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Effects of Mustard Seed Extract on Physicochemical and Storage Characteristics of Dry-aged Pork Loin Ham

Abstract

This study investigated the effects of mustard seed extracts on physicochemical and storage characteristics of dry-aged pork loin ham during the aging period. In experiment 1, antioxidant activity was assessed for mustard seed extracted with varying ethanol concentrations and the results showed high antioxidant activity at 25%, 50%, and 75% ethanol concentrations. In experiment 2, pork loin was treated with mustard seed extracts obtained using different ethanol concentrations: not treated (control), 25% (MS25), 50% (MS50), and 75% (MS75). Physicochemical and storage characteristics of pork loin ham were measured in wk 0, 2, 4, and 6. The pH, a_w, yellowness, thiobarbituric acid reactive substances and volatile basic nitrogen values were lower in treated samples compared to the control ($p<0.05$). In conclusion, applying mustard seed extracts, particularly MS75, in the dry-aged pork loin ham production process could enhance storage stability and improve color attributes without having negative impacts on product quality.

Keywords: Mustard seed · Natural antioxidant · Pork loin ham · Dry-aging · Storage characteristics

Introduction

During the production of dry-aged pork loin ham, proteins and lipids that make up the meat are broken down into free amino acids and free fatty acids by the action of enzymes such as calpain, cathepsin, and lipase (Toldrá et al., 1997). The oxidation of these fatty acids contributes to the flavor formation of meat products. On the other hand, if oxidation occurs in an environment where appropriate temperature and humidity conditions are not met, the risk of meat spoilage and rancidity increases (Morrissey et al., 1998). To ensure storage stability of dried meat products, manufacturers are using oxidation prevention (butylated hydroxytoluene: BHT, butylated hydroxyanisole: BHA) called synthetic antioxidants (Oswell et al., 2018).

Recently, consumer awareness of the potential carcinogenicity of synthetic antioxidants has led to an increase in research to replace BHT and BHA (Karre et al., 2013). Natural additives with antioxidant activity, such as rosemary, berries, and cruciferous plants, have been identified as having antioxidative effects (Lorenzo et al., 2018; Ramirez et al., 2020; Sebranek et al., 2005). In particular, the high phenol and flavonoid content of cruciferous plants has led to research on the possibility of replacing synthetic antioxidants (Ramirez et al., 2020).

Mustard (*Brassica juncea*), which belongs to the cruciferous, has been reported to have high contents of glucosinolates and phenolic compounds (Nicácio et al., 2021; Sharma et al., 2018). And the plant enzyme myrosinase hydrolyzes the glucosinolates to isothiocyanates (ITC; Barba et al., 2016). ITC, which contribute to the pungency of mustard, are functional additives with anticancer and antimicrobial properties (Lin et al., 2000). The antimicrobial and antioxidant activities of these glucosinolates and phenolic compounds are greatly influenced by the ratio of water to ethanol during the extraction process for additives (Moudache et al., 2016).

Mustard seeds have been widely researched as natural antioxidants due to their proven efficacy. However, studies comparing the storage enhancement effects of different extraction solvents in dried meat products are difficult to find. Therefore, we compared the antioxidant capacities of various ethanol concentrations extracted from ground mustard seeds and selected the treatment group with superior antioxidant capacity for addition during the curing process of dry-aged pork loin ham. Subsequently, we analyzed the storage characteristics of the dry-aged pork loin ham during 6 wk of drying. This research aimed to provide foundational data for understanding the changes in antioxidant capacities of the substances contained in mustard seeds depending on the extraction solvent and their functions within meat.

Materials and Methods

Experiment I: Antioxidant activity of mustard seed extract

Mustard seed extraction

Yellow mustard seeds (*Brassica juncea*, bb Royal, India) were ground by grinder (DP-5800BL, Guangdong Xinbao Electrical Appliances Holdings Co., Ltd., China) for 5 min at room temperature (23°C). The solvents which used for extraction were distilled water (DW) and ethanol, 5 different ratios (DW:ethanol; 0:100, 25:75, 50:50, 75:25, 100:0, v/v). The ground seeds were mixed with each solvent separately at a ratio of 1:10 and stirred for 24 h at room temperature. After centrifugation at 5000 rpm for 30 min (Supra R22, Hanil, Daejeon, Korea), the filtrate of supernatant was stored at -80°C to freeze it before being freeze-dried by a freeze-dryer (FD12008, ilShinBioBase, Dongducheon, Korea). The 5 groups of freeze-dried extracts were dissolved in each solvent to make a stock (20%, w/v) used for experiments and loin ham manufacturing.

Extraction yield measurement

The ground seeds were weighed before extraction (initial weight), and the freeze-dried extracts were weighed again (final weight). The extraction yield percentage was calculated using the following formula:

$$\text{Extraction yield} = \frac{\text{final weight}}{\text{initial weight}} \times 100$$

Antioxidant activity measurement

Sample preparation

The 5 groups of stocks were used in the antioxidant experiment, which were made from extracts of mustard seeds that had been extracted with 25%, 50%, 75%, 100% ethanol, and 100% DW (0% ethanol). The most suitable dilution factor was determined through preliminary experiments conducted in this study, and each extract was finally diluted 100 times and used for experiments (Amarowicz et al, 1996).

