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Running Title (within 10 words)	Relationship between lipid oxidation and proteolysis on the quality properties of dry-cured loin
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Abstract

The aim of this study is to compare the quality characteristics of dry-cured loins with different levels of proteolysis and lipid oxidation and to investigate the relationship between these factors on quality characteristics. The dry-cured loins were divided into four groups (HH, HL, LH, and LL) based on the proteolysis index and 2-thiobarbituric acid reactive substances (TBARS). Moisture, protein, and fat content were all significantly influenced by proteolysis and lipid oxidation ($p<0.05$). The total fatty acid content in the high proteolysis groups (HH and HL) was significantly lower than that in the low proteolysis groups (LH and LL) ($p<0.05$). For total free amino acid content, HH was the highest, and LL was the lowest ($p<0.05$). On the other hand, there was no significant difference between HL and LH ($p>0.05$). In the amount of total volatile compounds, there was no significant difference between HH and HL ($p>0.05$), but LH and LL significantly differed ($p<0.05$). In conclusion, proteolysis and lipid oxidation can influence the quality characteristics of dry-cured loin. Additionally, proteolysis might be as influential in generating volatile compounds as lipid oxidation.

Keywords: dry-cured loin, proteolysis, lipid oxidation, volatile compound, quality properties

Introduction

Lipid oxidation and proteolysis are factors that cause significant biochemical changes in the quality characteristics of dry-cured meat. Previous studies have well-established the relationship between quality characteristics and these factors (Toldrá, 1998). The majority of volatile compounds originate from the chemical or enzymatic oxidation of unsaturated fatty acids. These compounds can then further interact with proteins, peptides, and free amino acids. In the case of lipid oxidation, fatty acids generated through lipolytic enzymes in dry-cured meat generate volatiles through oxidation reactions (Toldrá, 2006; Gilles, 2009). Regarding proteolysis, amino acids released from meat proteins form volatile compounds due to Strecker degradation and Maillard reaction (Luo et al., 2021; Pérez-Santaescolástica et al., 2018; Toldrá, 1998). Volatile compounds generated by these two biochemical pathways are a key factor in aroma/flavor enhancement, and independent research on their effects on quality characteristics has been conducted by researchers. Górska et al. (2017) divided 21 dry-cured loins into three groups according to the characteristics of volatile compounds through principal component analysis (PCA) and evaluated the correlation between volatile compounds and sensory characteristics through sensory evaluation of each group. In addition, the relationship between volatile compounds and sensory properties as well as the relationship between physicochemical properties has been demonstrated (Marušić Radovčić et al., 2016; Petričević et al., 2018). Luo et al. (2021) mentioned that 3-methylbutanal is a compound produced by the interaction between leucine and some branched amino acids, and Villalobos-Delgado et al. (2014) reported that benzene acetaldehyde is produced through Strecker degradation from phenylalanine. In addition, it has been reported that furan and non-heterocyclic sulfur compounds are produced (Flores, 2018). Consequently, amino acids produced by proteolysis not only act as flavor precursors

49 themselves but also affect various flavor/aroma-related compounds through additional
50 biochemical reactions. Therefore, a better understanding of lipid oxidation and proteolysis during
51 manufacturing may contribute to generating the volatile compounds in final products.
52 Many previous studies have reported the effects of lipid oxidation or proteolysis levels on
53 changes in the quality characteristics of dry-cured meat. Pérez-Santaescolástica et al. (2018)
54 compared quality characteristics in three dry-cured ham groups (low, proteolysis index < 32%;
55 medium, 32% ≤ proteolysis index ≤ 36%; high, proteolysis index > 36%) according to the
56 proteolysis level, and the authors reported that an increase in proteolysis led to a rise in texture
57 profile analysis (TPA) adhesiveness and a decrease in the quantity and quality of volatile
58 compounds. They argued that the processing conditions conducive to lipid oxidation, such as
59 increased salt content, typically, restrict the function of proteolytic enzymes. On the other hand,
60 Marušić Radovčić et al. (2021) identified that during the manufacturing period, the biceps
61 femoris, where proteolysis was high, exhibited a greater increase in volatile compounds than the
62 semimembranosus. In addition, the authors mentioned that a moderate degree of lipid oxidation
63 could have a positive effect on the flavor enhancement of dry-cured ham and that a high degree
64 of lipid oxidation caused damage to the nutritional, functional, and sensory properties. Previous
65 studies have shown that not only volatile substances but also other quality characteristics (texture
66 properties, color, fatty acids, amino acids, etc.) could be changed by lipid oxidation or
67 proteolysis.
68 Although the effect of lipid oxidation or proteolysis on quality characteristics is also
69 important, understanding the relationship between them is essential for a better understanding of
70 the quality characteristics. However, research related to elucidating the relationship between lipid
71 oxidation and proteolysis is very limited. A discussion on the relationship between lipid oxidation

and proteolysis can only be found in one paper. Harkouss et al. (2015) reported a quantitative correlation between lipid oxidation and proteolysis and established a linear regression model with a high coefficient of determination, regardless of muscle type, from 0 to 21 weeks during the entire manufacturing period of dry-cured ham (R^2=0.92). The scarcity of this information is likely due to the inability in the manufacturing process to selectively initiate chemical reactions associated solely with protein or lipid oxidation or degradation. To overcome this limitation, in this study, the opposite of the experimental group was confirmed through a statistical procedure for the study group based on the TBARS and proteolysis index of the generally prepared dry-cured loin. Furthermore, this study has the advantage of considering not only proteolysis but also lipid oxidation at the same time.

Lipid oxidation and proteolysis-related changes are biochemical reactions that occur simultaneously. Therefore, the relationship between the two reactions needs to be clearly established. Our hypothesis suggests that proteolysis may substantially impact quality characteristics considering the approximate composition of raw meat. Additionally, the effect of lipid oxidation may be greater when it is a limited volatile compound. The purpose of this study was to compare the quality characteristics of dry-cured loins by grouping them according to the levels of lipid oxidation and proteolysis, and to discuss the relationship between lipid oxidation and proteolysis on quality characteristics.

