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Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef

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ABSTRACT

Beef rumps (*middle gluteal*) were dry aged for 28 days using different air flow velocities of 0, 2.5, and 5 m/s (DA0, DA2.5, and DA5, respectively). The microbial composition, physicochemical traits (moisture, pH, and shear force), flavor compounds (inosine 5′-monophosphate, reducing sugar, free amino acid, and free fatty acid), and electronic tongue profile were analyzed at day 0, 14, and 28. No molds or yeasts were detected until day 14. On day 28, *Pilaira anomala* was found to be the most abundant in DA0, whereas DA2.5 and DA5 showed increased composition of *Debaryomyces hansenii*. With that, the significant changes in physicochemical traits and flavor compounds occurred. In addition, the pattern of flavor compounds and taste attributes from DA0, which had different mold and yeast compositions, were discriminable from DA2.5 and DA5. Therefore, our results suggest that air flow can affect microbial composition on the crust, possibly resulting in different sensory properties of dry-aged beef.

1. Introduction

Dry aging is a traditional method of aging which exposes 'unpackaged meat' to controlled temperature, relative humidity, and air flow velocity (Khan, Jung, Nam, & Jo, 2016; Kim et al., 2019). Its application for meat had been reduced due to high weight loss through moisture evaporation and trimming. However, in recent years, it has attracted consumers' attention due to its characteristic beefy and roasted flavor (Lee et al., 2018).

In general, the development of meat flavor is attributable to the reaction between flavor compounds (taste-related compounds and aroma volatiles) during aging and/or cooking (Jayasena, Ahn, Nam, & Jo, 2013a, 2013b; Mottram, 1998). The main taste-related compounds are inosine 5′-monophosphate (IMP), reducing sugar, and free amino acids (FAAs), whereas several hundred aroma volatiles are derived from the oxidation of lipids [e.g. triglycerides, phospholipids, and free fatty acids (FFAs)] and/or the Maillard reaction between reducing sugar and FAAs. Consequently, the amounts of such compounds in meat at the point of cooking decide its flavor. In terms of dry-aged beef, the concentration of flavor compounds by moisture evaporation has been suggested as a main contributor to its flavor (Kim, Kempa, & Samuelsson, 2016; Lee et al., 2019). The moisture evaporation effect on the concentration of FAAs and reducing sugar and final flavor of dry-aged beef was previously investigated and reported that there may be another factor affecting dry-aged flavor over the concentration of flavor compounds (Lee et al., 2019).

During dry aging, there were two significant changes in dry-aged beef: i) moisture evaporation, which results in the formation of dried surface (crust) and ii) mold and/or yeast growth on the crust (Dashdorj, Tripathi, Cho, Kim, & Hwang, 2016; Lee et al., 2019). The occurrence of unknown mold/yeast on the crust of dry-aged beef was also observed in our previous study. As the role of microorganisms in flavor development is well known, in association with their proteolytic and/or lipolytic activities, we suggested their role in flavor development of dry-aged beef as well. In addition, the typical resultants of microbial growth - including increased pH and trimethylamine content - were observed in dry-aged beef flesh without additional microbial growth on the inside (Lee et al., 2019), indicating that microbial growth on the crust may affect the sensory properties of dry-aged beef.

Meanwhile, although the counts of mold/yeast on the crust were similar, different features in their appearance were observed by the presence of air flow in the preliminary study. Therefore, we

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hypothesized that different air flow velocities may change microbial composition (e.g. mold/yeast and bacteria) on the crust during dry aging, possibly resulting in different sensory properties of the final dry-aged beef product. Furthermore, the identification of microorganisms in dry-aged beef, depending on different air flow velocities is worth investigation as its effect has not been studied. Therefore, the objectives of this study were to investigate i) the mold/yeast composition of dry-aged beef crust and ii) changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef.

2. Material and methods

2.1. Raw material and aging process

Twenty-one beef rumps (*middle gluteal*) from different carcasses (Holstein steer, quality grade 3; Jo, Cho, Chang, & Nam, 2012), with initial pH 5.63 ± 0.02 (the InLab® Solids Pro IP67 probe; Mettler-Toledo Inti., Inc., Schwerzenbach, Switzerland), were obtained from a local slaughter house. The rumps were transferred to a meat processing plant (Seoul, Korea) and three rumps were randomly assigned to serve as controls (non-aged, day 0). The remaining 18 rumps were randomly assigned to one of six dry-aging treatments ($n = 3$ for each treatment): air velocity of 0 m/s (DA0) aged 14 or 28 days; air velocity of 2.5 m/s (DA2.5) aged 14 or 28 days, and air velocity of 5 m/s (DA5) aged 14 or 28 days. All dry-aged groups were aged at 4 °C and 75% relative humidity.