Total phenolic contents (TPC)

To determine total phenolic contents (TPC), a method using the Folin-Ciocalteu reagent was adapted from Choi et al. (2022). Each stock (40 μL) and 80 μL of 2 N Folin-Ciocalteu reagent were mixed by vortex mixer (SVM-10, SciLab Korea, Seoul, Korea) and incubated for 3 min. Then, 800 μL of 20% Na_2CO_3 (w/v) was added to the mixture, and incubated for 30 min at 37℃ in the dark. The absorbance was measured at 765 nm using multi-mode microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA, USA). Gallic acid solutions (0-150 μg/mL) were used for the standard curve and the results were expressed as mg GAE (gallic acid equivalents)/g.

Total flavonoid contents (TFC)

The method proposed by Woisky and Salatino (1998) was chosen for measuring the total flavonoid contents (TFC). In this process, 100 μL of 1 N NaOH and 1 mL of diethylene glycol were mixed with 100 μL of each stock respectively. The mixture was then vortexed using a vortex mixer (SVM-10, SciLab Korea) and incubated in a darkroom at 37℃ for 1 h. The absorbance was measured at 420 nm (SpectraMax iD3, Molecular Devices). The standard curve was generated using naringin (0-150 μL/mL), and the results were expressed as mg NE (naringin acid equivalents)/g.

2, 2-Diphenyl-1-picrylhydrazy (DPPH) free radical scavenging activity

The method described by Choi et al. (2022) was chosen for measuring the DPPH free radical scavenging activity. This involved mixing each stock (500 μL) with an equal volume of DW, followed by the addition of 1 mL of 0.2 mM DPPH solution. And reacted the mixture in a darkroom for 30 min at 23℃. The absorbance was measured at 517 nm (SpectraMax iD3, Molecular Devices). For the standard curve, Trolox (0-600 μg/mL) was used, and the results were expressed as mg TE (Trolox equivalents)/g

Experiment II: Effects on dry-aged pork loin ham of mustard seed extract

Sample preparation

Pork loin (*M. longissimus dorsi*) was obtained 24 h after slaughter from I-homemeat (Seoul, Korea). The excess fat and connective tissues of the pork loins were removed, and the loins were cut into portions of approximately 500 g each. The portions were then randomly divided into 4 groups. For each group, 1% (w/v) of mustard seed extract stock, extracted using different ethanol concentrations (25%, 50%, 75%), was added to the curing solution to create the experimental groups (MS25, MS50, MS75). Pork loins cured without added

antioxidants served as the control. Each pork loin was weighed and packed in a polyethylene bag (WJpackage, Seoul, Korea) before being immersed in a 100% curing solution (w/w) containing 3.5% salt and 2% sugar (Table 1). After 7 d of curing at 4℃, the pork loins were placed on a tray to allow for a 2 h period of exudate release. To ensure uniform distribution of the curing solution, the polyethylene bags containing the loins were flipped once a day during the curing process. Finally, pork loins were dried in a dry-aging refrigerator (DA-45, Korea Alesso, Seoul, Korea) for 6 wk at 12℃ with a relative humidity of 60-70%.

Microbial analysis

Aerobic bacteria (AB), *Staphylococcus* spp. (ST), and *E. coli* (EC) were selected to evaluate the microbial population. Dry-aged pork loin ham sample (25 g) was mixed with 50 mL of sterile saline in a sterile bag and then homogenized. This homogenate was diluted by adding 1 mL of it to 9 mL of sterile saline, and further dilutions were made as required. The diluted solution was then plated onto Tryptic Soy Agar (TSA) for AB, Mannitol Salt Agar (MSA) for ST, and 3M™ Petrifilm (3M, Saint Paul, MN, USA) for EC and incubated (37℃, 24 h). Cultured colonies were counted and their numbers were expressed as Log CFU/g.

Color

The dry-aged pork loin ham samples were cut in half and allowed to bloom for 30 min prior to color measurement. The measurements were performed using a colorimeter (CR-10, Minolta, Tokyo, Japan), calibrated with a white standard plate (CIE L*: +97.83, CIE a*: –0.43, and CIE b*: +1.98) under an 8-lx illumination angle.

Proximate compositions

The proximate compositions of dry-aged pork loin ham samples were analyzed as per the guidelines set forth by association of official analytical chemists (AOAC, 2010). Each content was measured through the following methods:

- The moisture content: oven-drying at 105℃ (AOAC 950.46)
- The protein content: Kjeldahl method (AOAC 928.08)
- The fat content: Soxhlet method (AOAC 991.36)
- The ash content: dry ashing method at 550℃ (AOAC 920.153)

Aging loss

Each dry-aged pork loin ham sample was weighed following the respective aging periods (wk 2, 4, 6). All aging loss measurements were expressed as a percentage of the weight before aging (wk 0), and the percentage was calculated using the following formula:

$$\text{Aging loss (\%)} = \frac{\text{weight before aging (g)} - \text{weight after aging (g)}}{\text{weight before aging (g)}} \times 100$$

pH

For the pH analysis, dry-aged pork loin ham samples were mixed with DW (1:4, v/v). The mixture was then homogenized. After homogenization, a pH meter (Model S220, Mettler-Toledo, Schwerzenbach, Switzerland) was utilized to determine the pH of the samples.

