



# Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses<sup>☆</sup>

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## ABSTRACT

The role of moisture evaporation in the taste attributes of dry- and wet-aged beef was determined in this study. A total of 30 striploins (*longissimus lumborum*) were dry or wet aged for 28 days and analyzed for moisture content, taste-active compounds [free amino acids (FAAs), inosine 5'-monophosphate (IMP), and reducing sugars], and taste attributes by an electronic tongue. After the completion of aging process, higher amounts of FAAs and reducing sugars were found in dry-aged beef ( $P < .05$ ) in negative correlations with moisture content ( $r^2 = -0.9$  and  $-0.9$ , respectively), which were not detected in wet-aged beef. However, the different taste attributes of dry- and wet-aged beef were observed by the electronic tongue from day 14, whereas their moisture content was significantly different only at day 28. Consequently, although the moisture evaporation during dry aging process contributed to the increased flavor of dry-aged beef, there are other factors affecting flavor development including microbial activity on the surface crust.

## 1. Introduction

Aging is defined as storing meat for a certain period of time to improve its eating quality attributes, including tenderness, flavor, and juiciness (Lee et al., 2017; Oh et al., 2017). In general, aging can be divided into two different methods: dry and wet. Dry aging is the aging method exposes raw meat to ambient conditions under controlled temperature ( $-1$  to  $4$  °C), relative humidity (RH, 65–85%), and air flow velocity (0.2–5 m/s) (MLA, 2018; NIAS, 2018), whereas wet aging stores vacuum-packaged meat (Y. H. B. Kim et al., 2018). For the past decades, dry aging has largely been replaced by wet-aging in vacuum packaging, due to the relatively low saleable yield and more complicated supply chain logistics associated with the former (Khan, Jung, Nam, & Jo, 2016; Lee et al., 2018).

In recent years, however, the demands for dry-aged beef have been increased due to its desirable and characteristic flavor (Dashdorj, Tripathi, Cho, Kim, & Hwang, 2016). According to previous studies, the concentrated taste compounds, especially free amino acids (FAAs), of dry-aged beef are considered as the main contributor to its flavor (Kim, Kempa, & Samuelsson, 2016; Lee et al., 2017). This phenomenon could

be related to the moisture evaporation (Kim et al., 2016), which is a typical result of dry aging process by the exposure of meat to ambient conditions (Dashdorj et al., 2016; Khan et al., 2016). However, the scientific evidence for the effects of moisture evaporation on the flavor development of dry-aged beef is limited. In addition, the changes in other important taste-active compounds, such as inosine 5'-monophosphate (IMP) and associated reducing sugars, of dry-aged beef have not been clearly reported.

Meanwhile, there have been conflicting results in flavor difference between dry- and wet-aged beef. Most studies agreed that dry-aged beef has more intense beefy and roasted flavor (Campbell, Hunt, Levis, & Chambers IV, 2001; Kim et al., 2016; Warren & Kastner, 1992). However, some consumers could not detect the flavor difference between dry- and wet-aged beef (Dikeman, Obuz, Gok, Akkaya, & Stroda, 2013; Li et al., 2014; Li, Babol, Wallby, & Lundstrom, 2013; Smith et al., 2014). Therefore, it may be difficult to investigate when and how the taste attributes of dry-aged beef could be discriminable from that of wet-aged beef with a human sensory panel. To overcome this limitation, we applied an electronic tongue, which was previously developed for discriminating the taste of foods by the detection of chemical

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substances with a high sensitivity (Tahara & Toko, 2013). Therefore, in this study, we determined, 1) the role of moisture evaporation in taste-active compounds and 2) the changes in the taste attributes of dry- and wet-aged beef were compared by chemical and electronic tongue analyses were determined.

## 2. Materials and methods

### 2.1. Raw material and aging process

A total of 30 striploins (*longissimus lumborum*) from both sides of 15 different beef carcasses (Holstein steer, quality grade 3; Jo, Cho, Chang, & Nam, 2012) were obtained from a local slaughterhouse and transferred to a meat processing plant (Seoul, Korea). The initial pH of all samples ( $5.51 \pm 0.01$ ) was measured directly by a pH meter with the InLab® Solids Pro IP67 probe (SevenGo, Mettler-Toledo Inti., Inc., Schwerzenbach, Switzerland), prior to the aging process. The striploins from the same sides of different carcasses were randomly arranged for each dry- and wet-aged group. Before the aging process, the wet-aged group was vacuum-packaged (HFV-600 L, Hankook Fujee Co., Ltd., Siheung, Korea) with a low density polyethylene/nylon bag (oxygen permeability of 22.5 mL/m<sup>2</sup>/24 h atm at 60% RH/ 25 °C and water vapor permeability of 4.7 g/m<sup>2</sup>/24 h at 100% RH/25 °C). Both groups were aged for 0, 7, 14, 21, and 28 days under different conditions (dry aging at 4 °C, 75% RH, and 2.5 m/s air flow velocity or wet aging at 4 °C after the vacuum-packaging). At the sampling stage, the crust of dry-aged beef was trimmed off and both dry- and wet-aged meat samples were vacuum-packaged (HFV-600 L, Hankook Fujee Co., Ltd.) and stored at –70 °C until the analyses.

