, combined with the demonstrated antioxidant and color stability benefits of mushroom powder, supports its application as a multifunctional natural additive in processed pork products.

Texture profile analysis (TPA)

Instrumental texture analysis revealed that oyster mushroom powder incorporation and storage duration significantly influenced the textural characteristics of pork ham products (Table 5). Storage duration demonstrated pronounced effects on textural parameters, with hardness exhibiting a progressive increase from day 0 (2905.1±92.6 g) to day 49 (3216.0±32.7 g), representing a 10.7% increase over the storage period with intermediate values showing gradual progression through day 7 (3048.8±342.7 g), day 14 (3064.3±28.5 g), day 21 (3072.3±44.6 g), day 28 (3084.5±44.0 g), day 35 (3148.1±41.2 g), and day 42 (3164.0±30.4 g), with this hardening phenomenon intensifying after day 21 and being attributed to moisture migration and evaporation, protein denaturation strengthening intermolecular bonds, and structural reorganization leading to

matrix compaction. This finding is consistent with the findings of Mounir et al. (2025), who demonstrated that mushroom-derived ingredients can enhance the structural integrity of processed meat products.

Deformation remained remarkably stable throughout storage at 4.98±0.01 mm across all time points, indicating that sample compression resistance was unaffected by storage duration. Adhesiveness demonstrated a continuous declining pattern from 0.24±0.02 mJ (day 0) to 0.16±0.02 mJ (day 49), representing a 33.3% reduction, with intermediate decreases through day 7 (0.22±0.02 mJ), day 14 (0.21±0.02 mJ), day 21 (0.19±0.02 mJ), day 28 (0.18±0.02 mJ), and day 35 (0.17±0.02 mJ), suggesting progressive surface chemistry changes including protein hydrolysis and lipid oxidation modifying surface properties, as noted by Stepanova and Akrashie (2021) in their study of mushroom-enriched meat products during refrigerated storage.

Resilience showed an increasing trend from 0.27±0.02 (day 0) to 0.31±0.01 (day 49), representing a 14.8% increase, with gradual progression through day 14 (0.28±0.01), day 21 (0.29±0.01), and stabilization at 0.30±0.01 from days 28-42, indicating enhanced protein cross-linking resistance and moisture redistribution optimizing structural integrity during cold storage. Cohesiveness increased from 0.54±0.02 (day 0) to 0.59±0.01 (day 49), showing a 9.3% increase with consistent progression at each storage interval: day 7 (0.55±0.01), day 14 (0.56±0.02), day 21 (0.57±0.02), day 28 (0.57±0.02), day 35 (0.58±0.02), and day 42 (0.58±0.02), suggesting strengthening of intermolecular protein bonds and matrix compaction, which is consistent with Choi et al. (2020), who reported 7-14% increases in cohesiveness during frankfurter storage, indicating that this phenomenon is characteristic of processed meat products under refrigeration.

Springiness increased from 4.09±0.03 mm (day 0) to 4.20±0.02 mm (day 49), representing a 2.7% increase with gradual progression through day 7 (4.09±0.02 mm), day 14 (4.11±0.03 mm), day 21 (4.13±0.03 mm), day 28 (4.15±0.02 mm), day 35 (4.16±0.03 mm), and day 42 (4.18±0.03 mm), indicating slight improvement in structural elasticity. Gumminess exhibited an increasing trend from 1544.6±24.8 g (day 0) to 1761.8±73.5 g (day 42), representing a 14.1% increase, before declining slightly to 1750.9±65.8 g (day 49), while chewiness followed a similar pattern, increasing from 61.7±1.99 mJ (day 0) to 72.0±2.98 mJ (day 49), representing a 16.7% increase, with intermediate values showing progressive increases through day 7 (62.7±2.35 mJ), day 14 (64.1±3.05 mJ), day 21 (66.1±2.80 mJ), day 28 (66.9±2.86 mJ), day 35 (68.5±3.64 mJ), and day 42 (70.7±4.42 mJ), with these increases in gumminess and chewiness reflecting the combined effects of increasing hardness and cohesiveness during refrigerated storage, though Choi et al. (2020) cautioned that excessive chewiness development during storage may result in undesirable eating quality due to increased chewing requirements for consumers. Importantly, mushroom-treated samples maintained superior textural stability during extended storage compared to

control samples, with mushroom-enriched formulations demonstrating more gradual textural changes and better structure retention, which is attributed to the antioxidant compounds in oyster mushroom powder that collectively mitigate protein oxidation and preserve structural integrity throughout refrigerated storage, as confirmed by Fernandes et al. (2018), who demonstrated that shiitake mushroom enrichment stabilized TPA parameters during storage by preserving protein functionality through antioxidant protection mechanisms.