Material and Methods

Sample preparation

Sixty pork loins at 2 d postmortem from the right side of the carcass were employed for this study. Pork loin was purchased six times at two-month intervals from a local butcher block in

Jinju, Korea. At the time of each purchasing, the pork loins were assigned a sample code (random 4-digit code). The coded pork loins were left in a cooler until 3 d postmortem before manufacturing dry-cured loins. The fresh pork loin was salted after all connective tissues were removed. For salting, purified sodium chloride was used and 4% of the pork loin weight was rubbed on the surface. Then, it was vacuum-packed and stored in a refrigerator at 4℃ for 7 d. The surface of the cured loin was washed in tap water and the water on the surface was removed with a paper towel. The cured loins were placed in a permeable fibrous casing and dried at 9℃ (50~55% RH) for 14 d, then the temperature was raised by 1℃ every 3 d, and finally aged at 18℃ (65~70% RH). The total production period was 55 d. The dry-cured loins were removed from the casing and stored at -80℃ until analysis. To assign samples based on lipid oxidation and proteolysis, the dry-cured loins were measured for lipid oxidation using 2-thiobarbituric acid reactive substances (TBARS) and for proteolysis using proteolysis index (PI), as described in Section 2.2. After dividing the samples into four groups based on TBARS and PI, detailed information is presented in Table 1.

2-thiobarbituric acid reactive substances and proteolysis index

The TBARS of dry-cured loin was determined with Bozkurt and Erkmen (2004) with some modifications. A 3 g of sample was homogenized with 27 mL of 3.86% perchloric acid using homogenizer (T25 Basic, IKA, Wilmington, NC, USA). The homogenate stood in a cold room for an hour. After standing, the homogenate was centrifuged at 2000×g for 10 min and filtered with Whatman No. 1 filter paper. The 2 mL of filtrate was mixed with 2 mL of 20 mM TBA solution and left for 16 hours at room temperature with dark conditions. For the blank, 2 mL of deionized water was used to replace for filtrate. The TBARS was measured at 531 nm

using Cary 60 spectrometer (Agilent Technologies, Santa Clara, CA, USA). A standard curve was calculated using 1,1,3,3-tetraethoxypropane.

The total nitrogen content (TN) was measured with 0.5 g of sample using the nitrogen analyzer (TruMac CNS analyzer, Leco, MI, USA).

The non-protein nitrogen content was determined according to Pérez-Santaescolástica et al. (2018) with some modifications. A 3 g minced sample was homogenized with 25 mL of deionized water, and 12 mL of 20% trichloroacetic acid (TCA) were introduced into the homogenate. The mixture was thoroughly agitated and then left to stabilize for an hour at room temperature. Subsequently, it was centrifuged again at 2000×g for 10 min. Post centrifugation, the supernatant was filtered. A 15 mL of the filtrate was then employed to determine the nitrogen content, using the same procedure as was used for determining total nitrogen content by Kjeldahl method. Finally, the proteolytic index (PI) was calculated using the formula (non-protein nitrogen/total nitrogen)×100, as described by Pérez-Santaescolástica et al. (2018).

Moisture, protein, and fat content

Moisture was performed by drying method the samples to a constant weight at 103℃ (method 934.01, AOAC, 2005). The total nitrogen content was determined as described in section 2.2. The protein content was calculated by multiplying the total nitrogen content by 6.25. The fat content was conducted with Soxhlet extraction method with diethyl ether (method 920.39, AOAC, 2005).

Instrumental color, pH, and texture profile analysis (TPA)

The dry-cured loins were cut to the same size [8 cm (W) × 5 cm (L) × 3 cm (H)], and three pieces were used for instrumental color. Each piece was measured at different locations five times. The instrumental color was analyzed by colorimeter (CR-400, Konica Minolta, Tokyo, Japan) with an 8 mm aperture using an illuminant with D65 and 2° standard observer after calibrating with a calibration plate (Y = 81.2; x = 0.3191; y = 0.3263). The Commission Internationale de l'Eclairage (CIE) L^* (lightness), a^* (redness), and b^* (yellowness) were used as color parameters. The mean value was read 5 readings for each piece and took the average of 3 pieces.

The 3 g of minced sample was homogenized with 27 mL of deionized water using homogenizer (T25 Basic, IKA). The pH of the sample was measured with a pH meter (S20 SevenEasy^{TM}, Geifensee, Switzerland) after calibrating with 7.00, 4.01, and 9.21 using pH buffer.

The TPA was performed using EZ-SX (Shimadzu Corp., Kyoto, Japan) with a flat bottom (5 mm diameter) probe. The 3 pieces of the sample was used after measuring the instrumental color. A double compression test was carried out with 50% compression of the original sample height. Force-time curves were expressed with a 500 N load cell applied at a 100 mm/min crosshead speed. The texture parameters were calculated according to Bourne (1982). The mean value was taken from two random points in each sample piece.

Free amino acid composition

Free amino acid composition was extracted as described by Aro et al. (2010) with some modifications. Briefly, a 3 g of sample and 27 mL of deionized water were homogenized for 30 s and 10 mL of 10% TCA solution was added and shaken at 250 rpm for 1 h at room temperature. After an hour, centrifugation was performed at 10,000×g for 10 min and the supernatant was

filtered using Whatman No.1. Finally, it was filtered with a 0.2 µm syringe filter, transferred to a 1.5 mL vial, and used for analysis. A 20 µL of sample was injected into amino acid analyzer (Biochrom 30, Biochrom, Cambridge, UK). Amino acids were derivatizated with 6-aminoquinolyl-Nhydroxysuccinimidyl carbamate (Waters AccQ-Fluor reagent kit) and used the standard with 40 amino acids (Physiology standard, Biochrom, Cambridge, UK). The results were expressed as mg of free amino acid/100 g of sample.