Water activity (a_w)

The a_w was carried out at 25℃ with a LabMaster-aw neo instrument (Novasina AG, Lachen, Switzerland). Measurement results are expressed in terms of %

Thiobarbituric acid reactive substances (TBARS)

TBARS were measured by the method described by Jeong et al. (2022). Dry-aged pork loin ham sample (10 g) was homogenized with 97.5 mL of DW and 200 μL of 0.3% BHT. The homogenized sample was then transferred into a round-bottom flask and 2.5 mL of 4N HCl, 1 mL of anti-foaming agent, 3 boiling stones were added and the homogenized sample was steam-distilled. Following this, the distillate was combined with an equal volume of 0.02 M TBA solution and then heated at 100℃ for 35 min. The absorbance was measured at 538 nm. 1,1,3,3-Trethoxypropane was used for preparing a standard curve to calculate the amount of malondialdehyde (MDA). The TBARS value was expressed as mg MDA/kg.

Volatile basic nitrogen (VBN)

VBN was determined using the method of Choi et al. (2018). Dry-aged pork loin ham sample (10 g) and 30 mL of DW were homogenized. Then, brought to a final volume of 100 mL with DW and filtered, and 1 mL of filtrate was filled to the outer compartment of the Conway dish and 1 mL of 0.01 N H_3BO_3 was filled to the inner compartment. Then the inner compartment was added with 100 μL of Conway reagent, while the outer compartment added 1 mL of 50% K_2CO_3 and the Conway dish was sealed. The sealed dish was incubated at a temperature of 37℃ for 2 h. After incubation, H_3BO_3 in the inner compartment underwent titration with 0.02 N H_2SO_4 and the resulting data was then processed by the subsequent formula:

$$\text{VBN (mg \%)} = (X - Y) \times (f \times 0.02N \times 0.14 \times 100 \times d)/S$$

X, Volume of sulfuric acid consumed for the sample titration (μL); Y, Volume of sulfuric acid consumed for the blank titration (μL); f, factor of reagent; N, normality; d, dilution factor; S, sample weight (g).

Statistical analysis

Each experiment was conducted a minimum of 3 times to collect the data. All data were presented as the mean value and standard deviation (SD), and processed using the General Linear Models procedure for one-way analysis of variance (ANOVA) in the SAS software (version 9.4 for Windows, SAS Institute, Cary, NC, USA). One-way ANOVA was performed separately for each of the two factors: the presence of mustard seed extract and the dry-aging period. To discern significant differences among the data, Duncan's multiple range test was utilized with a significance level of $p<0.05$.

Results and Discussion

Experiment I: Antioxidant activity of mustard seed extract

Extraction yield and antioxidant activity

Table 2 shows the extraction yield, TPC, TFC, and DPPH free radical-scavenging activity of mustard seed extracts with different extraction solvents. The highest extraction yield of the mustard seed was in 0% ethanol (100% DW) at 24.28% ($p<0.05$), and the yield then decreased significantly with an increase in ethanol concentration in the solvent. The lowest extraction yield was 11.43% in 100% ethanol ($p<0.05$). The extraction yield of mustard seed depends upon the polarity of its constituents (Nawaz et al., 2020). Mustard seeds contain a variety of substances such as glucosinolates, phenolic compounds, and other polar compounds (Szydłowska-Czerniak et al., 2015). The high extraction yield observed in 100% DW is owing to the increased solubility of these polar substances in DW, which is a highly polar solvent. This indicates that mustard seeds contain a high proportion of hydrophilic substances.

The TPC, TFC and DPPH free radical scavenging activity of the mustard seed extracts increased with an increase in ethanol concentration in the extraction solvent from 0% to 75% ($p<0.05$), but the lowest phenol and flavonoid content and DPPH radical-scavenging capacity

281 was obtained at 100% ethanol concentration ($p<0.05$). DW and ethanol are mainly used to
282 extract antioxidants such as phenol and flavonoids (Hikmawanti et al., 2021). We believe that
283 higher TPCs obtained in mixed solvents as phenolic compounds are generally hydrophilic but
284 the main phenolic compound in mustard seeds is sinapic acid (Nicácio et al., 2021), which is
285 soluble in both water and ethanol (Shakeel et al., 2016). Flavonoids are known to exhibit a
286 higher extraction efficacy in mixed solvents than in pure ethanol or DW, indicating that they
287 are both hydrophilic and hydrophobic (Moudache et al., 2016). The DPPH is proportional to
288 TPC (Muzolf-Panek and Waśkiewicz, 2022), and we observed similar results in this
289 experiment. Therefore, the antioxidant capacity of the extract does not always match the
290 extraction yield, and the extraction efficacy of antioxidants can be reduced when DW is used
291 for extraction (Moudache et al., 2016). Szydłowska-Czerniak et al. (2015) reported that
292 mustard seed's antioxidants showed a high extraction efficacy in mixed solvents, as observed
293 in this study. We observed that mustard seed extracts in 25%, 50%, and 75% ethanol had a
294 higher antioxidant capacity than those in 0% and 100% ethanol. Therefore, we selected 25%,
295 50%, and 75% ethanolic extracts in the pork loin-manufacturing process in the current study.
296

297 **Experiment II: Effects on dry-aged pork loin ham of mustard seed extract**

298 **Microbial analysis and color**

299 Table 3 shows the AB and ST as well as color measurement results at 0, 2, 4, and 6 wk.
300 We did not detect EC in both mustard seed extract-treated and control samples. AB and ST
301 increased significantly in the control samples during the dry-aging period ($p<0.05$). The
302 mustard seed extract-treated samples showed a significant increase in AB and ST until wk 4
303 ($p<0.05$) but no significant change between wks4 and 6. We observed significantly higher
304 levels of AB and ST at all wk in control samples compared to those in treated samples, except
305 at wk 0 ($p<0.05$). At wk 6, MS50 and MS75 had lower AB levels than MS25. ST was lower