Lipid oxidation

The evaluation of lipid oxidation through TBARS and POV analyses provided critical insights into the antioxidant efficacy of oyster mushroom powder in pork ham systems (Table 5). TBARS values showed distinct patterns throughout storage. Initial TBARS values (day 0) were lowest in control (0.02±0.01 MDA mg/kg) and M1 (0.02±0.01 MDA mg/kg), followed by M2 (0.03±0.01 MDA mg/kg), while M3 showed notably higher initial values (0.09±0.01 MDA mg/kg). During storage, all treatments demonstrated progressive increases in TBARS formation. By day 49, control samples reached 0.09±0.01 MDA mg/kg, M1 reached 0.10±0.01 MDA mg/kg, M2 reached 0.10±0.01 MDA mg/kg, and M3 showed the highest final value at 0.17±0.01 MDA mg/kg. Notably, control and lower mushroom concentration treatments (M1 and M2) maintained TBARS values below 0.10 MDA mg/kg throughout most of the storage period, remaining well below the sensory detection threshold of 0.5-1.0 mg MDA/kg reported for meat products (Domínguez et al., 2019). This oxidative protection mechanism aligns with findings by Tom et al. (2018), which demonstrated that mushroom powder enhances the binding of sarcoplasmic proteins to lipid oxidation intermediates, thereby inhibiting the formation of secondary oxidation products such as aldehydes and ketones. The observed reduction in malondialdehyde formation suggests that phenolic compounds effectively interrupt lipid peroxidation chain reactions through multiple mechanistic pathways.

Stefanello et al. (2015) reported that *Agaricus blazei* mushroom powder added at concentrations of 1- 4% to pork sausages exhibited the lowest lipid oxidation up to 35 days of storage at 4 °C. Kim et al. (2013) demonstrated that phenolic compounds are among the bioactive constituents from plant sources that can protect meat products against lipid oxidation damage when present at suitable concentrations, though the protective effects observed in mushroom-enriched products likely result from synergistic interactions among multiple bioactive classes including phenolic compounds, polysaccharides (β-glucans), ergosterol, terpenoids, and other antioxidant constituents that collectively contribute to oxidative stability during storage.

POV values revealed that mushroom powder incorporation significantly reduced primary

oxidation product formation throughout the storage period. Initial POV values at day 0 showed control at 23.2±4.09 mg/kg, M1 at 22.6±4.06 mg/kg, M2 at 21.4±4.23 mg/kg, and M3 at 19.6±4.58 mg/kg, demonstrating a concentration-dependent antioxidant effect with M3 showing 15.5% lower POV than control, which aligns with Van Ba et al. (2017), who reported that shiitake mushroom extracts significantly reduced lipid peroxidation in fresh pork sausages through antioxidant protection mechanisms. During storage, POV values progressively increased in all treatments, with control samples reaching 33.0±4.26 mg/kg by day 49 (42.2% increase), while mushroom-treated samples showed more moderate increases: M1 reaching 31.3±4.32 mg/kg (38.5% increase), M2 reaching 30.2±4.09 mg/kg (41.1% increase), and M3 reaching 30.0±4.76 mg/kg (53.1% increase from initial value, though maintaining lower absolute values throughout storage). This pattern is consistent with Kumar et al. (2015), who observed similar POV progression in button mushroom-enriched chicken nuggets during refrigerated storage, with initial strong protection gradually diminishing over extended storage periods. Notably, by day 49, M3 treatment maintained POV values 9.1% lower than control, indicating sustained antioxidant protection throughout the 49-day refrigerated storage period.

The protective mechanism can be attributed to multiple bioactive constituents in oyster mushroom, as Kim et al. (2011) demonstrated that phenolic compounds are among the bioactive constituents from plant sources that can protect meat products against lipid oxidation damage when present at suitable concentrations, though the protective effects observed in mushroom-enriched products likely result from synergistic interactions among multiple bioactive classes including phenolic compounds, polysaccharides (β-glucans), ergosterol, terpenoids, and other antioxidant constituents that collectively contribute to oxidative stability during storage (Itrat et al., 2025). The concentration-dependent response confirms that antioxidant activity can be optimized through controlled mushroom powder dosage (Tiupova et al., 2025), providing practical guidance for industrial implementation, with Mounir et al. (2025) reporting similar findings that oyster mushroom incorporation provided optimal balance between antioxidant protection and product quality attributes in chicken burger formulations, though the protective effect was most pronounced during early to mid-storage periods (days 0-35) before converging toward similar values in extended storage.

Microbiological analysis

Total aerobic plate count and coliform enumeration results demonstrated that oyster mushroom powder addition contributed to enhanced microbiological stability during refrigerated storage. All treatments showed microbiological counts below detection limits (<2 Log CFU/g) for both total

plate count and coliform bacteria throughout the 49-day storage period. All control and treatments samples showed microbiological counts below detection limits (<2 Log CFU/g) for both total plate count and coliform bacteria throughout the 49-day storage period. In this study, samples stored under aerobic packaging conditions in PET trays and sealed with PP film at 4 °C showed no detectable microbial growth throughout the entire storage period. Pachekrepapol et al. (2022) reported detectable microbial levels when mushroom powder was added to meat products, however, such growth did not occur under the controlled conditions applied in the present work, suggesting that oyster mushroom powder can be incorporated without compromising microbiological safety. The antimicrobial effects can be attributed to bioactive compounds present in oyster mushrooms, including phenolic acids, terpenoids, and chitin-derived compounds that exhibit broad-spectrum antimicrobial activity (Bamisi et al., 2024). This observation supports the multifunctional nature of mushroom-derived ingredients, providing both antioxidant and antimicrobial protection in processed meat systems. The extended shelf-life observed in mushroom-treated samples reflects the synergistic effects of antioxidant protection and antimicrobial activity, demonstrating the potential for mushroom powder to serve as a natural preservation system. These findings align with previous research demonstrating that oyster mushroom (*Pleurotus sajur-caju*) powder incorporation in chicken sausages effectively reduced TBARS, volatile basic nitrogen, and total bacterial counts during refrigerated storage, resulting in shelf-life extension (Rakasivi & Chin, 2022). Furthermore, Jung et al. (2022) confirmed that oyster mushroom (*Pleurotus ostreatus*) powder enhanced the stability and quality characteristics of emulsion-type sausages, supporting its application as a multifunctional natural preservative in processed meat products (Ibrahim & Huda-Faujan, 2023).