After the completion of dry aging at each sampling day, the crust was trimmed off and used for microbial analysis. The microbial analysis of non-aged control group was also conducted using surface portion of meat at the beginning of dry aging. For the other analyses, the internal meat was ground and used, excluding approximately 100 g for the measurement of shear force. Microbial and physicochemical traits (moisture content, pH, and shear force) were assessed immediately to avoid adverse effect from freezing, whereas the other samples for flavor compounds and electronic tongue profile were vacuum-packaged (HFV-600 L, Hankook Fujee Co., Ltd., Siheung, Korea) and stored at −70 °C until the analyses.

2.2. Microbial analysis

2.2.1. Microbial isolation

Microbial isolation and identification were conducted according to the method of Oh et al. (2016) with a few modifications. In brief, meat surface or trimmed-off crust samples (25 g) were randomly obtained from non- and dry-aged groups, respectively, and enriched with 0.1% peptone water (Difco Laboratories, Detroit, MI, USA). The enrichments were blended at 8 stroke/s for 60 s using a laboratory blender (Bag-Mixer 400 P, Interscience, Saint-Nom la Bretèche, France) and the diluents were spread onto the agar plates of potato dextrose agar and tryptic soy agar (PDA and TSA, respectively; Difco Laboratories) for mold/yeast and bacteria, respectively. PDA plates were incubated at 25 °C for 5 days, whereas TSA plates were kept at 37 °C for 2 days. After incubation, the colonies on the agar plates were harvested for PCR analysis.

2.2.2. Identification and pyrosequencing

The colonies on each PDA and TSA were identified using the ITS3-ITS4 and 16S rDNA sequencing for mold/yeast and bacteria, respectively. DNA was extracted (Powersoil® DNA isolation kit; Mo Bio Laboratories, Carlsbad, CA, USA) and amplified (EF-Taq; Solgent, Daejeon, Korea) as follows: i) initial denaturation at 95 °C for 2 min, ii) denaturation at 95 °C for 1 min, iii) annealing at 55 °C for 1 min, iv) extension 72 °C for 1 min with 35 cycles, and v) final extension at 72 °C for 10 min. The amplified DNA was analyzed by an ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA).

2.3. Physicochemical traits

2.3.1. Moisture content

The dry oven method was used to analyze moisture content (Park, Yong, Choe, & Jo, 2018). The weight of each sample was weighed before and after drying at 110 °C for 16 h in a dry oven (DS-520 L, Daewon, Gyeonggido, Korea) and calculated as described by Park et al. (2018).

2.3.2. pH

Ground meat samples (1 g) were homogenized with deionized distilled water (DDW, 9 mL) at Lv. 6 wheel scale for 30 s (T10 basic, Ika Works, Staufen, Germany). The homogenates were centrifuged at 2265 × g for 10 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea) and filtered (Whatman No. 4, Whatman PLC., Kent, UK) to obtain the supernatants. pH was measured using a pH meter (SevenGo, Mettler-Toledo Inti., Inc.) attached with the InLab® Expert Pro probe (Mettler-Toledo Inti., Inc.). The pH meter was pre-calibrated with standard buffers at 4.01, 7.00, and 9.21 (Mettler-Toledo Inti., Inc.).

2.3.3. Shear force

Non-ground internal meat samples (approximately 100 g in amount) were vacuum-packaged and boiled in a water bath for 30 min until a core temperature of 72 °C was reached. Cooked samples were cut parallel to the muscle fiber into six cylindrical shapes (1.27 cm × 4 cm, diameter × length) using a cork borer (Korea Ace Scientific, Seoul, Korea), and placed under a Warner-Bratzler shear probe, perpendicularly to the muscle fiber. Shear force was measured using a texture analyzer (TA1, Lloyd Instruments Ltd., Fareham, UK) with a cell load of 0.1 N and a cross-head speed of 200 mm/min. Average value from the six rounded cores were considered as one replicate.

2.4. Flavor compounds

2.4.1. IMP

IMP was obtained from the samples based on the method of Lee et al. (2017). The extract was filtered (0.2 μm membrane filter; Whatman PLC.) into a glass vial and injected into a high performance liquid chromatography system (HPLC; Ultimate 3000, Thermo Fisher Scientific Inc., Waltham, MA, USA). 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^2, 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).