### 2.2. Measurement of moisture evaporation

The moisture content of dry- and wet-aged beef was analyzed using the dry oven method (Lee, Jo, Nam, & Lee, 2016) to measure moisture evaporation. The weight of ground meat (5 g) was measured before and after the drying process in a dry oven at 110 °C for 16 h (DS-520 L, Daewon, Bucheon, Korea). Moisture content was expressed as the ratio of weight from the samples before and after the drying process.

$$\text{Moisture content (\%)} = \frac{(\text{Weight before drying} - \text{Weight after drying})}{\text{Weight before drying}} \times 100$$

### 2.3. Taste-active compounds

#### 2.3.1. FAAs

The samples for FAAs were prepared by the method of Schwarz, Roberts, and Pasquali (2005) and injected into the high performance liquid chromatography system (HPLC, Ultimate 3000, Thermo Fisher Scientific Inc., Waltham, MA, USA) with pre-column derivatization. The analytical conditions were as follows: mobile phase, 40 mM sodium phosphate dibasic buffer (pH 7.8) and distilled water (DW)/acetonitrile/methanol (10:45:45, v/v); flow rate and time, 1.5 mL/min for 35 min; column, VDSpher 100 C18-E (4.6 × 150 mm<sup>2</sup>, 3.5 μm particles, VDS optilab Chromatographie Technik GmbH, Berlin, Germany); and detector, UV/Vis detector at 266 and 340 nm. Chemical reference standards for each analyte (Agilent technologies, Santa Clara, CA, USA) were used to generate a standard curve for calculation of the peak areas.

#### 2.3.2. IMP and reducing sugars

IMP was extracted from both dry- and wet-aged samples based on the method of Lee et al. (2017). The extract was filtered through a membrane filter (0.2 μm; Whatman PLC., Kent, UK) into a glass vial and injected into the HPLC system (Ultimate 3000, Thermo Fisher Scientific

Inc.). The analytical conditions were as follows: injection volume, 10 μL; mobile phase, 20 mM potassium phosphate monobasic (pH 5.5); flow rate and time, 1.0 mL/min for 25 min; column, Synergi Hydro-RP (250 × 4.6 mm<sup>2</sup>, 4 μm particles; Phenomenex Inc., Seoul, Korea) at 30 °C; and detector, UV/Vis detector at 254 nm. The peak area was calculated from a standard curve obtained using a standard IMP (Sigma-Aldrich, St. Louis, MO, USA).

Reducing sugars were determined by the method of Jayasena et al. (2015). Each extract was dissolved with deionized distilled water (DDW, 1 mL) and added to 2 mL of dinitrosalicylic solution (0.5 g of dinitrosalicylic acid, 8.0 g of sodium hydroxide, and 150 g of rochelle salt in 500 mL of DDW) in a 15-mL test tube and heated at 90 °C for 10 min. The mixture was cooled under running water and its absorbance was measured at 550 nm (X-ma 3100, Human Co., Ltd., Seoul, Korea). The standard glucose (Sigma-Aldrich) was used to calculate amount of the reducing sugars.

### 2.4. Electronic tongue

Taste attributes of dry- and wet-aged beef were detected using an electronic tongue (Astree, Alpha MOS, Toulouse, France) with seven sensors (AHS, PKS, CTS, NMS, CPS, ANS, and SCS) and 1 reference electrode (Ag/AgCl). The ground meat samples (100 g) were homogenized (T25, Ika Works) with DW (200 mL) and centrifuged at 2265 × g for 10 min (Continent 512R, Hanil Co., Ltd.). Then, the supernatants were obtained from the samples and used for electronic tongue analysis. During the analysis, the sample vials were kept at 20 °C. The data was reported as the pattern discrimination index (%) and taste attributes of dry- and wet-aged beef based on the AlphaSoft program (Alpha MOS).

### 2.5. Trimethylamine (TMA) content

For the analysis of TMA, ground meat (5 g) was placed in a 20 mL vial and volatiles from the headspace were injected into the gas chromatography-type electronic nose (Heracles II, Alpha MOS) equipped with dual columns (MXT-5 and 1701, Restek, Bellefonte, PA, USA). The analytical conditions were as follows: 10 min headspace generation at 80 °C; 5 mL injection volume; 40 °C and 240 °C the initial and final trap temperature, respectively; and flame ionization detector. The column oven temperature was 40 °C for 5 s, increased to 150 °C by 0.5 °C/s, 260 °C by 5 °C/s, and held for 30 s. The peak area was integrated using the AlphaSoft program (Alpha MOS) and reported TMA content from the samples.