306 in MS50 and MS75 than in MS25 from wk 4 onwards ($p<0.05$). When the cell membrane of
307 mustard seed collapses due to physical shock, glucosinolates are released, which are
308 hydrolyzed into ITC by myrosinase (Barba et al., 2016). ITC is known to inhibit the growth
309 of pathogenic microorganisms, including *S. aureus* and EC, by disrupting cellular respiration,
310 collapsing cell membranes, and inhibiting enzymatic activity (Lin et al., 2000). Mustard has a
311 high glucosinolate content that can be useful for producing ITC (Sharma et al., 2018). The
312 polarity of the extraction solvent influences the extraction efficiency of glucosinolates
313 (Nawaz et al., 2020), as these have a higher extraction efficacy in mixed solvents (i.e.,
314 solvents with higher ethanol ratio) (Doheny-Adams et al., 2017). Therefore, MS50 and MS75
315 exhibited a superior bactericidal capacity compared to MS25 owing to their higher extraction
316 efficacy.
317 Across all samples, there was a significant decrease in lightness, redness, and yellowness
318 during the dry-aging period ($p<0.05$). However, no significant difference was observed in
319 lightness between control and the mustard seed extract-treated samples throughout the dry-
320 aging period. The decrease in lightness, seen during the dry-aging process, was owing to
321 reduced light scattered by the meat surface due to a decrease in moisture (Hughes et al.,
322 2020). Redness and yellowness did not differ significantly between the control and treated
323 samples at wk 0. However, when compared to the control, a higher redness value in all
324 treated samples after wk 2 and a lower yellowness value in all treated samples after wk 4
325 ($p<0.05$) were observed. Reduction in redness is associated with the production of
326 metmyoglobin, which exhibits brownness due to oxidation of myoglobin (Wang et al., 2021).
327 Myoglobin is known to form ferrylmyoglobin, which appears green, due to ferryl oxidation
328 and reaction with hydrogen peroxide, causing a decrease in redness but increase in
329 yellowness (Reeder et al., 2002). This result suggests that the more modest decline in redness
330 within the treatment groups, as compared to the control groups, results from the enhanced

antioxidant activity by the addition of mustard seed extract. This increased activity is thought to have decelerated the redness reduction and impeded the yellowness increase. Furthermore, green sulfmyoglobin produced by the reaction of myoglobin and hydrogen sulfide generated during proteolysis by microorganisms increases the yellowness of meat (Liu et al., 2022). The increased bacterial count seen in the control groups, compared to that in treatment groups, is consistent with the above observations. The addition of mustard seed extract improves pork colors, such as increasing redness of dry-aged pork loin ham and decreasing yellowness, by promoting antioxidant and antibacterial activity.

Proximate compositions and aging loss

Table 4 shows the proximate compositions of dry-aged pork loin ham at wk 0, 2, 4, and 6. All the samples demonstrated a significant decrease in moisture content ($p<0.05$), while their protein, fat, and ash content displayed a significant increase during the dry-aging period ($p<0.05$). We did not observe any significant differences between treated and control samples over the dry-aging period. Moisture content was negatively correlated with protein, fat, and ash content. This is mostly due to a relative increase in the dry-matter content resulting from decreased moisture content (Seong et al., 2015). Kim and Lee (2003) demonstrated that fat and water contents of meat are inversely proportional, as observed in the present study as well.

Figure 1 illustrates the aging loss of dry-aged pork loin ham samples at wk 2, 4, and 6 compared to the wk 0. The amount of aging loss increased significantly in all the samples throughout the dry-aging period ($p<0.05$), and the primary reason for this is believed to be the reduction in moisture content. Over the dry-aging period, the control and the mustard seed extract-treated samples showed no significant difference in the amount of aging loss. Similar to the results of the present study, Andrés et al. (2017) reported that adding pomegranate,

grape, and tomato extract did not affect the weight loss in lamb patties (*Longissimus thoracis*). While manufacturing dry-aged pork loin ham, high reduction of weight during aging can lead to economic loss and decline in quality (Bonfatti and Carnier, 2020). In this study, mustard seed extracts did not affect the composition change or yield during the dry-aging of pork loin ham. Therefore, we believe that the addition of mustard seed extracts can improve storage without any decline in quality of dry-aged pork loin ham.

pH and a_w

Table 5 shows the pH and a_w of dry-aged pork loin ham samples at wk 0, 2, 4, and 6. The pH of the control and MS25 samples tended to increase with the passage of the dry-aging period, and the highest pH value was measured at wk 6 ($p<0.05$). MS50 and MS75 showed no significant change in pH over all wk. The mustard seed extract-treated samples and control samples did not show a significant difference in pH at wk 0, but significantly higher pH values were observed in control starting from wk 2 ($p<0.05$). The lowest pH was observed in MS50 and MS75 samples at wk 4 and 6 ($p<0.05$). Increased pH in meat products signifies decay or growth of pathogenic microorganisms (Sujiwo et al., 2018), which is consistent with the microbial content trends observed in the control and MS25 samples. Conversely, AB tended to increase in MS50 and MS75 over the dry-aging period even though pH decreased. This might be due to the inhibition of pathogenic microorganisms and delayed changes in pH because of the lactic acid produced by lactic acid bacteria (Leroy and De Vuyst, 2004). The curing solution used for preparing the dry-aged pork loin ham in this experiment (Table 1) contained sugar. The lactic acid bacteria present in meat might have used this sugar for metabolism (Gänzle, 2015).