2.4.2. Reducing sugar

Reducing sugar was concentrated from dry-aged beef according to the method outlined by Jayasena et al. (2015). Each concentrate was dissolved in DDW (1 mL) and added to 2 mL of the color developing reagent (0.5 g of dinitrosalicylic acid, 8.0 g of sodium hydroxide, and 150 g of potassium sodium tartrate in 500 mL of DDW) in a 15-mL test tube and heated in a water bath 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 amount of reducing sugar was estimated using a glucose standard curve (Sigma-Aldrich).

2.4.3. FAAs

FAAs analysis was conducted using a 600 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) as described by Kim et al. (2019). The FAAs were extracted using 0.6 M perchloric acid and freeze-dried under vacuum (−50 °C and < 1 μmHg; PVTFD-10 K, Ilshin Lab, Dongducheon, Korea). The freeze-dried extract was reconstituted with phosphate buffer (pH 7.0) and treated by deuterium oxide, containing 1 mM

3-(trimethylsilyl)-propionate (TSP; Sigma-Aldrich). The NMR analytical conditions were as follows: spectral width, 7813 kHz; the number of points, 128 k; the number of dummy scan and scan, 2 and 128, respectively. One-dimensional NMR spectrum was identified based on the Human Metabolome Database and calculated using an internal standard (1 mL TSP).

2.4.4. FFAs

Total lipids were extracted from the samples based on the method of Folch, Lees, and Sloane Stanley (1957). The extract (0.1 g) was added to 1 mL chloroform and the internal standard (1 mg triundecanoate/mL isooctane, Sigma-Aldrich) in a 15-mL test tube. Triglycerides were removed from the mixture by chloroform: 2-propanol (2:1. *v*/v) and FFAs were extracted using 2% acetic acid in diethyl ether with the solid phase extraction kit (Sigma-Aldrich). The extract was evaporated using nitrogen gas (99.999%) and heated in a water bath at 85 °C for 10 min. For methylation, 2 mL of 14% boron trifluoride-methanol solution was added after cooling and heated again in a water bath at 85 °C for 10 min. After cooling, 2 mL of isooctane and 1 mL of saturated sodium chloride were added and centrifuged at 1573 ×*g* for 3 min (Continent 512R, Hanil Co., Ltd.). The upper layer containing FFAs methyl esters was dehydrated with anhydrous sodium sulfate and injected into a gas chromatography (HP 7890, Agilent Technologies, Santa Clara, CA, USA) with a split ratio at 10:1. The GC analytical conditions were as follows: injection volume, 1 μL; carrier gas, nitrogen gas (99.999%); flow rate and time, 4.0 mL/min for 1 h; column, DB-23 (60 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte, PA, USA); and detector, flame ionization detector. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The column oven temperature was 50 °C for 1 min, increased to 130 °C by 25 °C/min, 170 °C by 8 °C/min, 215 °C by 2 °C/min, and held for 10 min. Each FFAs methyl ester was identified using external standards (Supelco® 37 FAME mix and CLA mix, Sigma-Aldrich) and calculated as outlined by the Korean Food Standards Codex (MFDS, 2018).

2.5. Electronic tongue profile

Taste attributes of non- and dry-aged groups were detected using an electronic tongue with seven different sensors (*GPS*, *SRS*, *STS*, *UMS*, *SPS*, *SWS*, and *BRS*) and one Ag/AgCl electrode as reference (Alpha MOS, Toulouse, France). Each *SRS*, *STS*, *UMS*, *SWS*, and *BRS* sensor represents sourness, saltiness, umami, sweetness, and bitterness, respectively. The ground meat samples (100 g) were homogenized (T25, Ika Works) with distilled water (200 mL) and centrifuged at 2265 ×*g* for 10 min (Continent 512R, Hanil Co., Ltd.). Then, the supernatants (100 mL) from the samples were used for electronic tongue profile analysis. During the analysis, sample vials were maintained at 20 °C. The data obtained was also reported as pattern discrimination index (%) based on the AlphaSoft program (Alpha MOS).

2.6. Statistical analysis

A completely randomized design was used to investigate changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. The one-way analysis of variance was applied for the effect of air flow velocity and aging 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 based on Tukey's multiple range test at a confidence level of $P < .05$. Differences in physicochemical quality traits and flavor compounds of DA0, DA2.5, and DA5 were also calculated before and after the occurrence of mold and yeast (between days 0 and 14 or days 14 and 28), using paired *t*-test (SAS Institute Inc.), to observe changes occurred before and after the appearance of mold/yeast on the crust. Principal component analysis (PCA) was applied for four different flavor compounds (IMP, reducing sugar, FAAs, and FFAs) in non- and dry-aged groups (DA0, DA2.5, and DA5).

Table 1
The compositions of mold/yeast and bacteria isolated from the crust of beef rump dry aged for 28 days with different air flow velocities (0, 2.5, and 5 m/s) using next generation sequencing.