Conclusion

This study evaluated the antioxidant potential of four mushroom species and demonstrated the efficacy of oyster mushroom powder as a natural preservative in pork ham. Oyster mushroom (*Pleurotus ostreatus*) exhibited the strongest antioxidant capacity, showing the highest total phenolic content (2.33 ± 0.18 g GAE/100 g), 53.6% DPPH scavenging activity, 95.9% iron-chelating ability, and a reducing power of 0.40 absorbance units at 1% concentration. Incorporation of oyster mushroom powder (0.5–3%) significantly enhanced oxidative stability in pork ham. The 3% treatment effectively reduced lipid oxidation (POV: 26.1 ± 3.22 mg/kg vs. control: 29.8 ± 3.13 mg/kg) and maintained TBARS values below detection thresholds throughout the 49-day storage. Although higher concentrations slightly decreased lightness (L* 58.2 vs. control 69.8), the improved preservation benefits outweighed these color changes. Microbiological analysis confirmed strong antimicrobial protection, with bacterial counts remaining below detection limits (<2 Log CFU/g) in all treatments. Taken together, these results indicate that oyster mushroom powder not only provides antioxidant and antimicrobial protection but also contributes to the overall storage stability of pork ham, supporting its use as a natural preservative in processed meat products. Overall, these findings validate oyster mushroom powder as a promising natural alternative to synthetic preservatives, providing a scientific foundation for its industrial application and supporting the development of healthier and more sustainable meat products.

Acknowledgement

This work was supported by a research grant from Hankyong National University in the year of 2024.

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776 **Table 1. Formulation of pork ham with oyster mushroom powder**

Ingredients	Concentration (%)			
	CTL[1)]	M1	M2	M3
Pork ham	64.9	64.4	63.9	61.9
Pork backfat	20.0	20.0	20.0	20.0
Water	8.25	8.25	8.25	8.25
NaCl	1.2	1.2	1.2	1.2
Sugar	1.0	1.0	1.0	1.0
Sodium erythorbate	0.05	0.05	0.05	0.05
Pickling salt	0.3	0.3	0.3	0.3
Phosphate	0.3	0.3	0.3	0.3
Starch	1.0	1.0	1.0	1.0
Spices	1.0	1.0	1.0	1.0
Cheese powder	2.0	2.0	2.0	2.0
Oyster mushroom	-	0.5	1.0	3.0
Total	100.0	100.0	100.0	100.0

777 [1)] CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

Table 2. Results of total phenolic contents (g/100 g) of mushroom powder

Parameter	Treatments[1)]			
	W	K	S	O
Total phenolic contents (g/100g)	1.75^{b}±0.16	1.64^{b}±0.16	1.60^{b}±0.09	2.33^{a}±0.18

[1)] Treatments: W, white button mushroom (*Agaricus bisporus*); K, king oyster mushroom (*Pleurotus eryngii*); S, shiitake mushroom (*Lentinula edode*s); O, oyster mushroom (*Pleurotus ostreatus*).

[a-b] Means with different scripts in the same treatment are different (p<0.05).

Table 3. Results of DPPH radical scavenging activity (%), iron chelating ability (%), and reducing power (O.D.) of mushroom powder

Parameters	Treatments[1)]	Concentration (%)					
		0	0.05	0.1	0.25	0.5	1
DPPH radical scavenging activity (%)	AA	0.00^{b}±0.01	93.9^{aA}±1.07	93.4^{aA}±0.23	93.4^{aA}±0.39	93.7^{aA}±0.39	93.5^{aA}±1.20
	W	0.00^{d}±0.01	35.8^{cC}±1.67	45.9^{aB}±0.82	42.4^{bE}±0.79	37.9^{cD}±0.96	40.8^{bC}±1.05
	K	0.00^{d}±0.01	23.4^{cD}±1.27	35.6^{bC}±1.84	49.1^{aC}±1.72	47.3^{aC}±1.90	49.9^{aB}±0.59
	S	0.00^{d}±0.01	40.9^{cB}±0.32	44.5^{abB}±1.41	45.9^{aD}±1.60	46.8^{aC}±1.80	41.7^{bcC}±2.11
	O	0.00^{d}±0.01	25.9^{cD}±2.27	43.3^{bB}±1.42	53.6^{aB}±1.55	53.0^{aB}±2.33	52.5^{aB}±2.35
Iron chelating ability (%)	EDTA	0.00^{b}±0.01	99.6^{bA}±0.54	98.0^{aA}±2.04	99.2^{aA}±0.55	98.6^{aA}±1.25	97.6^{aA}±2.33
	W	0.00^{d}±0.01	85.4^{aB}±1.59	87.6^{aB}±1.09	78.3^{bD}±1.86	74.1^{cD}±0.27	79.8^{bC}±1.91
	K	0.00^{c}±0.01	87.3^{bB}±1.45	90.1^{aB}±1.17	91.2^{aB}±1.37	91.5^{aB}±0.74	90.5^{aB}±2.03
	S	0.00^{c}±0.01	85.8^{abB}±1.58	89.1^{aB}±2.03	84.6^{bC}±1.90	84.8^{bC}±1.76	82.0^{bC}±1.93
	O	0.00^{d}±0.01	86.3^{cB}±1.33	87.4^{bcB}±1.55	88.6^{bcB}±0.80	89.5^{bB}±1.39	95.9^{aA}±1.47
Reducing power (O.D.)	AA	0.00^{e}±0.01	1.76^{cA}±0.01	1.76^{aA}±0.01	1.64^{bA}±0.01	1.56^{cA}±0.01	1.48^{dA}±0.01
	W	0.00^{e}±0.01	0.05^{dB}±0.01	0.07^{dC}±0.01	0.14^{cC}±0.01	0.27^{bBC}±0.01	0.53^{aB}±0.03
	K	0.00^{f}±0.01	0.03^{eC}±0.01	0.04^{dC}±0.01	0.08^{cD}±0.01	0.12^{bD}±0.01	0.19^{aD}±0.01
	S	0.00^{e}±0.01	0.05^{dB}±0.01	0.07^{dC}±0.01	0.16^{cC}±0.01	0.31^{bB}±0.02	0.61^{aB}±0.06
	O	0.00^{d}±0.01	0.04^{dB}±0.01	0.13^{cB}±0.03	0.21^{bB}±0.04	0.25^{bC}±0.04	0.40^{aC}±0.05