Lipid extract and fatty acid profile

Lipid extraction was carried out according to Folch et al. (1957). Saponification, methylation, and gas chromatography learning conditions were employed by Seo et al. (2021). The fatty acid methyl etsers (FAME) were separated with gas chromatography (7890B, Agilent Technologies, CA, USA) equipped with a Supelco SP® -2560 capillary gas chromatography (GC) column (100 m × 0.25 mm × 0.20 µm). The individual FAMEs were identified based on a standard mixture of Supelco 37 Component FAME Mix (Sigma Aldrich, MO, USA). The amount of individual fatty acids and fatty acid groups identified was calculated with internal standard (C11:0, undecanoic acid). The fatty acid was expressed as g/100 g tissue.

Volatile compound extraction and injection

The SPME fiber was used after pre-conditioning at 270℃ for 30 min according to the manufacturer's manual before use. Briefly, 2.5 g of the minced sample was placed in a 20 mL screw-capped vial and sealed with a screw cap fitted with PTFE septa. The adsorption of volatiles was carried out for 55 min in an incubator at 45℃ using 50/30 µm DVB/CAR/PDMS SPME fiber (57328-U, Supelco, PA, USA). After adsorption, the SPME fiber was immediately injected

into the GC inlet and desorbed for 3 min in pressure splitless mode (30 psi). At this time, the GC inlet temperature was 240℃. Before adsorbing the next sample, pre-conditioning was performed for 8 min while flowing carrier gas (Helium, 99.999%) at 240℃ at 100 mL/min for overall SPME fiber cleaning.

GC-mass spectrometry

The GC machine was 7890B (Agilent Technologies, CA, USA). Helium was used as the carrier gas and a constant flow rate of 1.0 mL/min was used. The GC column was a Supelcowax 10 capillary column (60 m × 0.32 mm × 0.25 μm, Supelco, Bellefonte, PA, USA). The oven temperature program is described in Domínguez et al. (2019) and analyzed for a total of 49.5 min. A mass spectrometer of 5975C (Agilent Technologies, CA, USA) was used, and the temperatures of the mass transfer line, ion source, and quadrupole were 260℃, 230℃, and 150℃, respectively. Mass spectra were obtained at 70 eV electric shock and EMV of 1080, and data were collected in the m/z range of 40-450 at a scan speed of 3.4 times per second. Compounds were identified by comparing their mass spectra with those contained in the NIST 20/Wiley 12th library. The results are expressed as area units (AU) × 10^5/g of sample.

Statistical analysis

All quality characteristics were taken an average of three replication on each measurement for further statistical analysis. The distribution of sample groups was expressed as a score plot using principal component analysis using data from physicochemical variables by MetaboAnalyst (version 5.0; https://www.metaboanalyst.ca/). Mixed model was used to confirm the difference in physicochemical properties in the sample groups according to the TBARS value

and PI. The mixed model was performed using the mixed procedure of SAS 9.4. The statistical models were used to evaluate the quality characteristics (moisture, instrumental color, pH, texture profile analysis, fatty acid content, free amino acid content, and volatile compounds) of the dry-cured loin. These models included a fixed effect for the sample group and a random terms for processing batch and sample replication. Least squares means for all traits of interest were separated (F test, $p<0.05$) by using least significant differences analyzed by the PDIFF option in SAS.

Results and Discussion

Sample construction

Table 1 and Fig. 1 show the sample descriptions used in this study. PI and TBARS have been widely used as indicators of proteolysis and lipid oxidation in many previous studies, respectively (Marušić Radovčić et al., 2021). The sample groups used in this study showed significant differences in proteolysis and lipid oxidation levels ($p<0.05$). Sample grouping was also performed using principal component analysis (PCA) (Fig. 2). As a result of the PCA, PC1 and 2 from sample groups were explained 73% variance, which may consider a clear distribution between the groups. Therefore, the grouping of samples based on PI and TBARS appears to be highly appropriate.

In dry-cured ham, the PI reflecting excellent quality was reported in previous studies as 33-36% (Spanish style) and 22-30% (Italian style) (Careri et al., 1993; Pérez-Santaescolástica et al., 2018). However, in products using pork loin, there is no mention of the PI range for good quality yet. The PI values in the current study ranged from 7% to 14%, consistent with data

232 reported by other authors (Abellán et al., 2018; Stadnik & Dolatowski, 2013). This difference
233 between dry-cured ham and dry-cured loin is due to manufacturing conditions such as raw
234 material, salting period, and drying-ripening period. Our TBARS (0.6~1.1 mg MDA/kg sample)
235 results are consistent with those reported by Muriel et al. (2007) and Soto et al. (2008). In
236 addition, Hoz et al. (2007) reported that the overall acceptability of dry-cured loins with 0.87 to
237 1.24 mg of TBARS was higher than that of dry-cured loins with higher TBARS. The results
238 suggest that the dry-cured loin utilized in this study can be characterized as exhibiting normal
239 quality. Therefore, despite the absence of a definitive statement regarding the specific ranges of
240 PI and TBARS, dividing these values into four groups based on the median value from the
241 present study may be a somewhat compelling approach.
242
243 *Physicochemical properties*
244 Table 2 presents the results of physicochemical properties for the four dry-cured loin
245 groups based on the levels of proteolysis and lipid oxidation. Moisture content exhibited a
246 significant difference between groups ($p<0.05$), with the HH group showing the highest moisture
247 content at around 51%, and the LL group exhibiting the lowest at around 47%. In protein content,
248 on the other hand, high proteolysis groups (HH and HL) were significantly lower than low
249 proteolysis groups ($p<0.05$). Also, the fat content was observed same significant result as for
250 protein content ($p<0.05$). Redness showed a significant difference among the sample groups, with
251 HH and HL exhibiting higher values, while LH and LL showed relatively lower values ($p<0.05$).
252 TPA hardness and cohesiveness showed significant differences across groups ($p<0.05$). HH and
253 HL exhibited lower TPA hardness than LH and LL, while TPA cohesiveness was higher. Thus,
254 the level of proteolysis and lipid oxidation did not significantly affect the lightness, yellowness,

and pH ($p>0.05$), but they notably influenced the other variables, with proteolysis seen to giving a more substantial effect than lipid oxidation.