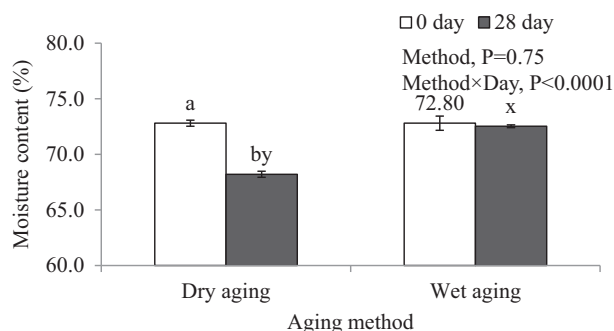
### 2.6. Statistical analysis

A complete randomized design was applied to compare taste-active compounds and electronic tongue analysis of dry- and wet-aged beef. Dry- ( $n = 15$ ) and wet-aged beef ( $n = 15$ ) were assigned for 0, 7, 14, 21, and 28 days of aging ( $n = 3$  for each aging period). The general linear model was analyzed for the effects of aging method and period and the results were reported as mean values with standard error of the means (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Significant differences among the mean values were determined on the basis of the Tukey's multiple range test at a level of  $P < .05$ . The correlation coefficient ( $r^2$ ) between moisture content and taste-active compounds of dry- and wet-aged beef was calculated (SAS Institute Inc.).

## 3. Results and discussion

### 3.1. Moisture content

Based on this study, the changes in moisture content of dry- and wet-aged beef was attributed to the interaction effect of aging method and days ( $P < .0001$ ) (Fig. 1). The moisture content of dry-aged beef was not lower than that of wet-aged beef up to day 21 but lower only at



**Fig. 1.** Moisture content (%) of beef striploins aged with different aging methods after 28 days (mean ± standard deviation).

<sup>a,b</sup>Different letters indicate a significant difference within the same aging method ( $P < .05$ ).

<sup>x,y</sup>Different letters indicate a significant difference within the same aging period ( $P < .05$ ).

day 28 ( $P < .05$ ), which is the point that the highest amounts of FAAs and reducing sugars was found in dry-aged beef (Tables 1 and 2). However, the concentration effect of taste-active compounds in dry-aged beef by the moisture evaporation could not completely explain the flavor of dry-aged beef as it was not proportionally changed with taste-related compounds. In other words, the concentration effect by the moisture evaporation would be a partial reason for the higher amounts of taste-active compounds found in dry-aged beef than wet-aged counterpart. In addition, Kim et al. (2016) suggested a relatively higher rate of proteolysis as another contributor to FAAs contents in dry-aged beef in comparison to wet-aged beef.

### 3.2. Taste-active compounds

#### 3.2.1. FAAs

The significant increase in all FAAs was shown during the first 7 days (approximately two-fold increase on average) then slowly increased thereafter until 21 days of aging, regardless of aging method ( $P < .05$ , Table 1 and Fig. 2). Then, the amount of FAAs in dry-aged beef was significantly higher at days 21 and 28 when compared to those in wet-aged beef. This phenomenon occurred as most of FAAs (17 out of 18 components) in wet-aged beef were maintained or decreased at the later stage of aging process, whereas FAAs (12 out of 18 components) in dry-aged beef were continually increased ( $P < .05$ ). Therefore, the change in total FAAs content from days 21 to 28 was relatively higher in dry-aged beef compared to wet-aged beef, showing that dry aging may promote further increase in FAAs content at the later stage of dry aging process. This is important as the concentration of taste-active FAAs can directly increase flavor intensity (Frank et al., 2016) and also serve as substrates for aroma volatiles through the Maillard reaction and Strecker degradation (Mottram, 1998). Similarly, the continuous increase in glutamic acid content of dry-aged beef was reported until 50 days of dry aging process in a previous study (Iida et al., 2016). Kim et al. (2016) demonstrated that the amounts of FAAs were more abundant in dry-aged beef (e.g. glutamic acid, isoleucine, leucine, phenylalanine, tryptophan, tyrosine, and valine) when compared to those in wet-aged beef, probably due to the relative concentration of protein composition by moisture evaporation and/or a higher rate of proteolysis during dry aging process. However, there was no clear evidence for either of these pathways.

In this study, the role of moisture evaporation in the concentration of FAAs in dry- or wet-aged beef determined using the Pearson's correlation coefficient between moisture and total FAAs content (data not shown). All FAAs in dry-aged beef had a significantly negative correlation to moisture content ( $r^2 = -0.9$  in average), as a consequence, its total FAAs content ( $r^2 = -0.9$ ,  $P < .0001$ ) was more affected by