The a_w of dry-aged pork loin ham samples were decreased significantly during the dry-aging period ($p<0.05$). The samples did not show a significant difference in a_w at wk 0. But at

wk 6, significantly lower a_w was measured in the mustard seed extract-treated samples compared to the control sample ($p<0.05$). In the proximate composition analysis, all the samples had 73-74% moisture at wk 0 and this significantly decreased over time to 26–27% at wk 6, indicating that the major factor in a_w reduction was the dry-aging process. Furthermore, the mustard seed extract was added to the pork loin in the form of a stock dissolved in a solvent, including ethanol. Ethanol has been reported to potentially influence microbial metabolism inhibition and the reduction of a_w (Hallsworth and Nomura, 1999). This could have had an impact on the a_w measurements in this study. a_w is an indicator of the moisture level that can be used for growth of microorganisms. Maintaining the a_w of the final dried meat product below 70% effectively inhibits the proliferation of harmful bacteria and ensures stability during storage (Syamaladevi et al., 2016). We observed that addition of the mustard seed extracts in dry-aged pork loin ham reduced microorganisms and a_w.

TBARS and VBN

Table 6 shows the TBARS and VBN of dry-aged pork loin ham samples at wk 0, 2, 4, and 6. All the samples had significantly higher levels of TBARS at wk 6 than at wk 0 ($p<0.05$), but there was no significant change in TBARS after wk 2. While there was no significant difference in TBARS between the mustard seed extract-treated samples, they consistently exhibited lower TBARS than the control every wk ($p<0.05$). Continuous exposure of lipids to oxygen results in accumulation of malondialdehyde in meat due to an oxidative reaction, which is assessed by measuring TBARS levels (Zhao et al., 2020). The lack of significant difference in TBARS levels, observed in the different treatment samples during the experimental duration, is most likely due to the low-fat content of the meat (Fuentes et al., 2014). All the treated samples had lower TBARS levels than the control sample owing to the antioxidant activity of the mustard seed extracts (Nicácio et al., 2021).

VBN increased continuously in all the samples throughout the dry-aging period ($p<0.05$). The control sample showed significantly higher VBN than all the treated samples continuously from wk 0 to 6 ($p<0.05$). We observed the lowest VBN in MS50 from wk 0 to 2 ($p<0.05$). MS50 and MS75 samples had significantly lower VBN compared to the control sample and MS25 from wk 4 onwards ($p<0.05$). During the dry-aging period, the level of VBN increases due to protein degradation and metabolism of microorganisms (Sujiwo et al., 2018). Mustard seed extract-treated samples had significantly lower VBN levels due to the inhibitory action of mustard seeds against microorganisms (Kanemaru and Miyamoto, 1990). Therefore, levels of VBN tended to be consistent with the results of microbial analysis. In summary, the mustard seed extracts effectively inhibited lipid oxidation and protein deterioration during the dry-aging process of the pork loin, and the greatest effect was observed in MS50 and MS75.

Conclusion

This study evaluated the effects of mustard seed extracts on storage characteristics of dry-aged pork loin ham during the aging period. Based on the study conducted, mustard seed extracts, especially those obtained from 50% and 75% ethanol, positively influenced the physicochemical and storage characteristics of dry-aged pork loin ham. These extracts significantly inhibited bacterial growth, stabilized pH levels, and reduced water activity, contributing to overall improved storage stability. In terms of color attributes, treatments with mustard seed extracts resulted in higher redness and lower yellowness compared to the control. Additionally, the levels of TBARS and VBN were lower in samples treated with mustard seed extracts. Therefore, these extracts could serve as an effective natural alternative to synthetic antioxidants, promoting enhanced safety, color, and storage longevity of dry-

aged pork loin ham. This contributes towards the development of healthier and more naturally preserved dry-aged pork products.

Conflicts of interest

The authors declare no potential conflict of interest.

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Ethics approval

Given that no human or animal subjects were involved in this study, IRB/IACUC approval was not necessary for this article.

Author contributions

Conceptualization: Kim HY

Data curation: Cho HG

Formal analysis: Kim HY, Cho HG

Methodology: Kim HY

Software: Cho HG, Kim HY

Validation: Cho HG

Investigation: Cho HG, Kim HY

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558

559 **Table 1.** Formulation of dry-aged pork loin with mustard extracts stock

Ingredients (%)		Treatment			
		Control	MS25	MS50	MS75
Main	Meat	100	100	100	100
Curing solution	Water	100	100	100	100
	Salt	3.5	3.5	3.5	3.5
	Sugar	2.0	2.0	2.0	2.0
	BHT	-	-	-	-
	Et25[1)]	-	1	-	-
	Et50[2)]	-	-	1	-
	Et75[3)]	-	-	-	1

560 [1)] Et25, mustard extract with 25% ethanol.
561 [2)] Et50, mustard extract with 50% ethanol.
562 [3)] Et75, mustard extract with 75% ethanol.
563 Control, pork loin without mustard extract; MS25, pork loin with mustard seed extract with
564 25% ethanol; MS50, pork loin with mustard seed extract with 50% ethanol; MS75, pork loin
565 with mustard seed extract with 75% ethanol.
566