The current results of moisture, protein and lipid content in dry-cured loin were agree with previous studies (Bermúdez et al., 2015; Ortiz et al., 2020; Seong et al., 2015). Moisture content is an important factor in determining the quality of dry-cured meat products, as the level of moisture dehydration directly affects tissue properties and various enzyme activities (Harkouss et al., 2015). In addition, moisture is one of the technological traits that determine not only dryness but also storage stability of dry-cured meat (Seong et al., 2015). HH exhibited a higher moisture content than HL, indicating weaker dehydration in HH than in HL. Accordingly, lipid oxidation and proteolysis were more active in HH than in HL. Moreover, the difference in moisture content between HH and HL was only about 1%, whereas HH and LH showed a difference of about 3%. Therefore, proteolysis may have a more significant influence than lipid oxidation on moisture, protein and fat content in dry-cured loin. Additionally, both the protein and fat contents changed due to variations in moisture content brought about by dehydration. In particular, the protein content increased, reaching levels similar to that of the water content. Such changes can significantly impact subsequent biochemical reactions, potentially altering the reaction products.

Our findings align with Pérez-Alvarez et al. (1999) report of high a^* values in dry-cured ham muscles with high moisture content, citing nitrosomyoglobin formation as the cause. The a* value may be influenced by the denaturation of nitrosomyoglobin due to the production of lactic acid during ripening (Lorenzo et al., 2013). Therefore, it can be hypothesized that proteolysis affected nitrosomyoglobin and production of lactic acid. However, this explanation alone does not sufficiently clarify why the sample group with higher proteolysis exhibited stronger redness.

Thus, additional research is needed on the relationship between proteolysis and color in dry-cured loin.

The observed hardness and cohesiveness cannot be entirely explained by moisture content alone, as proteolysis and lipid oxidation effects must also be considered. In HH and HL, the dry matter's interference in TPA measurement is reduced due to high moisture content, while LH and LL, with low moisture content, increase the interference of dry matter, resulting in the current hardness result. Dry-cured loin shrinks gradually during manufacturing, which may result in increased cohesiveness. LH and LL, which were highly dehydrated, contracted more strongly, and cohesiveness increased as the dehydrated empty space naturally decreased. Seo et al. (2021) also reported similar findings to ours.

Fatty acid profile

Table 3 shows the fatty acid content in relation to the levels of proteolysis and lipid oxidation. No significant difference was observed in saturated fatty acids (SFA) between the sample groups ($p>0.05$). However, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) exhibited significant differences between the groups with strong significance levels of $p<0.05$ and $p<0.001$, respectively. Concerning individual fatty acids, significant differences were found between sample groups for SFA at C10:0, C12:0, and C20:0 ($p<0.05$). For MUFA and PUFA, there were no significant differences between sample groups in C18:1 n-7 and C20:4, respectively, but significant differences were observed in other fatty acids ($p<0.05$). In particular, there was a significant difference for C16:1, C18:2 n-3, C18:3 n-6, C20:1, C20:2 and C20:3 ($p<0.05$). The amount of MUFA was significantly lower in HH than other sample groups ($p<0.05$). On the other hand, there was no significant difference between LH and LL in

MUFA($p>0.05$). Therefore, MUFA was affected by lipid oxidation in the high proteolytic sample group but not affected by lipid oxidation in the opposite case. In terms of the PUFA, HH and HL were significantly lower than LH and LL ($P<0.05$). These results indicated that higher proteolytic levels were associated with lower amount of PUFA, regardless of the degree of lipid oxidation. Conversely, lower proteolytic levels were linked to higher amount of PUFA, regardless of the degree of lipid oxidation. Additionally, there does not appear to be a noticeable effect of lipid oxidation on changes in PUFA. These results were reflected in the total fatty acid content, and HH and HL had a significantly lower total fatty acid content than LH and LL ($p<0.05$). Therefore, it may be suggested that proteolysis contributes more to changes in fatty acid content than lipid oxidation.

Previous studies have reported that the content of SFA, MUFA, and PUFA decreases in the final product compared to the initial stage (Gilles, 2009; Andres et al., 2005; Salazar et al., 2016). During this time, mainly neutral lipids and phospholipids decrease (Gilles, 2009; Andres et al., 2005; Salazar et al., 2016). In the present study, proteolysis induced more changes in fatty acid content than lipid oxidation, and these changes are mainly attributable to PUFAs. The most probable hypothesis that can be inferred is the interaction between protein and lipid oxidation. Oxidation reactions may easily lead from fat and protein, causing various changes in food. Among these changes, the loss of enzyme activity, which can directly affect the quality of dry-cured meat products, is a representative example (Viljanen et al., 2004). Considering that our dry-cured loin had a significantly higher protein content than fat content on a dry matter basis (Table 2) and that the oxidation reaction is a chain reaction, the following conclusions can be drawn. Fat-initiated oxidation can induce a cascade of oxidation reactions and promote the oxidation of proteins. Carbonylated proteins may be more susceptible to enzyme-induced proteolysis

(Nyström, 2005). Gan et al. (2019) also mentioned the relationship between proteolysis and protein oxidation, arguing that protein oxidation can cause changes to protein structure, leading to unfolded proteins that are more reactive forms for proteases. Consequently, the final products such as hydroperoxide from protein-lipid-derived oxidation may have induced changes in fatty acids (Viljanen et al., 2004; Lund et al., 2011). Among fatty acids, PUFA is known to undergo the most severe changes due to oxidation, with n-6 type fatty acids being the most severely reduced as oxidation proceeds (Sampaio et al., 2012). Therefore, in the group with high proteolysis, the content of PUFAs may have decreased, which may have affected the volatile compounds.

