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ARTICLE

Effects of Aging Methods and Periods on Quality Characteristics of Beef

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Abstract The objective of this study was to determine effects of aging methods (wet-aged, dry-aged, and packaged dry-aged) during 60 d on quality traits and microbial characteristics of beef. Wet-aged beef was packed by vacuum packaging and stored in a 4°C refrigerator. Dry-aged beef was used without packaging. Packaged dry-aged beef was packaged in commercial bags. Dry-aged and packaged dry-aged samples were stored in a meat ager at 2°C–4°C with 85%–90% relative humidity. Meat color, crust thickness, aging loss, cooking loss, Warner-Bratzler shear force (WBSF), texture profile analysis, Torrymeter, meat pH, water activity, volatile basic nitrogen (VBN), thiobarbituric acid reactant substances (TBARS), and microbial analysis were measured or performed every 15 d until 60 d of aging time. Meat color changed significantly with increasing aging time. Differences in meat color among aging methods were observed. Aging losses of dry-aged and packaged dry-aged samples were higher than those of wet-aged samples. Wet-aged beef showed higher cooking loss, but lower WBSF than dry-aged and packaged dry-aged beef. VBN and TBARS showed an increasing tendency with increasing aging time. Differences of VBN and TBARS among aging methods were found. Regarding microbial analysis, counts of yeasts and molds were different among aging methods at the initial aging time. Packaged dry-aged and dry-aged beef showed similar values or tendency. Significant changes occurred during aging in all aging methods. Packaged dry aging and dry aging could result in similar quality traits and microbial characteristics of beef.

Keywords dry aging methods, long-term aging, microbial safety, packaged dry-aged beef

Introduction

Meat is consumed worldwide due to its high contents of nutrients and high palatability to consumers. Meat with guaranteed tenderness and flavor can be sold at a premium price (Holman et al., 2019). Postmortem aging can improve the tenderness and enhance the flavor of meat, consequently improving meat palatability and enhancing

consumer preference (Kim et al., 2016). Meat aging process includes proteolysis, lipolysis, moisture loss, interaction with microbes, and so on (Khan et al., 2016; Kim et al., 2016; Koohmaraie, 1996; Lee et al., 2019a). There are two traditional methods for meat aging, wet (vacuum packed) aging and dry (unpacked) aging under controlled conditions [temperature, relative humidity (RH), and air velocity (Lee et al., 2018)]. Different conditions between wet aging and dry aging can result in different final quality. It has been shown that dry-aged beef has more intensified flavor such as beefier and roasted flavor than wet-aged beef (Kim et al., 2016; Lee et al., 2017). However, dry aging can harden the surface called crust due to moisture evaporation during the aging period (Lee et al., 2019a). The crust is a non-edible and loss part of dry-aged beef because a lot of microorganisms including bacteria, yeast, and mold can grow on crust (Lee et al., 2019b). Thus, more attention must be paid to dry-aging conditions to reduce the risk of meat spoilage. In addition, wet aging (7–21 d) generally takes less time than dry aging (14–35 d). Thus, most of aged beef are sold as wet-aged or vacuum-packaged (Dashdorj et al., 2016).

Commercial bags for dry aging have been developed recently (Dashdorj et al., 2016). These commercial bags are highly moisture permeable and oxygen permeable. Thus, beef packaged with these bags can be aged easier, resulting in beef quality similar to beef aged by the traditional dry aging method. Beef aged in commercial aging bag showed equal or greater tenderness and juiciness compared to the beef with traditional dry aging without showing significant difference in flavor (Ahnström et al., 2006; Dashdorj et al., 2016; DeGeer et al., 2009; Li et al., 2014). However, there are a few studies on technological quality property and microbiological safety of beef aged by various methods including wet aging, dry aging, and packaged dry aging using commercial bags during a long aging time. Therefore, the purpose of this study was to compare changes of quality traits and microbial characteristics of beef aged by different aging methods (wet aging, dry aging, and packaged dry aging) during 60 d.

Materials and Methods

Meat samples

Beef samples were purchased from a commercial livestock market in Jeju Island, Korea. The current research used sirloin beef originated from castrated and 2 quality grade Hanwoo (Korean native cattle). Based on animal product traceability number, a total of six sirloin were used and cut six pieces, respectively. Slaughter day of cattle was set as 0 d of meat aging.

Meat aging procedure

Three meat aging methods (wet aging, dry aging, and packaged dry aging) were used in the research. Dry aging bag (30 cm×60 cm, UAMi Dry, Minneapolis, MN, USA) was purchased commercially. Commercial dry aging bag was made of polyethylene with moisture permeability of 8,000 g/15 μ/m^2/24 h and oxygen permeability of 2.3 mL/m^2/d at 38°C with 50% RH. Vacuum packaging bag for wet aging was made of nylon with oxygen permeability of 0.03 g/m^2/24 h and carbon dioxide permeability of 0.1 g/m^2/24 h. Beef samples of wet aging (vacuum packaging), dry aging (without packaging) and packaged dry aging (with commercial packing bag) were stored in a meat ager (LMP-1045DA, Daeyoung E&B, Ansan, Korea) at 2°C–4°C with 85%–90% RH, 0.5–2.0 m/s air flow rate.