Traits	Day 0	Day 28		
	None	DA0	DA2.5	DA5
Composition of mold and yeast (%)				
Candida	ND	ND	0.1	0.1
Debaryomyces hansenii	ND	0.2	15.9	14.7
Pilaira anomala	ND	99.8	83.7	85.2
Rhodotorula	ND	ND	0.3	ND
Composition of bacteria (%)				
Enterobacterium	0.1	6.2	6.2	2.2
Flavobacterium	1.7	0.2	2.1	5.9
Lactobacillus	55.5	0.9	5.3	7.8
Pseudomonas	42.7	92.7	86.3	84.2

Non-aged beef (None) and dry-aged beef with air flow velocities of 0 (DA0), 2.5 (DA2.5), and 5 m/s (DA5).
ND, not detected.

3. Results and discussion

3.1. Microbial analysis

3.1.1. Mold and yeast composition

Mold and yeast were not detected on the crust of dry-aged beef on day 14, however, different microbial features were found thereafter with different air flow velocities. On day 28, mold and yeast from the crust of dry-aged beef were isolated from each dry-aged group and identified (Table 1). In DA0, *Pilaira anomala* (99.8%) was the most abundant microorganism on the crust with a very small composition of *Debaryomyces hansenii* (0.2%), whereas DA2.5 and DA5 had the increased composition of *D. hansenii* (15.9% and 14.7%, respectively). In addition, *Candida* was found in both DA2.5 and DA5 (0.1% and 0.1%, respectively), while *Rhodotorula* (0.3%) was observed only in DA2.5. The presence of *D. hansenii* and *Rhodotorula* from the crust of dry-aged beef was also reported when beef *longissimus thoracis* and *biceps femoris* were dry aged at 1–4 °C and 80–90% relative humidity for 60 days (Ryu et al., 2018). These two microorganisms have been reported in several meat and meat products, especially dry-cured/fermented meat products (Dave & Ghaly, 2011). However, as far as we know, this is the first study to report *P. anomala* on the crust of dry-aged beef. It has only previously been reported in a Korean fermented soybean paste called *Doenjang* (Kim et al., 2009).

DA0, without air flow, had relatively more different mold and yeast compositions from those in DA2.5 and DA5. Based on this result, we postulated that the presence and/or extent of air flow can change the mold and yeast composition in dry-aged beef and this may affect its sensory properties as both microorganisms have different proteolytic and/or lipolytic activities (Kim et al., 2018). The effect of air flow velocity on microbial composition in meat and meat products is unknown so far. In fact, the application of air flow during dry aging was related with its final yield by moisture evaporation, not microbial growth (Dashdorj et al., 2016). Moisture content may also change microbial growth as it can affect water activity, (Dave & Ghaly, 2011). However, most molds and yeasts can be grown across a wide range of water activities (Beuchat, 1983). Also, in this study, the moisture content between days 14 and 28 was significantly decreased only in DA5, whereas DA0 and DA2.5 possessed similar amounts of moisture before and after dry aging ($P < .05$; Table 2). Therefore, moisture evaporation could not explain the difference in microbial composition of *P. anomala* and *D. hansenii* in dry-aged beef (Table 1). According to Garijo et al. (2008)

Table 2
Physicochemical traits from the dry-aged beef rump dry aged for 28 days with different air flow velocities.

Traits	None	Dry-aged			SEM[1]	Δ Days 14–0			Δ Days 28–14		
		DA0	DA2.5	DA5		DA0	DA2.5	DA5	DA0	DA2.5	DA5
Moisture (%)	71.67[a]	72.44[a]	70.72[a]	66.32[b]	1.280	−1.37	−0.45	−0.40	2.14*	−0.50	−4.95**
pH	5.63[b]	5.96[a]	5.60[b]	5.63[b]	0.024	−0.01	−0.06	−0.06	0.34**	0.03	0.04
Shear force (N)	84.84[a]	41.74[c]	58.86[b]	53.34[b]	2.992	−26.39**	−27.93***	−24.68**	−24.68**	4.73**	−8.53*

Non-aged beef (None) and dry-aged beef with air flow velocities of 0 (DA0), 2.5 (DA2.5), and 5 m/s (DA5).
The differences between days 0 and 14 (Δ Days 14–0) or days 14 and 28 (Δ Days 28–14) was calculated based on the occurrence of mold and yeast growth from the crust of beef rump.
a,b Means within the same row with different superscripts differ significantly ($P < .05$).
[1] Standard error of the means ($n = 12$).
* $P < .05$.
** $P < .01$.
*** $P < .0001$.

who investigated the occurrence of mold, yeast, and bacteria in the air of a Spanish winery, mold could be present everywhere either inside the winery or in the air, however, yeast got onto the subjects through air flow. Consequently, the presence of air flow and its velocity may have introduced and increased the *D. hansenii* composition of DA2.5 and DA5 in this study.