**Table 1**  
Free amino acid contents (mg/100 g) of beef striploins aged with different aging methods during 28 days.

Traits	Aging method	Aging period (day)					SEM <sup>1</sup>
		0	7	14	21	28	
Ala	Dry	22.19 <sup>c</sup>	44.09 <sup>b</sup>	44.18 <sup>b</sup>	59.64 <sup>ax</sup>	63.69 <sup>ax</sup>	0.879
	Wet	22.19 <sup>c</sup>	44.81 <sup>b</sup>	44.59 <sup>b</sup>	49.40 <sup>ay</sup>	42.39 <sup>by</sup>	0.967
	SEM <sup>2</sup>	0.192	0.752	0.725	1.465	0.999	
Arg	Dry	6.72 <sup>d</sup>	12.32 <sup>b</sup>	14.01 <sup>bx</sup>	19.43 <sup>ax</sup>	19.21 <sup>ax</sup>	0.261
	Wet	6.72 <sup>c</sup>	12.09 <sup>b</sup>	12.64 <sup>by</sup>	14.24 <sup>ay</sup>	11.75 <sup>by</sup>	0.215
	SEM <sup>2</sup>	0.019	0.164	0.188	0.348	0.320	
Asn	Dry	2.77 <sup>e</sup>	6.18 <sup>d</sup>	7.64 <sup>cy</sup>	10.59 <sup>b</sup>	13.80 <sup>ax</sup>	0.132
	Wet	2.77 <sup>d</sup>	6.47 <sup>c</sup>	8.27 <sup>bx</sup>	10.79 <sup>a</sup>	11.94 <sup>ay</sup>	0.256
	SEM <sup>2</sup>	0.015	0.125	0.067	0.424	0.087	
Asp	Dry	0.55 <sup>c</sup>	1.17 <sup>b</sup>	1.19 <sup>by</sup>	1.60 <sup>by</sup>	4.78 <sup>ax</sup>	0.108
	Wet	0.55 <sup>c</sup>	1.22 <sup>d</sup>	1.65 <sup>cx</sup>	3.52 <sup>bx</sup>	3.89 <sup>ay</sup>	0.044
	SEM <sup>2</sup>	0.010	0.051	0.031	0.087	0.151	
Glu	Dry	2.12 <sup>c</sup>	8.47 <sup>dx</sup>	14.08 <sup>c</sup>	23.09 <sup>by</sup>	32.75 <sup>ax</sup>	0.484
	Wet	2.12 <sup>d</sup>	5.76 <sup>sy</sup>	13.82 <sup>b</sup>	25.99 <sup>ax</sup>	23.83 <sup>ay</sup>	0.582
	SEM <sup>2</sup>	0.178	0.344	0.651	0.653	0.658	
Gly	Dry	6.57 <sup>c</sup>	9.48 <sup>b</sup>	10.52 <sup>b</sup>	13.86 <sup>a</sup>	13.38 <sup>ax</sup>	0.254
	Wet	6.57 <sup>d</sup>	9.51 <sup>c</sup>	9.95 <sup>bc</sup>	12.30 <sup>a</sup>	11.59 <sup>aby</sup>	0.396
	SEM <sup>2</sup>	0.140	0.336	0.274	0.544	0.225	
His	Dry	3.10 <sup>d</sup>	6.69 <sup>c</sup>	7.90 <sup>b</sup>	11.28 <sup>a</sup>	11.73 <sup>ax</sup>	0.223
	Wet	3.10 <sup>d</sup>	6.26 <sup>c</sup>	7.46 <sup>b</sup>	10.30 <sup>a</sup>	8.56 <sup>by</sup>	0.256
	SEM <sup>2</sup>	0.019	0.120	0.128	0.349	0.369	
Ile	Dry	2.99 <sup>e</sup>	8.01 <sup>d</sup>	9.97 <sup>c</sup>	15.05 <sup>bx</sup>	17.66 <sup>ax</sup>	0.283
	Wet	2.99 <sup>d</sup>	7.84 <sup>c</sup>	9.76 <sup>b</sup>	13.35 <sup>ay</sup>	13.27 <sup>ay</sup>	0.124
	SEM <sup>2</sup>	0.010	0.081	0.103	0.304	0.360	
Leu	Dry	5.51 <sup>e</sup>	14.95 <sup>d</sup>	17.87 <sup>c</sup>	26.54 <sup>bx</sup>	29.26 <sup>ax</sup>	0.422
	Wet	5.51 <sup>d</sup>	14.56 <sup>c</sup>	17.53 <sup>b</sup>	23.84 <sup>ay</sup>	23.01 <sup>ay</sup>	0.236
	SEM <sup>2</sup>	0.010	0.132	0.178	0.510	0.523	
Lys	Dry	4.10 <sup>d</sup>	11.84 <sup>c</sup>	13.93 <sup>c</sup>	24.93 <sup>bx</sup>	20.40 <sup>ax</sup>	0.573
	Wet	4.10 <sup>c</sup>	12.31 <sup>b</sup>	14.20 <sup>b</sup>	19.81 <sup>ay</sup>	12.92 <sup>by</sup>	0.446
	SEM <sup>2</sup>	0.073	0.236	0.323	0.794	0.722	
Met	Dry	2.57 <sup>e</sup>	6.44 <sup>d</sup>	8.02 <sup>c</sup>	11.50 <sup>bx</sup>	12.58 <sup>ax</sup>	0.206
	Wet	2.57 <sup>d</sup>	6.50 <sup>c</sup>	7.91 <sup>b</sup>	10.55 <sup>ay</sup>	10.34 <sup>ay</sup>	0.131
	SEM <sup>2</sup>	0.015	0.079	0.111	0.182	0.312	
Phe	Dry	3.74 <sup>d</sup>	8.72 <sup>d</sup>	10.72 <sup>c</sup>	15.60 <sup>bx</sup>	17.35 <sup>ax</sup>	0.231
	Wet	3.74 <sup>d</sup>	8.64 <sup>c</sup>	10.57 <sup>b</sup>	14.30 <sup>ay</sup>	14.13 <sup>ay</sup>	0.153
	SEM <sup>2</sup>	0.006	0.077	0.135	0.251	0.325	
Pro	Dry	3.57 <sup>d</sup>	7.08 <sup>cd</sup>	10.47 <sup>bcx</sup>	12.47 <sup>b</sup>	18.56 <sup>ax</sup>	0.971
	Wet	3.57 <sup>c</sup>	4.08 <sup>bc</sup>	6.88 <sup>abey</sup>	10.29 <sup>ab</sup>	13.15 <sup>ay</sup>	1.442
	SEM <sup>2</sup>	0.274	1.449	0.838	2.067	0.640	
Ser	Dry	6.36 <sup>c</sup>	14.56 <sup>d</sup>	16.98 <sup>c</sup>	21.26 <sup>b</sup>	25.98 <sup>ax</sup>	0.316
	Wet	6.36 <sup>d</sup>	14.59 <sup>c</sup>	16.72 <sup>b</sup>	20.57 <sup>a</sup>	22.33 <sup>ay</sup>	0.390
	SEM <sup>2</sup>	0.035	0.383	0.347	0.547	0.253	
Thr	Dry	4.25 <sup>e</sup>	10.26 <sup>d</sup>	12.08 <sup>c</sup>	17.23 <sup>b</sup>	20.24 <sup>ax</sup>	0.195
	Wet	4.25 <sup>d</sup>	10.28 <sup>c</sup>	12.37 <sup>b</sup>	16.02 <sup>a</sup>	15.85 <sup>ay</sup>	0.270
	SEM <sup>2</sup>	0.020	0.182	0.225	0.395	0.194	
Try	Dry	1.08 <sup>d</sup>	2.60 <sup>c</sup>	3.14 <sup>b</sup>	4.07 <sup>a</sup>	4.51 <sup>ax</sup>	0.106
	Wet	1.08 <sup>d</sup>	2.64 <sup>c</sup>	3.20 <sup>b</sup>	3.78 <sup>a</sup>	3.56 <sup>aby</sup>	0.113
	SEM <sup>2</sup>	0.020	0.060	0.111	0.080	0.194	
Tyr	Dry	3.49 <sup>d</sup>	10.96 <sup>c</sup>	12.17 <sup>bx</sup>	18.19 <sup>ax</sup>	18.89 <sup>ax</sup>	0.221
	Wet	3.49 <sup>c</sup>	10.73 <sup>b</sup>	10.08 <sup>by</sup>	12.27 <sup>ay</sup>	11.06 <sup>by</sup>	0.243
	SEM <sup>2</sup>	0.193	0.133	0.157	0.338	0.275	
Val	Dry	4.56 <sup>e</sup>	13.56 <sup>d</sup>	16.57 <sup>c</sup>	25.29 <sup>bx</sup>	28.67 <sup>ax</sup>	0.373
	Wet	4.56 <sup>d</sup>	13.25 <sup>c</sup>	17.08 <sup>b</sup>	23.26 <sup>ay</sup>	22.06 <sup>ay</sup>	0.257
	SEM <sup>2</sup>	0.019	0.204	0.225	0.342	0.551	