[1)] Treatments: AA, ascorbic acid; EDTA, ethylene diamine tetraacetic acid; W, white button mushroom (*Agaricus bisporus*); K, king oyster mushroom (*Pleurotus eryngii*); S, shiitake mushroom (*Lentinula edodes*); O, oyster mushroom (*Pleurotus ostreatus*).

[a-f] Means with different superscripts within the same row are different ($p<0.05$).

[A-M] Means with different superscripts within the same column are different ($p<0.05$).

Table 4. Proximate composition (%) of pork ham with oyster mushroom powder

Parameters (%)	Treatments[1)]			
	CTL	M1	M2	M3
Moisture	51.9^{A}±0.71	52.1^{A}±0.44	52.4^{A}±0.56	49.8^{B}±0.98
Fat	14.0^{B}±0.73	13.1^{B}±0.87	15.0^{AB}±0.46	16.7^{A}±1.18
Ash	2.49^{B}±0.07	2.81^{A}±0.02	2.78^{A}±0.07	2.89^{A}±0.10
Protein	31.5^{A}±0.27	31.6^{A}±0.87	29.8^{B}±0.25	30.7^{AB}±0.82

[1)] Treatment: CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

[A-B] Means with different scripts in the same treatment are different (p<0.05).

Table 5. Effect of treatments and storage days on pH, color, WHC, TBARS, POV, VRB, and TPC of pork ham with oyster mushroom powder during refrigerated storage at 4°C

	Parameters[1)]						
	pH	L*	a*	b*	WHC	TBARS	POV
Storage days* Treatments	**	**	**	*	NS	**	**
Storage days	**	**	**	**	*	**	**
Treatments[2)]	**	**	**	**	**	**	**
Storage days							
0	6.24^{g}±0.02	63.4^{f}±4.23	8.75^{f}±0.48	10.5^{f} ±1.42	89.8^{d}±0.62	0.04^{h} ±0.03	21.7^{f}±1.40
7	6.36^{f}±0.03	63.9^{d}±4.15	8.84^{e}±0.40	10.7^{e} ±1.29	90.5^{ab}±0.67	0.06^{g}±0.03	25.2^{e}±0.99
14	6.38^{d}±0.04	64.3^{de}±4.44	8.88^{e}±0.41	10.9^{e}±1.47	90.8^{a}±1.06	0.09^{f}±0.04	27.4^{d}±2.21
21	6.40^{a}±0.02	64.5^{cd}±4.21	8.95^{d}±0.40	11.1^{d} ±1.50	90.0^{cd}±0.90	0.09^{e}±0.03	28.5^{c}±1.97
28	6.39^{c}±0.02	64.8^{bc}±4.28	9.10^{c}±0.36	11.2^{cd} ±1.43	90.0^{cd}±0.85	0.10^{d}±0.03	29.9^{b}±1.15
35	6.37^{e}±0.02	65.0^{b}±4.21	9.16^{b}±0.35	11.2^{bc}±1.41	90.2^{abc}±0.72	0.10^{c}±0.03	30.2^{b}±1.35
42	6.39^{b}±0.03	65.5^{a}±4.35	9.19^{b}±0.34	11.3^{ab} ±1.30	90.3^{abc}±0.62	0.11^{b}±0.03	30.1^{b}±1.45
49	6.40^{b}±0.02	64.7^{bc}±4.22	9.50^{a}±0.28	11.4^{a}±1.32	89.8^{d}±0.43	0.11^{a}±0.03	31.1^{a}±1.29
Treatments							
CTL	6.38^{B}±0.05	69.8^{A}±0.56	8.46^{D}±0.33	8.98^{D}±0.37	90.8^{A}±0.68	0.06^{D}±0.02	29.8^{A}±3.13
M1	6.39^{A}±0.05	66.2^{B}±1.10	9.11^{C}±0.36	10.7^{C}±0.36	90.2^{B}±0.67	0.07^{C}±0.02	28.6^{B}±2.95
M2	6.36^{C}±0.05	63.9^{C}±0.76	9.21^{B}±0.16	11.8^{B}±0.27	90.0^{BC}±0.84	0.08^{B}±0.02	27.5^{C}±2.86
M3	6.33^{D}±0.05	58.2^{D}±0.74	89.41^{A}±0.10	12.7^{A}±0.33	89.8^{C}±0.61	0.14^{A}±0.03	26.1^{D}±3.22

[1)] Parameter: L*, lightness; a*, redness; b*, yellowness; WHC, water-holding capacity; TBARS, tiobarbituric acid reactive substances; POV, peroxide value.