3.1.2. Bacterial composition

Before dry aging, the bacterial composition of beef rump was the highest with *Lactobacillus* followed by *Pseudomonas*, Flavobacterium, and Enterobacterium (55.5%, 42.7%, 1.7%, and 0.1%, respectively; Table 1). After 28 days of dry aging with different air flow velocities, the compositions of *Pseudomonas* and Enterobacterium were increased in all dry-aged groups and, especially, DA0 had the highest compositions of both *Pseudomonas* and Enterobacterium. DA2.5 and DA5 had similar composition of *Pseudomonas*, whereas DA5 had the lowest Enterobacterium composition within the different dry-aged groups. Meanwhile, *Lactobacillus*, which was predominant in the non-aged group, was considerably decreased in composition possibly due to the increase in *Pseudomonas* during dry aging. Therefore, DA2.5 and DA5 had higher compositions of *Lactobacillus* and Flavobacterium, when compared with DA0. The growth of *Pseudomonas* and Enterobacterium during dry aging may be due to aerobic and refrigeration conditions, while *Lactobacillus* is comfortable with anaerobic conditions (Dave & Ghaly, 2011). Air flow itself does not help bacterial growth; however, it allows microorganism to travel through air in three different ways: i.e. a dust particle, droplet, and/or single particle (Garijo et al., 2008). Therefore, the presence of air flow in this study may help the settlement of bacteria on dry-aged beef crust.

3.2. Physicochemical traits

3.2.1. Moisture content

During dry aging period, moisture evaporation can be attributed to air flow (Dashdorj et al., 2016). It was previously suggested as a contributor to dry-aged beef flavor due to the concentration of flavor compounds, especially FAAs and reducing sugar (Lee et al., 2019). In this study, DA5, which had the fastest air flow, resulted in the lowest moisture content among the treatments as we expected ($P < .05$; Table 2). However, regardless of dry aging, the moisture content of DA0 and DA2.5 were not significantly different from non-aged group. This result shows that the development of dry-aged flavor in DA0 and DA2.5 may not be only related to the concentration of such compounds by moisture evaporation. In addition, if flavor and/or flavor compounds between DA0 and DA2.5 were different in the present study, it could not be explained by moisture evaporation alone.

3.2.2. pH

After 28 days of dry aging, DA0 had significantly higher pH than DA2.5 and DA5 due to a relatively higher increase in pH between days 14 and 28 ($P < .01$; Table 2). As Lee et al. (2019) and Ryu et al. (2018) suggested the effect of microorganisms from the crust of dry-aged beef, this could be attributed to the production of amine/ammonia (basic) by the outgrowth of *Pseudomonas* and less numbers of lactic acid-producing *Lactobacillus* (acidic) on the crust of DA0, when compared with microorganisms in DA2.5 and DA5 (Dave & Ghaly, 2011; Table 1). However, this may not completely explain the increased pH of DA0 as the other dry-aged groups also had high composition of *Pseudomonas* (84.2–86.3%) and *D. hansenii*, which is known to possess high proteolytic activity (Dura, Flores, & Toldra, 2004; Patrignani et al., 2007). Therefore, we hypothesize that *P. anomala*, which was predominant in DA0, may also induce an increase in pH due to the production of amine/ammonia, possibly from its high proteolytic activity. In a previous study, we had confirmed significantly higher proteolysis by *P. anomala* than *D. hansenii* using a skim milk-containing agar model system (Kim et al., 2018). Also, we spread *P. anomala* onto the surface of meat at the beginning stage and analyzed internal meat during 28 days of dry aging. As a result, its pH value was significantly higher than *D. hansenii*-spread one from day 14, which supports our hypothesis.

3.2.3. Shear force

DA0 had the lowest shear force, when compared to DA2.5 and DA5 ($P < .05$; Table 2). There was a significant decrease in shear force of all dry-aged groups throughout the aging period, especially at the early stage of dry aging. There were no significant differences in the extent of decrease in shear force among the dry-aged groups between days 0 and 14 of dry aging, therefore, there was no significant difference found with different air flow velocities. Meanwhile, between days 14 and 28, the decrease in shear force of DA0 was relatively higher than those of DA2.5 and DA5 ($P < .01$). This result indicates that another factor, possibly the occurrence of mold and yeast with different compositions (Table 1), may influence changes in shear force of DA0 at the later stage of dry aging (Table 2).