Table 2. Extraction yield and antioxidant measurements of mustard extracts with various levels of ethanol concentrations

Traits[1)]	Ethanol (%)				
	0	25	50	75	100
Extraction yield (%)	24.28±0.28[a]	17.72±0.40[b]	16.18±0.50[c]	13.46±0.79[d]	11.43±0.36[e]
TPC (mg GAE/g)	18.87±1.49[c]	19.85±0.56[bc]	21.06±0.36[b]	26.84±0.81[a]	16.18±1.16[d]
TFC (mg NE/g)	113.47±9.96[b]	142.18±18.36[b]	324.80±25.18[a]	334.66±27.43[a]	16.98±6.04[c]
DPPH (%)	49.75±1.93[d]	56.93±1.28[c]	83.23±0.12[b]	86.60±0.25[a]	17.32±2.72[e]

All values represented as mean±SD.

[1)] TPC, total phenolic contents; TFC, total flavonoids contents; DPPH, 2, 2-Diphenyl-1-picrylhydrazy free radical scavenging activity.

[a–e] Means in the same row marked with different letters denote significant differences ($p<0.05$).

Table 3. Microbial analysis (Log CFU/g) and color of dry-aged pork loin ham treated with mustard extract at different ethanol concentrations

Traits[1)]	Treatments	Aging period (wk)			
		0	2	4	6
AB (Log CFU/g)	Control	4.34±0.08^{dA}	5.15±0.13^{cA}	6.19±0.16^{bA}	6.55±0.12^{aA}
	MS25	4.28±0.17^{cA}	4.53±0.49^{bB}	5.33±0.46^{aB}	5.50±0.20^{aB}
	MS50	4.28±0.13^{cA}	4.64±0.11^{bB}	5.14±0.29^{aB}	5.19±0.20^{aC}
	MS75	4.23±0.15^{cA}	4.68±0.15^{bB}	5.26±0.23^{aB}	5.25±0.20^{aC}
ST (Log CFU/g)	Control	1.70±0.15^{cA}	3.70±0.20^{bA}	4.37±0.11^{aA}	4.33±0.17^{aA}
	MS25	1.69±0.21^{dA}	3.11±0.12^{cB}	3.71±0.1^{bB}	3.99±0.16^{aB}
	MS50	1.70±0.15^{cA}	2.83±0.23^{bC}	3.18±0.13^{aC}	3.24±0.19^{aC}
	MS75	1.67±0.24^{cA}	2.83±0.32^{bC}	3.23±0.27^{aC}	3.26±0.29^{aC}
EC (Log CFU/g)	Control	-	-	-	-
	MS25	-	-	-	-
	MS50	-	-	-	-
	MS75	-	-	-	-
CIE L*	Control	50.16±1.85^{aA}	47.33±1.19^{bA}	45.98±0.58^{cA}	44.18±1.08^{dA}
	MS25	50.11±1.48^{aA}	47.02±1.21^{bA}	46.37±0.87^{cA}	44.01±0.53^{dA}
	MS50	49.79±0.90^{aA}	46.92±0.94^{bA}	46.06±0.32^{cA}	43.66±0.47^{dA}
	MS75	49.65±0.62^{aA}	47.27±0.64^{bA}	45.99±0.36^{cA}	44.42±0.92^{dA}
CIE a*	Control	7.25±0.38^{aA}	5.93±0.39^{bB}	5.53±0.10^{cB}	4.34±0.29^{dB}
	MS25	7.41±0.27^{aA}	6.97±0.20^{bA}	6.28±0.39^{cA}	5.22±0.31^{dA}
	MS50	7.28±0.23^{aA}	6.91±0.15^{bA}	6.11±0.44^{cA}	5.10±0.28^{dA}
	MS75	7.34±0.18^{aA}	6.88±0.19^{bA}	6.19±0.43^{cA}	5.17±0.34^{dA}
CIE b*	Control	9.91±0.97^{aA}	8.57±0.23^{bA}	6.93±0.42^{cA}	5.93±0.33^{dA}
	MS25	9.63±0.61^{aA}	8.63±0.26^{bA}	5.73±0.30^{cB}	4.96±0.44^{dB}
	MS50	9.74±0.59^{aA}	8.48±0.43^{bA}	5.62±0.75^{cB}	4.81±0.23^{dB}
	MS75	9.81±0.57^{aA}	8.66±0.29^{bA}	5.87±0.40^{cB}	4.90±0.47^{dB}

All values represented as mean±SD.

[1)] AB, total aerobic bacteria; ST, *Staphylococcus* spp.; EC, *E. coli*.

[a–d] Means in the same row marked with different letters denote significant differences ($p<0.05$).

[A–C] Means in the same column marked with different letters denote significant differences ($p<0.05$).