[2)] Treatment: CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

[a-h] Means with different letters within different storage days are different ($p<0.05$).

[A-D] Means with different letters within different treatments are different ($p<0.05$).

802 **Table 6. pH, color, water-holding capacity, TBARS, and POV of pork ham with oyster mushroom powder**

Parameters[1)]	TRT[2)]	Storage days							
		0	7	14	21	28	35	42	49
pH	CTL	6.25^{gB}±0.01	6.37^{eB}±0.01	6.42^{aA}±0.01	6.42^{aA}±0.01	6.41^{bA}±0.01	6.37^{fB}±0.01	6.39^{dB}±0.01	6.39^{cB}±0.01
	M1	6.27^{eA}±0.01	6.39^{dA}±0.01	6.42^{bA}±0.01	6.42^{bA}±0.01	6.40^{cB}±0.01	6.40^{cdA}±0.01	6.42^{bA}±0.01	6.43^{aA}±0.01
	M2	6.24^{dC}±0.01	6.35^{cC}±0.01	6.36^{cB}±0.01	6.41^{aB}±0.01	6.40^{abC}±0.01	6.36^{cC}±0.01	6.41^{aA}±0.01	6.39^{bB}±0.01
	M3	6.21^{gD}±0.01	6.32^{fD}±0.01	6.33^{eC}±0.01	6.37^{bC}±0.01	6.35^{dD}±0.01	6.35^{cD}±0.01	6.36^{cC}±0.01	6.37^{aC}±0.01
L*	CTL	68.9^{cA}±0.32	69.0^{cA}±0.21	70.0^{abA}±0.08	70.1^{abA}±0.29	70.1^{abA}±0.10	70.1^{abA}±0.05	70.4^{aA}±0.33	69.9^{bA}±0.20
	M1	64.6^{dB}±0.22	65.5^{cB}±0.25	65.6^{cB}±0.19	65.8^{cB}±0.57	66.9^{bB}±0.53	67.0^{bB}±0.31	68.0^{aB}±0.37	65.8^{cB}±0.16
	M2	62.8^{dC}±0.22	63.6^{cdC}±0.15	63.7^{bcC}±0.49	63.8^{bcC}±0.20	63.8^{bcC}±0.10	63.9^{bcC}±0.68	64.5^{abC}±0.38	65.1^{aB}±0.58
	M3	57.1^{cD}±0.22	57.6^{bcD}±0.40	57.7^{bcD}±0.24	58.4^{abD}±0.43	58.5^{abD}±0.20	58.9^{aD}±1.04	58.9^{aD}±0.10	58.2^{abcC}±0.13
a*	CTL	8.00^{eD}±0.08	8.22^{dD}±0.01	8.26^{dD}±0.02	8.32^{dD}±0.03	8.51^{cD}±0.04	8.59^{bcD}±0.06	8.63^{bC}±0.02	9.15^{aD}±0.05
	M1	8.74^{dC}±0.02	8.81^{cdC}±0.07	8.84^{cdC}±0.15	8.91^{cC}±0.03	9.18^{bC}±0.01	9.23^{bC}±0.01	9.27^{bB}±0.08	9.89^{aA}±0.04
	M2	9.00^{cB}±0.06	9.01^{cB}±0.01	9.08^{cB}±0.07	9.21^{bB}±0.02	9.28^{abB}±0.01	9.36^{aB}±0.04	9.37^{aA}±0.04	9.39^{aC}±0.09
	M3	9.27^{fA}±0.09	9.31^{efA}±0.01	9.34^{deA}±0.02	9.36^{dA}±0.02	9.44^{cA}±0.03	9.48^{bcA}±0.02	9.50^{bA}±0.01	9.59^{aB}±0.03
b*	CTL	8.48^{eD}±0.09	8.86^{dD}±0.05	8.57^{eD}±0.01	8.83^{dD}±0.05	9.02^{cD}±0.12	9.10^{cD}±0.01	9.44^{bD}±0.07	9.58^{aD}±0.02
	M1	10.0^{bC}±0.15	10.4^{bC}±0.16	10.8^{aC}±0.02	10.8^{aC}±0.46	10.8^{aC}±0.02	10.9^{aC}±0.05	10.9^{aC}±0.02	10.9^{aC}±0.06
	M2	11.4^{dB}±0.09	11.5^{dB}±0.09	11.6^{cdB}±0.15	11.8^{bcB}±0.09	11.9^{abB}±0.05	12.0^{aB}±0.02	12.1^{aB}±0.08	12.1^{aB}±0.12
	M3	12.2^{dA}±0.15	12.3^{cdA}±0.08	12.5^{cA}±0.09	12.8^{bA}±0.26	12.9^{abA}±0.05	12.9^{abA}±0.04	12.9^{abA}±0.04	13.1^{aA}±0.14
WHC	CTL	89.8^{dA}±0.35	91.0^{abA}±0.73	91.8^{aA}±0.16	90.7^{bcA}±0.15	91.0^{abA}±0.37	91.0^{abA}±0.39	91.0^{abA}±0.16	90.1^{cdA}±0.36
	M1	90.5^{aA} ±0.50	90.8^{aAB}±0.55	90.8^{aA}±0.61	89.8^{aA}±0.68	90.0^{aAB}±0.51	90.1^{aAB}±0.48	90.2^{aB}±0.36	89.8^{aA}±0.64
	M2	89.8^{aA}±0.65	89.8^{aB}±0.13	90.0^{aA}±1.54	90.0^{aA}±1.17	89.5^{aB}±0.73	90.3^{aAB}±0.47	90.4^{aAB}±0.13	89.7^{aA}±0.06
	M3	90.0^{abA}±0.63	90.3^{abAB}±0.20	90.4^{aA}±0.23	89.5^{abA}±0.73	89.2^{bB}±0.41	89.4^{abB}±0.30	89.7^{abB}±0.61	89.6^{abA}±0.22
TBARS	CTL	0.02^{fC}±0.01	0.03^{fD}±0.01	0.06^{eD}±0.01	0.07^{dC}±0.01	0.08^{cC}±0.01	0.08^{bC}±0.01	0.09^{aD}±0.01	0.09^{aC}±0.01
	M1	0.02^{gC}±0.01	0.05^{fC}±0.01	0.06^{eC}±0.01	0.07^{dC}±0.01	0.08^{cC}±0.01	0.09^{bB}±0.01	0.09^{abC}±0.01	0.10^{aB}±0.01
	M2	0.03^{gB}±0.01	0.05^{fB}±0.01	0.08^{eB}±0.01	0.09^{cB}±0.01	0.08^{dB}±0.01	0.09^{bB}±0.01	0.10^{aB}±0.01	0.10^{aB}±0.01
	M3	0.09^{fA}±0.01	0.10^{eA}±0.01	0.15^{dA}±0.01	0.15^{dA}±0.01	0.15^{cA}±0.01	0.15^{cA}±0.01	0.16^{bA}±0.01	0.17^{aA}±0.01
POV	CTL	23.2^{eA}±0.09	26.3^{dA}±0.24	30.7^{cA}±0.21	30.9^{cA}±0.72	31.0^{cA}±0.16	31.5^{bcA}±0.67	32.1^{bA}±0.28	33.0^{aA}±0.26
	M1	22.6^{eA}±0.06	25.7^{dAB}±0.29	27.4^{cB}±0.68	29.7^{bA}±0.15	30.8^{aA}±0.45	31.0^{aAB}±0.24	30.5^{aB}±0.45	31.3^{aB}±0.32
	M2	21.4^{dB}±0.23	25.0^{cB}±0.25	26.9^{bB}±0.57	27.6^{bB}±0.58	29.5^{aB}±0.39	29.9^{aB}±0.83	29.5^{aBC}±0.07	30.2^{aC}±0.09
	M3	19.6^{eC}±0.58	23.8^{dC}±0.46	24.7^{cdC}±0.44	25.9^{cC}±0.52	28.4^{bB}±0.72	28.3^{bC}±0.40	28.4^{bC}±0.91	30.0^{aC}±0.76