Free amino acid

Table 4 presents the content of free amino acids. Except for taurine and anserine, there was a significant difference between groups overall ($p<0.001$). HH had the highest content at approximately 2309 mg and LL had the lowest content at 1799 mg ($p<0.05$). Furthermore, there was no significant difference between HL and LH, with both having a content between 1880 and 1895 mg ($p<0.05$). Aspartic acid, threonine, alanine, valine, and isoleucine showed the same results among sample groups, with HH having the highest content and LL having the lowest content ($p<0.05$). Similarly, serine, asparagine, glutamic acid, glycine, isoleucine, leucine, tyrosine, and phenylalanine showed the same trend, with the highest content in HH and the lowest content in LL ($p<0.05$). Additionally, there was no significant difference between HL and LH ($p<0.05$). These findings were consistent with those of Cittadini et al. (2020), who reported that free amino acid content was high in the group with high TBARS. In summary, proteolysis or lipid oxidation could influence the generation of free amino acids, with high levels of either

leading to an increase in the content of free amino acids. Furthermore, high levels of both could lead to the highest content of free amino acids.

As discussed earlier, free amino acids are considered as representative indicators of proteolysis. Therefore, the changes in free amino acid levels according to various conditions have been investigated in several previous studies. Garrido et al. (2012) reported that the total amino acid content decreased with an increase in salting time, as higher salt concentrations can inhibit proteolytic activity. Protein degradation through cathepsin B, L, and calpain activity promotes the production of small peptides and free amino acids. In particular, myosin heavy chain, C protein, and desmin are degraded by proteolytic enzymes, which can contribute to the generation of free amino acids (Zhou et al., 2017).

Lipid oxidation is a significant chemical reaction that occurs in meat and meat products. It is a breakdown of lipid components and is closely related to lipolytic activity, which releases free fatty acids (Muriel et al., 2007). Reactive oxygen species typically initiate lipid oxidation by reacting with fatty acids (Domínguez et al., 2019). Lipid oxidation products such as hydrogen peroxide, lipid-free radicals, and volatile secondary oxidation products can react with proteins to generate protein-centric free radicals (Hematyar et al., 2019). Furthermore, carbonylation of proteins is promoted by lipid oxidation, and carbonylated proteins are more sensitive to proteolytic enzymes (Nyström, 2005). Moreover, the folded structure of natural proteins forms a rigid structure that makes it difficult for proteolytic enzymes to act as a substrate (Fontana et al., 2004). However, oxidation-induced changes in the secondary and tertiary structures of proteins result in an unfolded state, leading to an increase in the site of action of proteases and protein degradation (Grune et al., 2004). This ultimately leads to an increase in the production of free amino acids released by proteolysis.

370

371 *3.5. Volatile compound*

372 The volatile compounds were analyzed by the sample group and shown in Table 5. A total
373 of 27 volatile compounds were detected, consisting of 11 alcohols, 4 alkanes, 6 ketones, and 6
374 aldehydes. Of these, 7 alcohols, 3 alkanes, 3 ketones, and 4 aldehydes showed significant
375 differences between groups ($p<0.05$). For ∑alcohol, ∑alkane, ∑ketone, and ∑total, HH and HL
376 were high, while LH and LL were low ($p<0.05$). However, ∑aldehyde showed the opposite result
377 ($p<0.05$). Therefore, the sample group with high protein degradation (HH and HL) produced a
378 large amount of total volatile compounds. Interestingly, there was no difference according to lipid
379 oxidation in the sample group with high protein degradation, but there was a significant
380 difference according to lipid oxidation in the opposite case ($p<0.05$).

381 An additional discussion was not conducted because the properties of individual volatile
382 compounds are widely known in dry-cured meat.

383 The formation of volatile compounds in dry-cured meat products is primarily considered a
384 result of lipid oxidation. Due to the lipolytic activity (enzymatic and oxidative) of dry-cured
385 meat, unsaturated fatty acids are converted into various compounds, mainly aliphatic compounds
386 such as alcohols, aldehydes, and ketones. In dry-cured meat, volatiles are mainly produced by
387 lipid oxidation in the initial processing step, whereas in the ripening step, both lipid and amino
388 acid degradation occur (Gilles, 2009). In fact, among the results of our study, the amount of the
389 PUFA and volatile compounds showed the same manner between groups, which is likely due to
390 the effect of proteolysis. Therefore, the change in PUFA was caused by proteolysis, and it could
391 be inferred that the current volatile compound result is also due to this. Earlier, it was explained
392 that oxidation was carried out by proteolysis and had an effect on PUFA. A previous study

reported that lipid oxidation is closely related to volatile compounds (Domínguez et al., 2014). Additionally, lipid oxidation usually occurs in polyunsaturated fatty acids, as the double bond site has a radical reactive site, making it more sensitive with more double bonds (Domínguez et al., 2019). Therefore, during the dry-aging process of dry-cured loin, polyunsaturated fatty acids are converted to volatile compounds due to lipid oxidation, generating alcohols, aldehydes, and ketones. Moreover, free amino acids are known to act as precursors of volatile compounds and are mainly produced by Strecker degradation and Maillard reaction, with heterocyclic compounds containing sulfur or nitrogen and aliphatic compounds being produced (Toldrá, 1998; Jurado et al., 2007).

Conclusion

The study investigated how proteolysis and lipid oxidation affect key quality parameters during the manufacturing of dry-cured loin. The results of this study demonstrated that varying levels of proteolysis and lipid oxidation can influence the physicochemical properties, total fatty acid content, total free amino acids content, and amount of volatile compounds in dry-cured loin. While lipid oxidation did not affect the fatty acid content, it did influence the free amino acid content between sample groups. Proteolysis, however, impacted both. Intriguingly, volatile compounds were influenced by the level of lipid oxidation when proteolysis was high but remained unaffected by lipid oxidation levels when proteolysis was low. These results implicate that lipid oxidation and proteolysis can directly cause changes in quality characteristics when manufacturing dry-cured loin. It also suggests that not only lipid oxidation but also proteolysis may play an important role in the formation of volatile compounds in dry-cured loin.