<sup>1</sup>Standard error of means (n = 15), 2(n = 6).

<sup>a-c</sup>Means within the same row with different superscript differ significantly ( $P < .05$ ).

<sup>x,y</sup>Means within the same column with different superscript differ significantly ( $P < .05$ ).

moisture content when compared to that of wet-aged beef ( $r^2 = -0.5$ ,  $P < .05$ ). It means that the amount of moisture evaporation had a strong correlation with the concentration of FAAs in dry-aged beef. However, not all FAAs (e.g. alanine, arginine, glycine, histidine, tryptophan, and tyrosine) in dry-aged beef were proportionally increased by the decrease in moisture content, especially from days 21 to 28 (Table 1 and Fig. 1), which is consistent with the result from Kim et al. (2016). This result indicates that the changes in FAAs content of dry-aged beef

**Table 2**  
Inosine 5'-monophosphate (mg/100 g) and reducing sugars contents (mM) of beef striploins aged with different aging methods during 28 days.

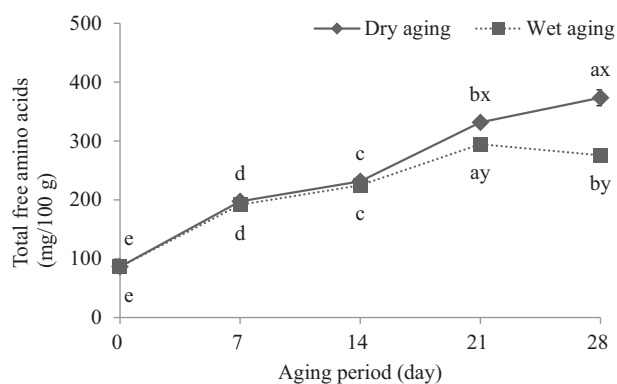
Traits	Aging method	Aging period (day)					SEM <sup>1</sup>
		0	7	14	21	28	
IMP	Dry	180.21 <sup>a</sup>	138.71 <sup>bx</sup>	126.24 <sup>cx</sup>	97.96 <sup>dx</sup>	55.07 <sup>ey</sup>	1.515
	Wet	180.21 <sup>a</sup>	130.78 <sup>by</sup>	115.78 <sup>cy</sup>	85.81 <sup>dy</sup>	87.14 <sup>dx</sup>	0.905
	SEM <sup>2</sup>	1.341	1.402	0.886	1.249	1.294	
Reducing sugars	Dry	9.05 <sup>d</sup>	10.84 <sup>cy</sup>	11.71 <sup>bc</sup>	12.91 <sup>ab</sup>	14.37 <sup>ax</sup>	0.328
	Wet	9.05 <sup>b</sup>	13.22 <sup>ax</sup>	11.80 <sup>a</sup>	13.06 <sup>a</sup>	10.16 <sup>by</sup>	0.330
	SEM <sup>2</sup>	0.340	0.412	0.216	0.451	0.063	

<sup>1</sup>Standard error of means (n = 15), 2(n = 6).