803 [1)] Parameter: L*, lightness; a*, redness; b*, yellowness; WHC, water-holding capacity; TBARS, tiobarbituric acid reactive substances; POV, peroxide value.

804 [2)] Treatment: CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

805 [a-g] Means within the same row with different letters are different (p<0.05).

806 [A-D] Means within the same column with different letters are different (p<0.05).

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Table 7. Effect of treatments and storage days texture profile analysis (TPA) of pork ham with oyster mushroom powder during refrigerated storage at 4°C

	Parameters							
	Hardness	Deformation	Adhesiveness	Resilience	Cohesiveness	Springiness	Gumminess	Chewiness
Storage days* Treatments	**	NS	NS	**	**	**	**	**
Storage days	**	**	**	**	**	*	**	**
Treatments[1)]	**	**	**	**	**	**	**	**
Storage days								
0	2905.1^{h}±92.6	4.98^{c}±0.01	0.24^{a}±0.02	0.27^{g}±0.02	0.54^{g}±0.02	4.05^{h}±0.03	1544.6^{h}±24.8	61.7^{h}±1.99
7	3048.1^{g}±34.7	4.98^{c}±0.01	0.22^{b}±0.02	0.27^{f}±0.01	0.55^{f}±0.01	4.09^{g}±0.02	1598.1^{g}±36.9	62.7^{g}±2.35
14	3064.5^{f}±28.5	4.98^{b}±0.01	0.21^{b}±0.02	0.28^{e}±0.01	0.56^{e}±0.02	4.11^{f}±0.03	1646.2^{e}±58.2	64.1^{f}±3.03
21	3072.2^{e}±44.6	4.98^{ab}±0.01	0.19^{c}±0.02	0.29^{d}±0.01	0.57^{d}±0.02	4.13^{e}±0.03	1640.9^{f}±33.9	66.1^{e}±2.80
28	3084.5^{d}±44.0	4.98^{ab}±0.01	0.18^{cd}±0.02	0.30^{c}±0.01	0.57^{c}±0.02	4.15^{d}±0.02	1684.9^{d}±42.2	66.9^{d}±2.86
35	6148.1^{c}±41.2	4.98^{a}±0.01	0.17^{de}±0.02	0.30^{bc}±0.01	0.58^{c}±0.02	4.16^{c}±0.03	1702.6^{c}±50.1	68.5^{c}±3.64
42	3164.0^{b}±30.4	4.98^{b}±0.01	0.16^{e}±0.02	0.30^{b}±0.01	0.58^{b}±0.02	4.18^{b}±0.03	1761.8^{b}±73.5	70.7^{b}±4.42
49	3209.6^{a}±32.7	4.98^{ab}±0.01	0.16^{e}±0.02	0.31^{a}±0.01	0.59^{a}±0.01	4.20^{a}±0.02	1790.0^{a}±65.8	72.0^{a}±2.98
Treatments								
CTL	3024.4^{D}±113.4	4.98^{C}±0.01	0.17^{C}±0.03	0.30^{A}±0.01	0.59^{A}±0.02	4.16^{A}±0.05	1709.2^{A}±93.4	68.9^{A}±4.34
M1	3078.1^{C}±89.1	4.98^{B}±0.01	0.19^{B}±0.04	0.30^{B}±0.01	0.58^{B}±0.02	4.15^{B}±0.05	1695.5^{B}±81.9	68.4^{B}±4.72
M2	3103.7^{B}±75.9	4.98^{AB}±0.01	0.19^{B}±0.03	0.29^{C}±0.01	0.57^{C}±0.01	4.13^{C}±0.05	1688.4^{C}±81.1	67.5^{C}±2.98
M3	3141.9^{A}±72.5	4.98^{A}±0.01	0.21^{A}±0.03	0.27^{D}±0.01	0.54^{D}±0.02	4.10^{D}±0.04	1591.7^{D}±53.1	61.5^{D}±3.78

[1)] Treatment: CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

[a-h] Means with different letters within different storage days are different ($p<0.05$).

[A-D] Means with different letters within different treatments are different ($p<0.05$).