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Author Contributions

Conceptualization: Seo JK, Yang HS. Data curation: Lee YS, Seo JK, Eom JU. Formal analysis: Seo JK, Eom JU. Methodolgy: Seo JK, Yang HS. Sofrware: Seo JK, Eom JU. Validation: Seo JK, Yang HS. Investigation: Seo JK, Yang HS, Lee YS. Writing – original draft: Seo JK, Lee YS. Wiriting – review & editing: Seo JK, Yang HS.

Conflicts of Interest

The authors have no conflicts of interest to report.

IRB/IACUC approval

This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

439

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551

552 **Figure legends**

553 Fig. 1. The values of PI (upper) and TBARS (below) for the sample group. HH, PI and TBARS
554 of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and TBARS of
555 high level; LL, PI and TBARS of low levels. Error bar represents the standard error. $^{a-d}$Different
556 small letters within different sample groups were significantly different ($p<0.05$).

557

558 Fig. 2. The score plot was expressed to sample distribution in one or two PC. HH, PI and TBARS
559 of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and TBARS of
560 high level; LL, PI and TBARS of low levels.

561

562 Table 1. Sample classification of dry-cured loin by PI and TBARS

Sample classification[1]	PI range	TBARS range	n
HH	$X \geq 10.6$	$X \geq 0.88$	16
HL	$X \geq 10.6$	$X < 0.88$	9
LH	$X < 10.6$	$X \geq 0.88$	18
LL	$X < 10.6$	$X < 0.88$	17

563 [1]HH, PI and TBARS of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and
564 TBARS of high level; LL, PI and TBARS of low levels.

565

566 Table 2. Physicochemical properties for sample groups determined by PI and TBARS

Item	HH[1]	HL	LH	LL	SEM[2]	p-value[3]
Moisture (%)	50.87a	49.54b	47.59c	46.65d	0.52	***
Protein (%)	42.04c	42.71b	43.69ab	43.88a	0.33	**
Fat (%)	5.59b	6.15b	7.02a	7.87a	0.10	*
Lightness	44.51	45.26	46.67	44.20	0.72	ns
Redness	7.63a	7.56a	6.57b	6.83b	0.27	*
Yellowness	5.11	5.66	5.82	5.61	0.24	ns
pH	5.96	5.99	6.01	6.07	0.03	ns
Hardness (N)	19.07b	18.81b	20.95a	21.23a	0.66	**
Cohesiveness	0.46a	0.46a	0.42b	0.42b	0.01	***

567 [1]HH, PI and TBARS of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and
568 TBARS of high level; LL, PI and TBARS of low levels.
569 [2]Standard error of the means.
570 [3]*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.
571 a-dDifferent small letters within different sample groups were significantly different ($p<0.05$).
572 ns, no significance.
573

574 Table 3. Fatty acid content for sample groups determined by PI and TBARS

Fatty acid content (mg/100g tissue)	HH[1]	HL	LH	LL	SEM[2]	p-value[3]
C10:0	28.33c	29.08c	33.92a	30.85b	0.98	*
C12:0	29.22c	31.80b	34.86a	33.65a	1.10	*
C14:0	154.06	169.91	162.98	167.31	11.57	ns
C16:0	2156.83	2208.81	2222.41	2202.15	20.29	ns
C16:1	330.25a	323.46a	331.62a	307.52b	9.86	*
C17:0	56.67	46.34	56.53	47.67	7.43	ns
C18:0	1100.55	1090.32	1124.87	1119.77	96.53	ns
C18:1 n-7	358.59	352.54	374.96	352.38	26.61	ns
C18:1 n-9	3493.79b	3572.62a	3629.91a	3639.72a	44.48	*
C18:2 n-3	42.50c	55.42b	64.06a	65.43a	3.52	***
C18:3 n-6	751.70c	846.82b	976.96a	979.57a	35.40	***
C20:0	31.87c	34.53b	39.57a	37.39a	1.62	**
C20:1	79.69b	89.04a	90.44a	90.67a	5.55	**
C20:2	43.38c	49.97b	55.58ab	57.95a	3.17	***
C20:3	38.96b	39.98b	48.99a	43.93b	2.23	*
C20:4	158.49	152.64	175.23	172.92	17.35	ns
SFA	3556.65	3611.69	3674.19	3636.92	56.91	ns
MUFA	4262.32b	4329.48a	4426.93a	4391.22a	67.95	*
PUFA	1035.03b	1144.84b	1320.82a	1318.86a	74.43	***
n-6/n-3	17.69	15.28	15.25	14.97	2.03	ns
Total	8854.00b	9086.00b	9421.00a	9347.00a	104.41	*

575 [1]HH, PI and TBARS of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and
576 TBARS of high level; LL, PI and TBARS of low levels.
577 [2]Standard error of the means.
578 [3]*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.
579 a-cDifferent small letters within different sample groups were significantly different ($p<0.05$).
580 ns, no significance.
581 SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
582

583 Table 4. Free amino acid content for sample groups determined by PI and TBARS

Free amino acid (mg/100g dry-cured loin)	HH[1]	HL	LH	LL	SEM[2]	p-value[3]
Taurine	105.45	103.70	87.96	89.94	5.89	ns
Aspartic	62.63a	42.11c	51.20b	42.73c	3.28	***
Threonine	130.14a	99.53c	102.69b	95.00c	4.84	***
Serine	77.63a	59.16b	61.28b	55.62c	2.76	***
Asparagine	87.54a	67.37b	69.55b	62.26c	2.95	***
Glutamic	248.69a	200.99b	198.03b	189.92c	7.96	***
Proline	71.22	63.36	60.74	69.51	5.49	ns
Glycine	118.05a	94.95b	94.42b	86.23c	4.20	***
Alanine	214.74a	164.56c	173.92bc	167.88c	6.74	***
Valine	132.67a	105.96c	114.05b	109.5c	4.98	**
Methionine	72.91a	61.71b	59.43c	54.71d	1.93	***
Isoleucine	104.36a	85.84bc	89.01b	84.76c	3.34	***
Leucine	175.04a	143.35b	145.22b	135.52c	5.46	***
Tyrosine	103.78a	85.95b	86.51b	81.75c	2.87	***
Phenylalanine	87.37a	75.42b	73.90b	68.82c	2.23	***
Ornithine	5.29b	4.06b	7.74a	6.29a	0.72	**
Lysine	246.63a	204.34b	209.20b	200.70b	8.55	***
Histidine	60.24a	53.74b	49.76c	48.47c	2.31	**
Tryptophan	16.18a	8.82b	6.57c	3.27d	1.65	***
Anserine	33.21	32.47	31.04	31.50	0.82	ns
Arginine	152.19a	122.13b	122.33b	114.75c	4.72	***
Total	2309.72a	1880.55b	1895.55b	1799.26c	78.51	***

584 [1]HH, PI and TBARS of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and
585 TBARS of high level; LL, PI and TBARS of low levels.