583 Control, dry-aged pork loin ham without mustard extract; MS25, dry-aged pork loin ham with
584 mustard seed extract with 25% ethanol; MS50, dry-aged pork loin ham with mustard seed
585 extract with 50% ethanol; MS75, dry-aged pork loin ham with mustard seed extract with 75%
586 ethanol.
587

588 **Table 4.** Proximate compositions of dry-aged pork loin ham treated with mustard extract at
589 different ethanol concentrations

Traits (%)	Treatments	Dry-aging periods (wk)			
		0	2	4	6
Moisture	Control	73.94±1.19^{a}	60.59±0.55^{b}	35.57±0.85^{c}	26.48±1.32^{d}
	MS25	73.77±0.54^{a}	60.78±0.77^{b}	34.84±1.42^{c}	26.12±1.04^{d}
	MS50	74.04±0.42^{a}	59.68±1.22^{b}	35.30±1.67^{c}	26.53±0.49^{d}
	MS75	74.13±0.48^{a}	60.24±1.02^{b}	34.57±1.66^{c}	26.20±0.46^{d}
Protein	Control	21.63±1.12^{d}	35.20±1.02^{c}	56.15±0.63^{b}	65.62±1.15^{a}
	MS25	21.61±0.65^{d}	34.92±1.78^{c}	55.74±0.77^{b}	64.95±0.77^{a}
	MS50	21.57±0.50^{d}	35.34±1.40^{c}	56.05±1.27^{b}	65.16±1.74^{a}
	MS75	21.47±1.03^{d}	34.29±1.23^{c}	55.86±0.90^{b}	65.26±1.73^{a}
Fat	Control	1.52±0.03^{d}	2.72±0.05^{c}	4.11±0.03^{b}	5.79±0.74^{a}
	MS25	1.49±0.05^{d}	2.71±0.04^{c}	4.07±0.27^{b}	5.71±0.88^{a}
	MS50	1.54±0.06^{d}	2.70±0.08^{c}	4.10±0.06^{b}	5.65±0.97^{a}
	MS75	1.50±0.03^{d}	2.73±0.04^{c}	4.08±0.07^{b}	5.41±1.23^{a}
Ash	Control	0.85±0.01^{d}	1.98±0.08^{c}	3.81±0.13^{b}	5.98±0.40^{a}
	MS25	0.85±0.02^{d}	2.02±0.05^{c}	3.82±0.09^{b}	5.88±0.28^{a}
	MS50	0.86±0.03^{d}	2.04±0.06^{c}	3.85±0.19^{b}	5.82±0.19^{a}
	MS75	0.83±0.04^{d}	2.03±0.07^{c}	3.78±0.07^{b}	5.93±0.18^{a}

590 All values represented as mean±SD.
591 $^{a–d}$ Means in the same row marked with different letters denote significant differences
592 ($p<0.05$).
593 No significant differences were observed between the means in the same column.
594 Control, dry-aged pork loin ham without mustard extracts; MS25, dry-aged pork loin ham
595 with mustard seed extracts with 25% ethanol; MS50, dry-aged pork loin ham with mustard
596 seed extracts with 50% ethanol; MS75, dry-aged pork loin ham with mustard seed extracts
597 with 75% ethanol.
598

599 **Table 5.** pH and a_w of dry-aged pork loin ham treated with mustard extract at different ethanol
600 concentrations

Traits	Treatments	Dry-aging periods (wk)			
		0	2	4	6
pH	Control	5.76±0.06^{cA}	5.82±0.02^{bA}	5.83±0.00^{bA}	5.96±0.04^{aA}
	MS25	5.76±0.05^{cA}	5.75±0.04^{cB}	5.81±0.01^{bB}	5.85±0.01^{aB}
	MS50	5.75±0.03^{aA}	5.75±0.02^{aB}	5.75±0.02^{aC}	5.76±0.02^{aC}
	MS75	5.76±0.04^{aA}	5.75±0.03^{aB}	5.75±0.02^{aC}	5.75±0.02^{aC}
a_w	Control	0.97±0.00^{aA}	0.92±0.00^{bA}	0.85±0.00^{cA}	0.77±0.00^{dA}
	MS25	0.97±0.00^{aA}	0.90±0.01^{bB}	0.84±0.00^{cB}	0.74±0.00^{dB}
	MS50	0.97±0.00^{aA}	0.90±0.00^{cC}	0.80±0.00^{cC}	0.74±0.00^{dB}
	MS75	0.97±0.00^{aA}	0.93±0.01^{bA}	0.84±0.00^{cB}	0.74±0.00^{dB}

601 All values represented as mean±SD.
602 $^{a–d}$ Means in the same row marked with different letters denote significant differences
603 ($p<0.05$).
604 $^{A–C}$ Means in the same column marked with different letters denote significant differences
605 ($p<0.05$).
606 Control, dry-aged pork loin ham without mustard extracts; MS25, dry-aged pork loin ham
607 with mustard seed extracts with 25% ethanol; MS50, dry-aged pork loin ham with mustard
608 seed extracts with 50% ethanol; MS75, dry-aged pork loin ham with mustard seed extracts
609 with 75% ethanol.
610

Table 6. Thiobarbituric acid reactive substances and volatile basic nitrogen of dry-aged pork loin ham treated with mustard extract at different ethanol concentrations

Traits[1)]	Treatments	Dry-aging periods (wk)			
		0	2	4	6
TBARS (mg MDA/kg)	Control	0.51±0.02^{bA}	0.67±0.04^{aA}	0.65±0.00^{aA}	0.66±0.03^{aA}
	MS25	0.43±0.02^{bB}	0.51±0.01^{aB}	0.50±0.00^{aB}	0.51±0.02^{aB}
	MS50	0.40±0.00^{bB}	0.49±0.04^{aB}	0.49±0.02^{aB}	0.48±0.02^{aB}
	MS75	0.40±0.02^{bB}	0.49±0.02^{aB}	0.49±0.04^{aB}	0.49±0.01^{aB}
VBN (mg %)	Control	9.18±0.81^{cA}	10.38±0.13^{bA}	14.90±0.43^{aA}	15.79±0.56^{aA}
	MS25	8.74±0.22^{dB}	9.58±0.21^{cB}	12.04±0.38^{bB}	12.99±0.18^{aB}
	MS50	6.72±0.22^{dD}	7.54±0.13^{cD}	11.14±0.28^{bC}	12.26±0.21^{aC}
	MS75	7.62±0.39^{dC}	8.59±0.26^{cC}	11.59±0.21^{bC}	12.60±0.38^{aBC}

All values represented as mean±SD.