Meat tenderization is dependent on endogenous proteolytic enzymes in animal muscle (e.g. calpains, cathepsins, proteasome, and caspase) (Huff-Loneran, Zhang, & Lonergan, 2010; Ouali et al., 2006) and/or derived from microorganisms (Flores & Toldra, 2011). In the present study, the decrease in shear force of all dry-aged groups at the early stage of the aging period could be affected by muscle proteinases. On the other hand, the different compositions of *P. anomala* and *D. hansenii* may affect the change in shear force at the later stage of dry aging as both microorganisms were detected on the crust of dry-aged beef after 14 days of dry aging (data not shown). Dashdorj et al. (2016) reported that proteolytic and collagenolytic enzymes from mold

Table 3
Flavor compounds from the dry-aged beef rump dry aged for 28 days with different air flow velocities.

Traits	None	Dry-aged			SEM[1]	Δ Days 14–0			Δ Days 28–14		
		DA0	DA2.5	DA5		DA0	DA2.5	DA5	DA0	DA2.5	DA5
IMP (mg/100 g)	164.59^{a}	10.16^{c}	35.26^{b}	27.21^{b}	1.855	−67.19**	−26.38**	−32.88**	−87.24**	−103.00**	−104.50***
Reducing sugar (mM)	15.84^{b}	12.75^{c}	23.39^{a}	22.89^{a}	0.849	−2.61	3.25*	3.64	0.47	4.30**	3.41
Glu (mg/100 g)	5.56^{c}	8.60^{b}	16.18^{a}	16.65^{a}	0.249	1.11*	2.04**	5.02**	3.04**	10.62**	11.09**
Ile (mg/100 g)	3.51^{b}	11.10^{a}	13.08^{a}	14.36^{a}	0.985	0.98	2.26*	2.29*	7.59*	9.57*	10.85*
Leu (mg/100 g)	6.57^{c}	12.25^{b}	14.84^{a}	14.59^{a}	0.141	0.16	1.23*	2.40**	5.68**	8.27**	8.02**
Phe (mg/100 g)	6.46^{c}	11.39^{b}	12.81^{ab}	13.34^{a}	0.359	−0.17	1.44	2.02	4.93**	6.34**	6.87**
Val (mg/100 g)	3.64^{b}	8.65^{a}	9.83^{a}	8.47^{a}	0.306	0.31	0.85**	1.47*	5.00**	6.18**	4.83**
Total FAAs (mg/100 g)	25.76^{c}	51.99^{b}	66.74^{a}	67.42^{a}	1.142	1.27	5.78*	8.17*	23.19**	30.36**	30.57**
C16:0 (mg/g)	5.92^{b}	10.86^{a}	8.06^{ab}	6.89^{b}	0.652	0.43	2.23	0.96*	4.94*	2.14*	0.97**
C16:1 (mg/g)	0.19^{b}	0.61^{a}	0.37^{b}	0.33^{b}	0.045	0.01	0.11	0.06	0.42*	0.18*	0.14**
C18:0 (mg/g)	4.10^{b}	5.79^{a}	4.30^{b}	3.86^{b}	0.243	0.09	0.73*	0.03	1.69*	0.20	0.24*
C18:1 (mg/g)	2.52^{b}	7.31^{a}	4.75^{b}	4.07^{b}	0.529	0.70	1.69	1.31*	4.78*	2.23*	1.55*
C18:2 (mg/g)	0.58^{b}	1.83^{a}	1.89^{a}	1.33^{a}	0.164	0.04	1.06*	0.80	1.25*	1.31**	0.76**
Total FFAs (mg/g)	13.31^{c}	26.39^{a}	19.37^{ab}	16.47^{bc}	1.443	1.27	6.04	3.31**	13.23*	6.32*	3.29*

Non-aged beef (None) and dry-aged beef with air flow velocities of 0 (DA0), 2.5 (DA2.5), and 5 m/s (DA5).
IMP, inosine 5′-monophosphate; FAAs, free amino acid; FFAs, free fatty acid.
The differences between days 0 and 14 (Δ Days 14–0) or days 14 and 28 (Δ Days 28–14) was calculated based on the occurrence of mold and yeast growth from the crust of beef rump.
a–c Means within the same row with different superscripts differ significantly ($P < .05$).
[1] Standard error of the means (n = 12).
* $P < .05$.
** $P < .01$.
*** $P < .0001$.