586 [2]Standard error of the means.

587 [3]*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

588 a-dDifferent small letters within different sample groups were significantly different ($p<0.05$).

589 ns, no significance.

590 TFAA, total free amino acid.

591

592 Table 5. Volatile compound for sample groups determined by PI and TBARS

Compounds (AU×10^5)	HH[1]	HL	LH	LL	SEM[2]	p-value[3]
Heptanol	5.72a	5.85a	4.38b	4.59b	0.24	***
Hexanol	31.36a	25.58b	18.65c	16.29c	1.57	***
Nonanol	0.65	1.76	2.21	1.16	0.53	ns
Octanol	5.63b	5.75b	6.30ab	7.07a	0.62	*
1-Octen-3-ol	72.78	86.01	84.78	76.82	3.98	ns
Pentanol	38.99a	34.28b	27.99c	26.86c	1.83	***
3-Pentenol	0.43	0.54	0.40	0.37	0.04	ns
2,3-Butanediol	0.23	0.36	0.02	0.06	0.10	ns
3-Methyl-1-butanol	18.49a	19.64a	8.47b	7.15c	1.27	***
Benzeneethanol	5.95a	1.80b	1.82b	1.33b	0.60	***
Ethanol	29.07d	30.33c	31.97b	33.32a	0.77	***
Alcohol	209.30a	211.90a	186.70b	175.02c	6.00	***
2,3,5,8-Tetramethyldecane	1.82	1.43	1.53	1.54	0.23	ns
Decane	7.56a	7.20a	5.99b	5.92b	0.21	***
Dodecane	7.58a	7.20a	6.13b	5.64c	0.20	***
Tetradecane	1.55a	1.42a	0.68b	0.83b	0.17	*
Alkane	18.51a	17.25a	14.38b	13.93b	0.62	***
2,3-Octanedione	2.45	2.56	2.51	2.00	0.25	ns
2-Heptanone	5.48a	5.66a	5.40a	2.97b	0.58	**
2-Octanone	0.38	0.43	0.47	0.45	0.03	ns
2-Propanone	15.27a	11.96b	10.21c	9.35d	0.61	***
3-Octanone	1.61	2.22	0.93	1.30	0.38	ns
3-Hydroxy-2-butanone	3.01a	2.92a	1.75b	1.77b	0.19	***
Ketone	28.20a	25.74b	21.26c	17.84d	1.09	***
Benzaldehyde	3.38a	3.67a	1.89b	2.37b	0.44	*
Decanal	1.58	1.42	1.08	1.31	0.24	ns
Heptanal	8.55c	10.32b	10.17b	12.03a	0.61	***
Hexanal	24.17a	22.72b	21.32c	20.96c	0.67	**
Nonanal	33.39b	32.28b	38.84a	38.55a	1.77	*
Octanal	9.15	9.72	10.18	9.84	0.32	ns
Aldehyde	80.22	80.12	83.48	85.06	1.91	ns
Total	336.24a	335.01a	305.82b	291.85c	7.06	***

593 [1]HH, PI and TBARS of high levels; HL, PI of high level and TBARS of low level; LH, PI of low level and
594 TBARS of high level; LL, PI and TBARS of low levels.

595 [2]Standard error of the means.

596 [3]*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

597 a-dDifferent small letters within different sample groups were significantly different ($p<0.05$).

598 ns, no significance.