<sup>a-e</sup>Means within the same row with different superscript differ significantly (P < .05).

<sup>x,y</sup>Means within the same column with different superscript differ significantly (P < .05).

IMP, inosine 5'-monophosphate.



**Fig. 2.** Total free amino acid contents (mg/100 g) of beef striploins aged with different aging methods during 28 days.

<sup>a-e</sup>Different letters indicate a significant difference within the same aging method (P < .05).

<sup>x,y</sup>Different letters indicate a significant difference within the same aging period (P < .05).

could not be completely understood with the concentration effect by the moisture evaporation alone. There may be other factors affecting the further increase in FAAs content of dry-aged beef especially at the later stage of aging process and it may have a significant role in the flavor development of dry-aged beef.

As mentioned earlier, proteolysis was suggested as one of the contributors to the higher FAAs content of dry-aged beef (Kim et al., 2016). In general, proteolysis in meat and meat products is occurred through two types of proteolytic enzymes, *endo*- (e.g. calpains and cathepsins) and *exo*-peptidases (e.g. peptidase and aminopeptidase) (Toldra & Flores, 1998). Among them, aminopeptidase is responsible for the generation of FAAs during the aging process and is derived from muscle and/or microorganisms (Flores & Toldra, 2011; Iida et al., 2016; Toldra, 1998). In this study, it was postulated that the further proteolysis of dry-aged beef may be more related to microorganisms during dry aging process as muscle enzymes could lose its activity with increasing aging period (Iida et al., 2016; Toldra, Flores, & Sanz, 1997) and/or its exposure to oxygen (Frank et al., 2017; Kim, Lonergan, & Huff-Lonergan, 2010). The growth of microorganisms (e.g. mold and yeast) is one of the characteristic changes in dry-aged beef as well as the moisture evaporation (Dashdorj et al., 2016; Khan et al., 2016; Ryu et al., 2018). Ryu et al. (2018) investigated the microorganisms on the surface crust during 50–60 days of aging and found molds and yeasts, such as *Penicillium camemberti* and *Debaryomyces hansenii*. We also observed the growth of mold and yeast on the crust of dry-aged beef with the increase of aging period (M. Kim et al., 2018). The effect of mold

and yeast could be varied with dry aging process, due to its low temperature and water activity. However, their role in dry-aged beef is certainly expected as the proteolysis and lipolysis microorganisms have been proven for decades in dry-cured/fermented meat products (Flores & Toldra, 2011). In the further study, we isolated microorganisms from the surface of dry-aged beef and found the different tendencies of small peptide (< 3 kDa) generated in intact or inoculated dry-aged beef, depending on the types of microorganisms (data not shown). As a consequence, the microorganisms in dry-aged beef may influence the activity of muscle aminopeptidase. Also, it may affect indirectly as its metabolites, including amine and ammonia, could increase the pH (Lee et al., 2018). Most of muscle aminopeptidases are active at neutral pH (Toldra et al., 1997). In this study, a significantly higher pH of dry-aged beef (5.72) was found than that of wet-aged beef (5.44), possibly by the higher trimethylamine content in dry-aged beef (Fig. 5). Iida et al. (2016) also reported that the activity of muscle aminopeptidase C and H were maintained until 60 days of dry aging process. It may suggest the possibility of microbial role in dry-aged beef with the higher pH. In other words, the growth of mold and yeast during dry aging may induce the further proteolysis of dry-aged beef from days 21 to 28, directly by their proteolytic enzymes and/or indirectly through the activation of muscle aminopeptidase with the pH increase.

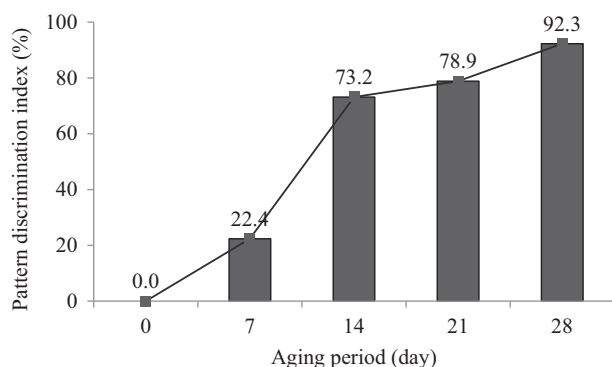
### 3.2.2. IMP and reducing sugars

IMP degradation is a well-known reaction during the aging process (Lee et al., 2017); however, there are only a few reports which present the changes in IMP of dry-aged beef and their comparison to wet-aged beef (Iida et al., 2016; Kim et al., 2016). In this study, IMP contents of dry- and wet-aged beef were significantly decreased with the increase of aging period, except for wet-aged beef from days 21 to 28 (Table 2). During this period, the continuous decrease of IMP was found only in dry-aged beef. As a result, dry-aged beef had a significantly lower IMP content (55.07 mg/100 g) than that of wet-aged beef (87.14 mg/100 g) after 28 days of aging, which is similar to the result from Kim et al. (2016) who reported 1.65-fold higher IMP content in wet-aged beef.