Thamnidium could break down muscle and connective tissue as it penetrates into meat. Lee et al. (2019) also suggested the role of microorganisms on the crust in dry-aged beef flesh based on its higher trimethylamine content. In this study, considering the results from pH and shear force tests (Table 2), the higher composition of *P. anomala* in DA0 (Table 1) may lead to higher proteolysis, when compared with those in DA2.5 and DA5 and it is supported by Kim et al. (2018). In addition, the endogenous proteolytic enzymes such as calpains in animal muscle are active at neutral pH (Huff-Loneran et al., 2010); DA0 may induce the activation of muscle proteinases during the entire dry aging period.

3.3. Flavor compounds

3.3.1. IMP and reducing sugar

IMP contents in all dry-aged groups were significantly decreased during 28 days of dry aging, especially in DA0 (Table 3). This phenomenon is mainly owing to the significant change in IMP content between days 14 and 28, which was relatively higher than that within the first 14 days. Further IMP breakdown could occur at a later stage of aging period, which was not reported in wet aging (Lee et al., 2019). In addition, the decrease in IMP generation could be attributed to the inactivation of enzymes involved in the dephosphorylation of nucleotides due to an increase in pH or proteolytic activity (Koutsidis et al., 2008; Reina, del Pulgar, Lopez-Buesa, & Garcia, 2014). For example, dry-cured loin with a slightly higher pH decreased the dephosphorylation rate of nucleotides (e.g. ATP, ADP, and IMP), therefore, lowered IMP and inosine contents (Reina et al., 2014). IMP degradation is pH dependent as it contains weak chemical bonds (e.g. glucoside and ester bonds) (Tikk et al., 2006). There are several factors affecting pH in meat and meat products; however, in terms of our study, the higher pH observed in DA0, when compared with DA2.5 and DA5, was possibly due to the degradation of protein to amine/ammonia by *P. anomala*. Therefore, in either ways, the lower IMP content in DA0 may be due to the proteolytic activity of *P. anomala*.

Meanwhile, during 28 days of dry aging, different changes in reducing sugar content were observed under various air flow conditions (Table 3). DA2.5 and DA5 had significantly higher reducing sugar content when compared to those in non-aged beef and DA0. We previously demonstrated that the amount of reducing sugar was significantly increased only with dry aging (2.5 m/s of air flow velocity), possibly by the further degradation of IMP (Lee et al., 2019). In the present study, however, there was a significant decrease in reducing sugar content of DA0. Based on the preliminary study, this could be attributed to the varying sugar utilization abilities of *P. anomala* and *D. hasnenii* (data not shown). Consequently, this result indicates that different air flow velocities could vary reducing sugar content by the different compositions of microorganisms, especially *P. anomala* (Table 1).

3.3.2. FAAs

FAAs is one of the main flavor compounds in meat and meat products and it also generates several aroma volatiles via the Maillard reaction with reducing sugar (Mottram, 1998). The FAAs components presented in Table 3 (e.g. isoleucine, leucine, phenylalanine, and valine) are related to the generation of cooked beef aroma, whereas glutamic acid possesses umami taste (Mottram, 1998; Shahidi, 1994; Toldra, Aristoy, & Flores, 2000). After 28 days, FAAs content was increased in all dry-aged groups ($P < .05$) and three out of five FAAs components (glutamic acid, leucine, and phenylalanine) in DA2.5 and DA5 were significantly higher than those in DA0. Such FAAs tended to be increased with aging period due to muscle aminopeptidase and/or microbial-derived enzymes (Flores & Toldra, 2011; Toldra, Flores, & Sanz, 1997). In this study, most FAAs components were increased, with relatively higher rates at the later stage of dry aging, possibly due to the occurrence of *P. anomala* and *D. hansenii* with different compositions (Table 1). Also, considering the results from our study, there may be different enzymes affecting the proteolytic activity of each *P. anomala* and *D. hansenii*. The enzymes involved in the proteolytic activity of *P. anomala* may be more effective with tenderization when compared to those of *D. hansenii*, whereas *D. hansenii* resulted in slightly higher proteolysis on FAAs generation.

3.3.3. FFAs

Regardless of different air flow velocities, major FFAs components (C16:0-C18:20) were significantly increased after 28 days of dry aging (Table 3). DA0 particularly had the highest amount of FFAs mainly by