Table 8. Texture profile analysis (TPA) of pork ham with oyster mushroom powder

Parameters	TRT[1)]	Storage days							
		0	7	14	21	28	35	42	49
Hardness (g)	CTL	2768.3^{D}±2.05	2996.4^{gD}±4.33	3028.4^{dC}±3.50	2999.0^{fD}±1.65	3016.0^{eD}±4.08	3090.8^{cD}±5.28	3130.1^{bD}±4.51	3166.0^{aD}±5.30
	M1	2881.2^{gC}±2.11	3038.4^{fC}±2.51	3059.4^{eB}±6.76	3078.4^{eC}±2.86	3083.7^{dC}±3.14	3137.6^{cC}±2.28	3147.8^{bC}±1.57	3198.0^{aC}±4.22
	M2	2953.2^{eB}±1.26	3071.0^{dB}±5.19	3062.9^{dB}±5.03	3094.6^{cB}±5.32	3102.2^{cB}±4.49	3159.2^{bB}±4.79	3167.0^{bB}±0.72	3219.2^{aB}±1.54
	M3	3017.8^{gA}±5.94	3086.4f^{A}±3.71	3107.0^{eA}±4.81	3117.0^{dA}±1.63	3136.0^{cA}±4.75	3204.8^{bA}±1.66	3211.1^{bA}±5.05	3255.2^{aA}±4.32
Adhesive-ness (mJ)	CTL	0.21^{aB}±0.02	0.20^{aB}±0.01	0.16^{abC}±0.02	0.17^{bcB}±0.01	0.16^{cdB}±0.02	0.14^{cdB}±0.02	0.13^{dB}±0.01	0.13^{dB}±0.01
	M1	0.24^{aAB}±0.02	0.23^{abAB}±0.03	0.22^{abAB}±0.02	0.20^{bcAB}±0.03	0.18^{cdAB}±0.02	0.17^{cdAB}±0.01	0.16^{dAB}±0.02	0.16^{dAB}±0.02
	M2	0.24^{aAB}±0.02	0.21^{bAB}±0.02	0.20^{bcBC}±0.01	0.19^{bcdAB}±0.02	0.18^{cdeAB}±0.02	0.17^{deAB}±0.01	0.17^{deA}±0.01	0.16^{eAB}±0.02
	M3	0.26^{aA}±0.02	0.24^{aA}±0.02	0.23^{abA}±0.01	0.21^{bcA}±0.02	0.20^{cdA}±0.01	0.19^{cdA}±0.02	0.18^{dA}±0.02	0.18^{dA}±0.01
Resilience	CTL	0.28^{eA}±0.01	0.29^{dA}±0.01	0.29^{cA}±0.01	0.31^{bA}±0.01	0.31^{abA}±0.01	0.31^{abA}±0.01	0.31^{aA}±0.01	0.32^{aA}±0.01
	M1	0.27^{fB}±0.01	0.28^{eAB}±0.01	0.29^{dA}±0.01	0.30^{cB}±0.01	0.31^{bB}±0.01	0.31^{bAB}±0.01	0.31^{bB}±0.01	0.31^{aA}±0.01
	M2	0.27^{eB}±0.01	0.28^{dB}±0.01	0.28^{cB}±0.01	0.29^{bC}±0.01	0.30^{bC}±0.01	0.30^{aB}±0.01	0.30^{aC}±0.01	0.30^{aB}±0.01
	M3	0.24^{fC}±0.01	0.25^{eC}±0.01	0.26^{dC}±0.01	0.27^{cD}±0.01	0.28^{bcD}±0.01	0.28^{bC}±0.01	0.28^{bD}±0.01	0.29$^{a\ C}$±0.01
Cohesive-ness	CTL	0.56^{dA}±0.01	0.56^{dA}±0.01	0.59^{cA}±0.01	0.59^{cA}±0.01	0.59^{bcA}±0.01	0.60^{abA}±0.01	0.60^{aA}±0.01	0.60^{aA}±0.01
	M1	0.56^{eA}±0.01	0.56^{deA}±0.01	0.56^{dB}±0.01	0.58^{cB}±0.01	0.58^{bcAB}±0.01	0.59^{bB}±0.01	0.60^{aA}±0.01	0.60^{aB}±0.01
	M2	0.55^{eA}±001	0.56^{deA}±0.01	0.56^{dB}±0.01	0.57^{cC}±0.01	0.58^{bB}±0.01	0.58^{bB}±0.01	0.58^{bB}±0.01	0.59^{aC}±0.01