Meat color measurement

Meat color was measured using a Minolta Chroma meter (CR-400, Konica Minolta, Osaka, Japan). Samples were put on a table for 20 min without any packaging to allow contact with air for the bloom (outside color). After removing the dried parts

of the aged meat (crust), inside meat color was measured. Calibration was performed using a standard plate (Y=91.7, x=0.3138, y=0.3200) produced by the manufacturer. Each measurement was done with five replications. The color of each sample was expressed as *Commission Internationale de l'Eclairage* (CIE) standard for CIE L*, CIE a*, and CIE b*.

Crust thickness measurement

Crust thickness was determined for the dried part of the aged meat after blooming without color change in the present research. Crust thickness was measured for dry aged and packaged dry aged beef samples.

Aging loss measurement

Aging loss was defined as weight loss during aging time. Aging losses after 15, 30, 45, and 60 d aging were calculated with the following Eq. (1):

$$\text{Aging loss (\%)} = \frac{\text{Initial weight (g)} - \text{Weight after aging (g)}}{\text{Initial weight (g)}} \times 100 \quad (1)$$

Cooking loss measurement

For cooking loss measurement, aged samples were cut (2 cm×4 cm×6 cm), weighed, and placed in a polyethylene bag without air. These samples were then cooked in a continuous boiling water bath (KMC-1205W1, Vision, Mukilteo, WA, USA) at 85℃ until the internal temperature reached 75℃. Cooked samples were taken out, cooled in an ice water for 20 min, and placed in a chilled condition (1℃–5℃) for equilibration. After cooling, polyethylene bags were removed. Samples were dried gently and weighed again. Cooking loss was calculated with the following Eq. (2):

$$\text{Cooking loss (\%)} = \frac{\text{Initial weight (g)} - \text{Weight after cook (g)}}{\text{Initial weight (g)}} \times 100 \quad (2)$$

Texture analysis

Warner-Bratzler shear force (WBSF) and texture profile analysis (TPA) were performed to measure texture properties of aged beef. Samples for texture properties were prepared with the same procedure used for cooking loss. Cooked samples were cut to 1 cm sticks along the muscle fiber direction for WBSF measurement. Other cooked samples were cut to cubes (1.5 cm×1.5 cm×1.5 cm) with muscle fiber axis for TPA measurement. A texture analyzer (Texture Analyzer CT3, AMETEK Brookfield, Middleborough, MA, USA) was used to measure both WBSF and TPA. Samples of WBSF were sheared perpendicular to the muscle fiber axis with a texture analyzer using a Warner-Bratzler shearing device. The peak force of the curve during shearing of the sample was WBSF. Muscle fiber axis of each cube sample was placed perpendicular to the direction of a cylindrical probe (3 mm diameter). The probe was moved at a constant speed of 2.0 mm/s (pre-test), 1.0 mm/s (test), or 4.5 mm/s (post-test). It compressed a predetermined percentage (75%) of sample thickness, came back to the initial point of contact with each sample, then stopped for a set time period (2 s) before beginning a second compression cycle. The force value was recorded every 0.01 s and plotted on a force-time plot. Hardness, adhesiveness, chumminess, and chewiness were calculated from the force-time plot of at least six cubes for each sample.

Volatile basic nitrogen (VBN)

VBN was measured based on the Korea Food Standards Codex of Ministry of Food and Drug Safety (MFDS, 2017). Ground beef samples (10 g each) were taken, added 90 mL of distilled water, and homogenized (D1000-E, Benchmark, Lodi, NJ, USA). Homogenates were centrifuged (Combi-514R, Hanil Science, Gimpo, Korea) at 1,360×g for 10 min at 4°C. The supernatant was filtered with a Whatman No.1 filter paper. One milliliter of filtrate was reacted with 1 mL of saturated K_2CO_3 (50 g/100 g) at 37°C for 2 h. After incubation, the reactant was titrated with 0.02 N H_2SO_4. VBN values of aged beef were expressed in mg%.

$$\text{VBN (mg\%)} = 0.28014 \times f \times (b - a) \times d \times 100/W,$$

where $0.28014 =$ VBN amount (mg) to equilibrium 1 mL of 0.02 N H_2SO_4.

a: titration (mL) of blank
b: titration (mL) of samples (mean of duplication)
W: weight of sample
d: dilution factor
f: titer of 0.02 N H_2SO_4

Meat pH measurement

Meat pH was measured directly using a portable pH meter (testo 206-pH2, Testo, Titisee-Neustadt, Germany) designed for meat. The pH meter had a built-in temperature compensation system.

Water activity (a_w) measurement

Aged beef samples were cut into cubes (1 cm^3) and analyzed in triplicate using a water activity analyzer (WA-160A, Amittari, Guangdong, China).