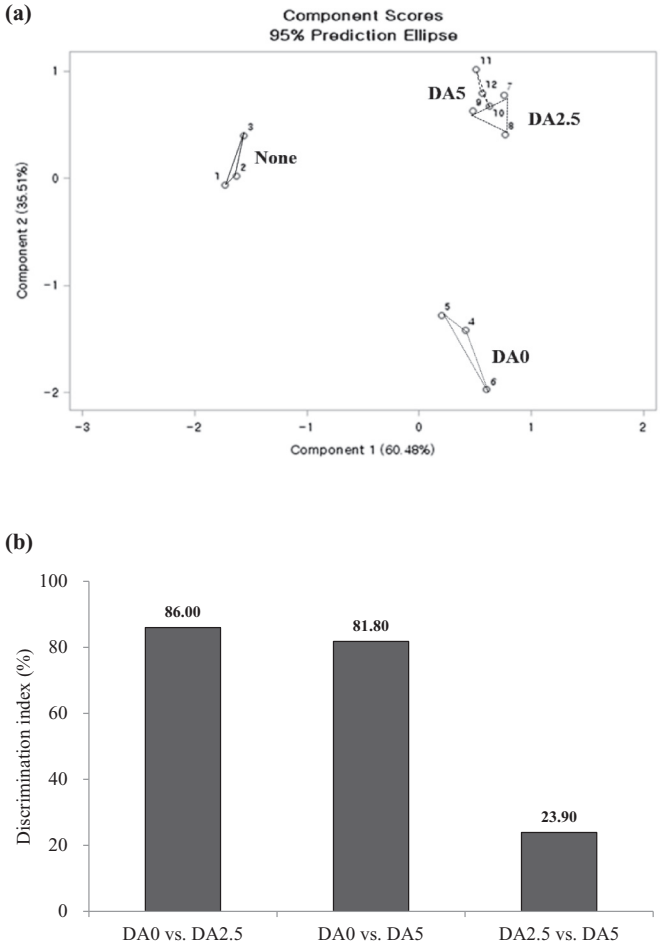

Fig. 1. The principal component analysis with four different flavor compounds (a) and the discrimination index of taste attributes (b) from dry-aged beef rump dry aged for 28 days with different air flow velocities (0, 2.5, and 5 m/s).
Non-aged beef (None) and dry-aged beef with air flow velocities of 0 (DA0), 2.5 (DA2.5), and 5 m/s (DA5).
Each circle in principal component analysis indicates None (1–3), DA0 (4–6), DA2.5 (7–9), and DA5 (10 − 12).

the change at the later stage of dry aging. The formation of FFAs is attributed to the hydrolysis of triglycerides and/or phospholipids and lipid oxidation by lipolytic enzymes in animal muscle and/or derived from microorganisms (Huang, Xiong, Kong, Huang, & Li, 2013). In this study, since the main change in FFAs content was observed after different compositions of *P. anomala* and *D. hansenii* were detected, the occurrence of *P. anomala* may have influenced lipolysis between days 14 and 28. Therefore, we assumed that *P. anomala* may have a higher lipolytic activity than *D. hansenii*, as shown in the agar model system (Kim et al., 2018). FFAs plays a significant role in the formation of aroma volatiles in meat and meat products (Mottram, 1998). It is highly susceptible to oxidation processes, as it is in the free form and affects the formation of aroma volatiles in meat and meat products (Huang et al., 2013). Consequently, the aroma volatiles in DA0 could be more abundant with a higher composition of *P. anomala*.

3.4. PCA for flavor compounds and taste attributes

PCA was applied for IMP, reducing sugar, total FAAs, and total FFAs (Fig. 1a). The results indicate that all dry-aged groups were significantly different from non-aged one. In addition, DA2.5 and DA5, which had similar mold and yeast composition, were shown to be similar, whereas DA0 was discriminable. Different taste attributes, including sourness, saltiness, umami, sweetness, and bitterness were analyzed using electronic tongue profile and their discrimination index was reported (Fig. 1b). Similar to the PCA data, the electronic tongue profile of DA0 with higher composition of *P. anomala* was discriminable from those of DA2.5 and DA5, which composed of approximately 15% of *D. hansenii*.

As the changes in microbial composition were relatively significant in mold and yeast (Table 1), we assumed *P. anomala* and *D. hansenii*, rather than bacteria, may have induced such changes in the sensory properties of dry-aged beef. In addition, the most significant changes in physicochemical traits and flavor compounds were occurred between days 14 and 28 (Tables 2 and 3), when the appearance of different mold/yeast features were observed (Table 1). Therefore, our results suggest that different microbial compositions on the crust could be attributed to the presence and/or extent of air flow, possibly resulting in different sensory properties of dry-aged beef.

4. Conclusion

We have shown that the presence and/or extent of air flow could vary microbial composition on the crust and resulted in the significant changes in physicochemical traits and flavor compounds of dry-aged beef. This is possibly due to the different proteolytic and lipolytic activities caused from the different compositions of microorganisms. Further investigation to determine the role of *P. anomala* and *D. hansenii* in dry-aged beef is now under way. We hypothesize that the presence of *P. anomala* and *D. hansenii* on the crust of dry-aged beef could vary proteolytic and lipolytic effects on meat flesh during dry aging.

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