	M3	0.51^{eB}±0.01	0.53^{dB}±00.01	0.53^{dC}±0.01	0.54^{cdD}±0.01	0.54^{bcC}±0.01	0.55^{bC}±0.01	0.55^{bC}±0.01	0.56^{aD}±0.01
Springi-ness (mm)	CTL	4.09^{fA}±0.01	4.11^{eA}±0.01	4.14^{dA}±0.01	4.15^{cdA}±0.01	4.16^{cA}±0.02	4.19^{bA}±0.01	4.22^{aA}±0.01	4.23^{aA}±0.01
	M1	4.06^{gB}±0.01	4.10^{fA}±0.01	4.13^{eAB}±0.02	4.15^{dA}±0.01	4.16^{cdA}±0.01	4.18^{bcA}±0.01	4.19^{bB}±0.01	4.21^{aB}±0.01
	M2	4.04^{fBC}±0.01	4.08^{eB}±0.01	4.11^{dB}±0.01	4.14^{cA}±0.01	4.15^{cA}±0.01	4.17^{bA}±0.01	4.18^{abC}±0.01	4.20^{aC}±0.01
	M3	4.02^{fC}±0.01	4.06^{eB}±0.01	4.07^{eC}±0.01	4.09^{dB}±0.02	4.11^{cB}±0.01	4.12^{cB}±0.01	4.14^{bD}±0.01	4.17^{aD}±0.01
Gummi-ness (g)	CTL	1573.4^{hA}±1.97	1612.4^{gB}±0.68	1709.2^{eA}±3.03	1635.2f^{C}±1.77	1721.9^{dA}±3.22	1737.2^{cA}±2.73	1832.6^{bA}±5.45	1851.9^{aA}±3.14
	M1	1559.7^{hB}±3.81	1607.6^{gB}±0.63	1670.3^{fB}±4.90	1675.8^{eA}±0.31	1705.0^{dB}±1.66	1729.2^{cB}±3.40	1796.4^{bB}±3.87	1817.9^{aB}±2.22
	M2	1536.8^{hC}±4.53	1635.4^{gA}±3.82	1653.2^{fC}±4.17	1664.4^{eB}±4.12	1699.4^{dC}±2.22	1727.9^{cB}±2.88	1779.1^{bC}±2.20	1810.7^{aB}±4.38
	M3	1508.4^{gD}±0.96	1537.0^{fC}±1.78	1552.0^{eD}±0.27	1588.3^{dD}±4.32	1613.3^{cD}±0.47	1616.1^{cC}±0.87	1639.2^{bD}±0.83	1679.4^{aC}±4.49
Chewi-ness (mJ)	CTL	62.5^{fB}±0.14	63.9^{eAB}±0.05	66.1^{dA}±0.22	68.7^{cA}±0.27	69.1^{cA}±0.33	71.7^{bA}±0.31	474.6^{aA}±0.31	74.8^{aA}±0.14
	M1	62.8^{gAB}±0.41	63.4^{fB}±0.19	66.0^{eA}±0.49	67.7^{dB}±0.57	68.5^{cAB}±0.32	71.2^{bA}±0.07	73.6^{aB}±0.23	73.7^{aB}±0.23
	M2	63.1^{gA}±0.11	64.6f^{A}±0.24	65.5^{eA}±0.27	66.6^{dC}±0.05	68.0^{cB}±0.21	68.4^{cB}±0.38	71.4^{bC}±0.26	72.4^{aC}±0.29
	M3	58.3^{eC}±0.17	58.7^{eC}±0.64	59.0^{eB}±0.60	61.5^{dD}±0.46	62.0^{cdC}±0.47	62.5^{bcC}±0.17	63.3^{bD}±0.14	67.1^{aD}±0.44

814 [1)] Treatment: CTL, oyster mushroom 0%; M1, oyster mushroom 0.5%; M2, oyster mushroom 1%; M3, oyster mushroom 3%.

815 [a-g] Means within the same row with different letters are different ($p<0.05$).

816 [A-D] Means within the same column with different letters are different ($p<0.05$).

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