To understand the decrease in IMP content of dry-aged beef, moisture content and enzyme activity involved in IMP degradation were firstly considered. However, in this case, moisture evaporation was excluded from the options as wet-aged beef, which had significantly higher moisture content (Fig. 1), had a higher IMP content when compared to that of dry-aged beef at day 28 (P < .05, Table 2). Instead, the lower IMP content in dry-aged beef could be attributed to the activation of enzymes involved in IMP degradation (Koutsidis et al., 2008). The hypoxanthine content of wet-aged beef was not significantly changed from days 21 to 28, whereas that of dry-aged beef was increased (P < .05, data not shown). Consequently, dry-aged beef had higher hypoxanthine content after 28 days of aging than that in wet-aged beef. This result indicates that IMP degradation was continued until the end of dry aging process not like that in wet-aged beef. Similarly, Iida et al. (2016) demonstrated that dry aging could significantly decrease IMP content of beef *longissimus thoracis* up to day 50. As IMP contributes to umami taste itself or together with glutamic acid (Shahidi, 1994), these results indicate that IMP would not be the major agent for flavor development of dry-aged beef.

In contrast, reducing sugars content of wet-aged beef (10.16 mM) was lower after 28 days of aging when compared to that of dry-aged beef (14.37 mM) (P < .05, Table 2). Both aging methods had a significant negative correlation to moisture content ( $r^2 = -0.9$ ,  $P < .0001$  and  $r^2 = -0.8$ ,  $P < .01$  for dry- and wet-aged beef, respectively) (data not shown). However, as the correlation of dry-aged beef was slightly stronger than that of wet-aged beef, a higher reducing sugars content of dry-aged beef may be more related to IMP degradation during the aging process. The enzymatic breakdown of IMP liberated ribose and ribose-5-phosphate (main reducing sugar components in animal muscle) through two different pathways after the slaughter: i) dephosphorylation of IMP to inosine and then breakdown inosine to





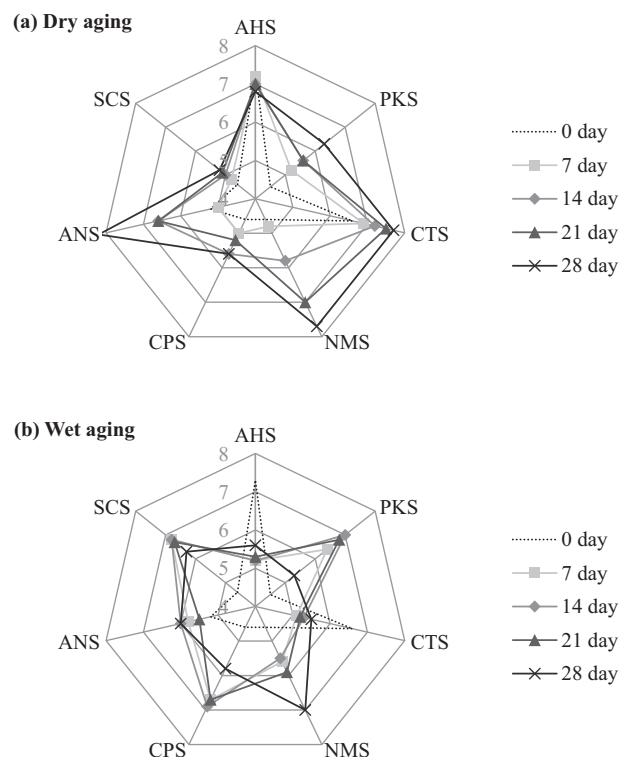
**Fig. 3.** Pattern discrimination index (%) from electronic tongue analysis of beef striploins aged with different aging methods during 28 days. The data was analyzed using the AlphaSoft program (Alpha MOS, Toulouse, France).

hypoxanthine and ribose or ii) IMP itself degraded to hypoxanthine and ribose-5-phosphate (Lee & Newbold, 1963). In this study, the changes in IMP and reducing sugars also had similar tendencies in both aging methods. As a consequence, this result shows that dry aging could increase reducing sugars content indirectly through the IMP degradation and the concentration effect by the moisture evaporation during dry aging may be partially responsible. The increased amount of reducing sugars during postmortem aging positively affect the beef flavor formation (Koutsidis et al., 2008) due to their sweet taste and as substrates in aroma formation through the Maillard reaction (Mottram, 1998; Shahidi, 1994). Hence, the increase in reducing sugars in dry-aged beef may have positively impact on the flavor formation in the present study.

### 3.3. Electronic tongue

The difference in taste attributes of dry- and wet-aged beef was analyzed using the electronic tongue (Figs. 2 and 3). The pattern discrimination index of dry- and wet-aged beef was increased with aging period and was discriminated after day 14 (> 73.17%) and it reached > 92.29% after 28 days of aging (Fig. 4). The increase in the distances between taste attributes of dry- and wet-aged beef may be related to the difference in saltiness, sourness, and umami taste detected by the sensors of ANS, CTS, and NMS, respectively (Fig. 3). These were gradually increased during 28 days of dry aging process, resulting in a relatively higher score in dry-aged beef than wet-aged beef at day 28.