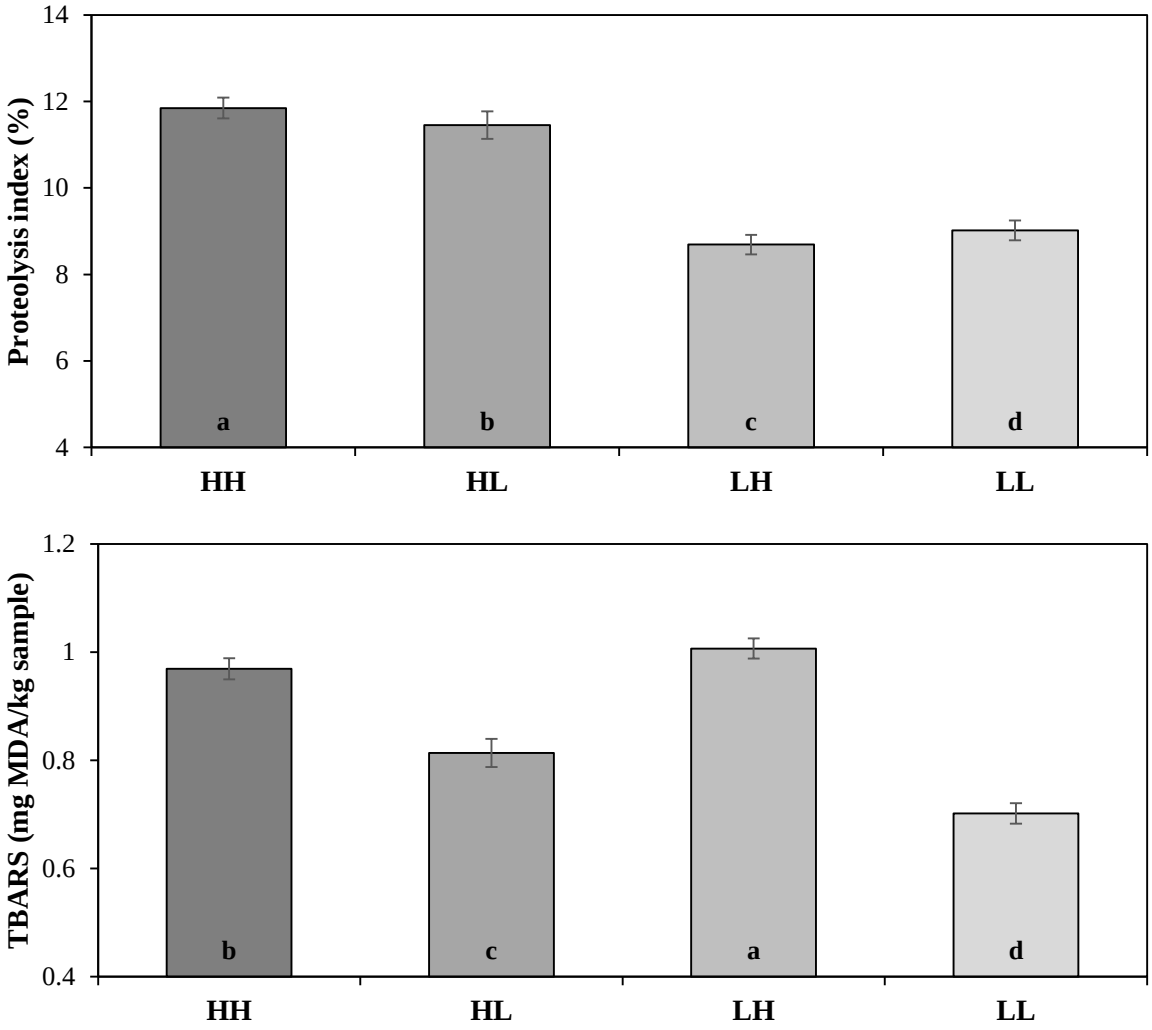
Proteolysis index (%)
14
12
10
8
6
4
a
b
c
d
HH
HL
LH
LL
TBARS (mg MDA/kg sample)
1.2
1
0.8
0.6
0.4
b
c
a
d
HH
HL
LH
LL

Fig. 1.

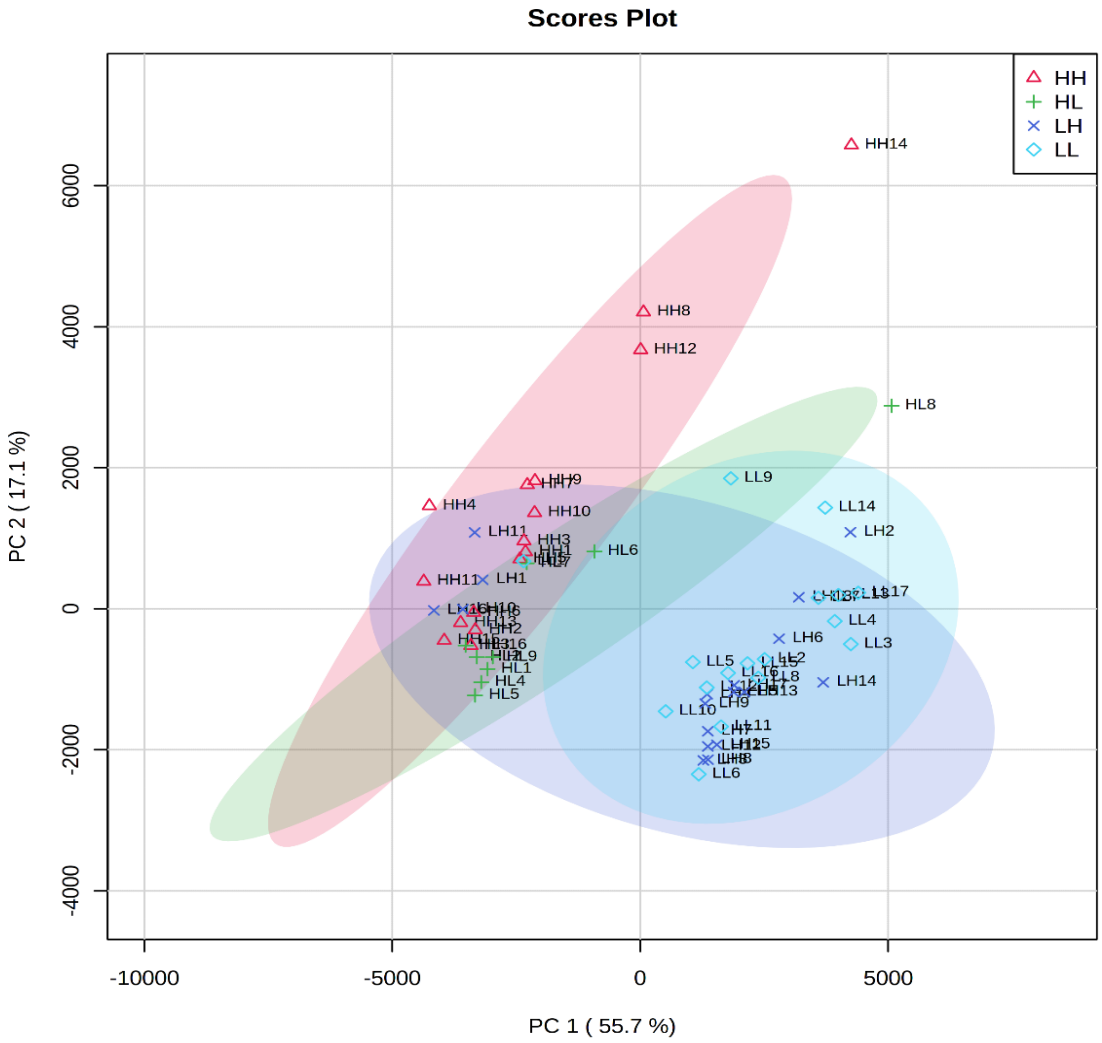

Fig. 2.