[1)] TBARS, Thiobarbituric acid reactive substances; VBN, volatile basic nitrogen.

$^{a–d}$ Means in the same row marked with different letters denote significant differences ($p<0.05$).

$^{A–D}$ Means in the same column marked with different letters denote significant differences ($p<0.05$).

Control, dry-aged pork loin ham without mustard extracts; MS25, dry-aged pork loin ham with mustard seed extracts with 25% ethanol; MS50, dry-aged pork loin ham with mustard seed extracts with 50% ethanol; MS75, dry-aged pork loin ham with mustard seed extracts with 75% ethanol.

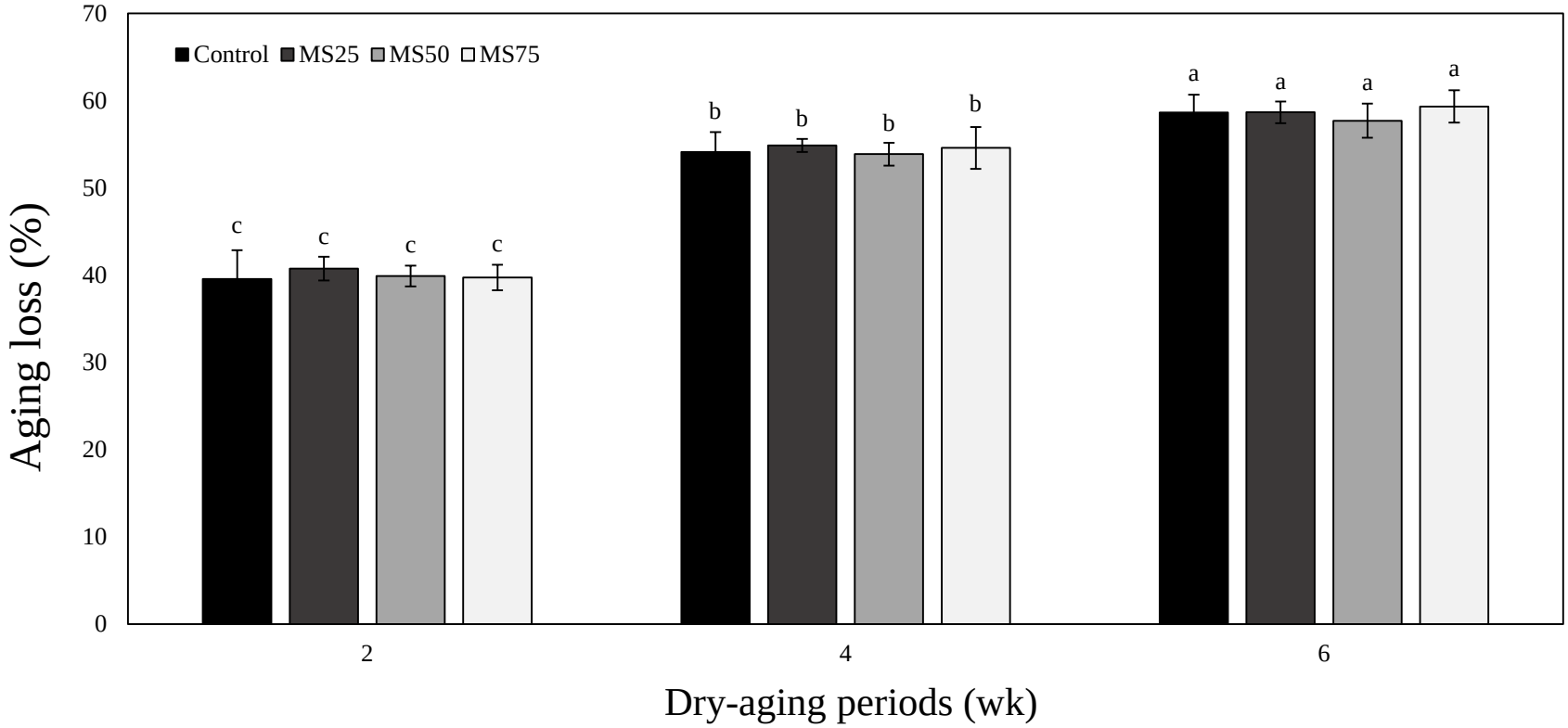

Figure 1. Aging loss of dry-aged pork loin ham treated with mustard extracts at different ethanol concentrations. [a-c] Means in the same color with different numbers are significantly different ($p<0.05$). No significant differences were observed between the means in the same wk. Control, dry-aged pork loin ham without mustard extracts; MS25, dry-aged pork loin ham with mustard seed extracts with 25% ethanol; MS50, dry-aged pork loin ham with mustard seed extracts with 50% ethanol; MS75, dry-aged pork loin ham with mustard seed extracts with 75% ethanol.