The differences in the concentration of taste-active compounds (FAAs, IMP, and reducing sugars) between dry- and wet-aged beef (Tables 1 and 2) may affect the flavor, although no sensory evaluation was conducted on these samples. FAAs themselves could contribute to sweet and bitter taste and also influence saltiness and sourness together with acids and inorganic/sodium salts, respectively (Shahidi, 1994). In addition, glutamic acid is one of the most important components for umami taste in meat (Zhao, Schieber, & Gänzle, 2016). In this study, the sum of FAAs which can be attributed to each taste (sweet, bitter, and umami) were significantly higher in dry-aged beef after 28 days of aging when compared to wet-aged beef (data not shown). Besides, aroma volatiles can be formed by FAAs with reducing sugars during cooking (Koutsidis et al., 2008; Mottram, 1998). Aroma volatiles were not analyzed in this study. However, based on previous study, it was assumed that significant differences in FAAs may cause different aroma development of dry- and wet-aged beef. For example, methionine could generate cooked beef aroma with a low threshold value, whereas isoleucine, leucine, phenylalanine, serine, threonine, and valine produce Strecker aldehydes and heterocyclic compounds, which possess the characteristic aroma in meat (Mottram, 1998; Toldra, Aristoy, & Flores, 2000).



**Fig. 4.** Spider plot for taste attributes from electronic tongue analysis of (a) dry- or (b) wet- aged beef striploins for 28 days. The data was analyzed using the AlphaSoft program (Alpha MOS, Toulouse, France).

Meanwhile, different IMP degradation/reducing sugars accumulation between dry- and wet-aged beef could contribute to different flavor of dry- and wet-aged beef. IMP and reducing sugars possess umami and sweet taste, respectively (Koutsidis et al., 2008). In addition, reducing sugars can generate aroma volatiles in meat through the Maillard reaction with amino acid (Mottram, 1998). However, further degradation of IMP in dry-aged beef may increase the accumulation of hypoxanthine, which may impart a bitter taste in meat and meat products (Shahidi, 1994; Tikki et al., 2006).

### 3.4. TMA content

As we hypothesized the greater proteolysis of dry-aged beef may also be related to microorganisms on crust, the amount of TMA from dry-aged beef flesh was determined to estimate the microbial metabolic activity on dry-aged beef (Fig. 5). At day 28, which showed the highest amounts of FAAs and reducing sugars, TMA content was significantly higher in dry-aged beef (approximately 2–3 fold) when compared to those in wet-aged beef ( $P < .05$ ). Due to the fact that TMA production is proportional to the microbial activity (Flores, Marcus, Nieto, Navarro, & Lorenzo, 1997; Pearson, 1968), the proteolytic activity by microorganisms in dry-aged beef could be a main reason for the characteristic flavor of dry-aged beef. Ryu et al. (2018) also suggested that microorganisms may play an important role in the flavor of dry-aged beef.

Table 3 summarizes the comparison on taste-active compounds and electronic tongue analysis between dry- and wet-aged beef based on the results from the present study.

## 4. Conclusion

Dry aging can produce meat with a higher concentration of taste-active compounds and volatile aroma precursors than wet aging. The differences in taste attributes between dry- and wet-aged beef could be

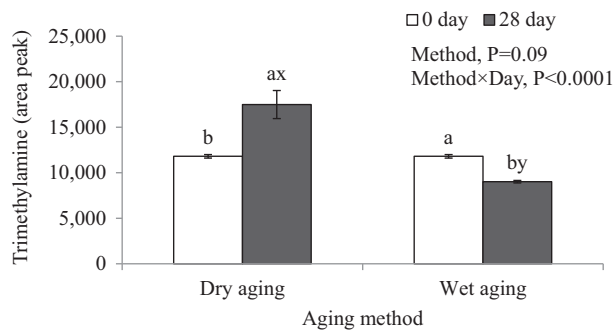


Fig. 5. Trimethylamine content (area peak) of beef striploins aged with different aging methods after 28 days (mean  $\pm$  standard deviation).

<sup>a,b</sup>Different letters indicate a significant difference within the same aging method ( $P < .05$ ).

<sup>x,y</sup>Different letters indicate a significant difference within the same aging period ( $P < .05$ ).

Table 3

Comparison on the taste attributes of dry- and wet-aged beef by chemical and electronic tongue analysis during aging period.

Taste attributes	Aging method		
FAAs	Dry	>	Wet
IMP	Dry	<	Wet
Reducing sugars	Dry	>	Wet
Saltiness	Dry	>	Wet
Sourness	Dry	>	Wet
Umami	Dry	>	Wet

This table was summarized based on the results from the present study.

FAAs, free amino acids; IMP, inosine 5'-monophosphate.

caused by two different reasons: i) mainly the further metabolic activities such as proteolysis and IMP degradation in dry-aged beef possibly by microorganisms on the crust and ii) partially the concentration effect by the moisture evaporation during dry aging process. We are currently conducting ongoing studies to confirm these hypothetical mechanisms.

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