Thiobarbituric acid reactive substance (TBARS)

TBARS were evaluated using the method of Inserra et al. (2014). Briefly, minced aged beef (2 g) was mixed 18 mL of perchloric acid. The mixture was added to 0.2 mL of butylated hydroxyanisol dissolved in 98% of ethanol at 3.75 mg/mL, homogenized (D1000-E, Benchmark) at 2,775×g for 1 min, and centrifuged (Combi-514R, Hanil Science, Korea) at 1,360×g for 10 min at 4°C. The supernatant was filtered with Whatman No. 1 filter paper. The filtrate (2 mL) was mixed with 2 mL of 20 mM TBA and incubated at room temperature in a dark room for 16–17 h. The mixture was measured at 531 nm using a spectrophotometer (Optizen™ POP, KLAB, Daejeon, Korea). Values of TBARS are expressed as mg of malonaldehyde (MDA)/kg of sample.

Freshness measurement using Torrymeter

Torrymeter (Fish Freshness Meter, Distell, East Kilbride, Scotland) was used to measure the freshness of aged beef. Outside and inside of aged beef were measured triplicated using Torrymeter in this study.

Microbial analysis

Each aged beef sample (25 g) was blended and homogenized with 225 mL of sterile saline (0.85% NaCl) using a

stomacher (LS-700A, BNF Korea, Gimpo, Korea) for 2 min. Serial dilutions were performed using sterile saline. Each diluent (1 mL) was spread onto the commercial microorganism count plates in triplicate. Following plates were used in this study; AC plates (3M™ Petrifilm™, 3M, St. Paul, MN, USA) for aerobic bacteria, LAB plates (3M™ Petrifilm™, 3M) for lactic acid bacteria, EC plates (3M™ Petrifilm™, 3M) for *Escherichia coli*, YM plates (3M™ Petrifilm™, 3M) for yeast and molds, and Pseudomonas Isolation Agar (Sigma-Aldrich, St. Louis, MO, USA) for *Pseudomonas*. AC and EC plates were incubated at 36℃ for 48 h. LAB and YM plates were incubated at 25℃ for 72 h. Pseudomonas Isolation Agar were incubated at 35℃ for 24–48 h. Microbial counts are expressed as Log CFU/g.

Statistical analysis

Statistical analysis was performed via general linear model procedure available in SAS software v. 9.4 (SAS Institute, Cary, NC, USA). Statistical models included effects of aging methods and aging days as fixed effects. Results are expressed as means and SDs. Significant differences in means were detected at $p<0.05$ by Duncan's multiple comparison.

Results and Discussion

Meat color

Appearance, texture, and flavor are three properties by which consumers judge meat quality. Appearance is an important factor that can influence consumer's decision to purchase meat. Consumers consider discolored meat as a lack of freshness with low value and price (Lee and Shin, 2019). The present study measured meat color for both the outside and inside of beef samples (Table 1). Differences in color and appearance between outside and inside of beef aged by dry aging and packaged dry aging could be observed easily by eyes. However, it was difficult to detect such differences between the outside and inside of wet aging beef. All color parameters (CIE L*, CIE a*, and CIE b*) on the outside of beef aged by dry aging and packaged dry aging were significantly ($p<0.001$) decreased when the aging time prolonged. CIE L* and CIE a* of the outside of wet-aged beef were significantly ($p<0.001$) increased with increasing aging time. Outside color indices (CIE L*, CIE a*, and CIE b*) of beef aged by dry aging with/without packaging showed significantly ($p<0.001$) lower values after 30 d of aging compared with those of beef aged by wet aging. Dried surface of dry aged beef might have contributed to this result due to moisture evaporation, myoglobin oxidation, and so on.

Only the CIE L* of the inside of dry-aged beef significantly ($p<0.001$) decreased. The inside CIE L* of packaged dry-aged beef was significantly decreased only after 45 d of aging. The inside CIE L* for wet-aged beef did not show any statistically significant change during aging. Inside CIE L* of beef aged by dry aging was significantly ($p<0.001$) higher than that of beef aged by wet aging after 45 d and 60 d of aging. Similarly, the inside CIE a* of wet aged beef did not show significant changes with increasing aging time. On the other hand, inside CIE a* of dry-aged beef and packaged dry-aged beef were decreased significantly ($p<0.001$) with increasing aging time. Dry-aged beef with/without packaging showed significantly lower inside CIE a* after 30 d of aging compared with wet-aged beef. Inside CIE b* of wet-aged beef did not change significantly according to aging time. However, significant ($p<0.001$) differences of inside CIE b* were observed for dry-aged beef and packaged dry-aged beef between before and after 45 d aging. Significantly ($p<0.001$) different inside CIE b* between wet-aged and dry-aged beef with/without packaging after 30 d, 45 d, and 60 d of aging were found. These results indicate that wet-aged beef and dry-aged beef show different color values during aging. In addition, color values of dry aged beef show similar changes regardless of packaging.

Table 1. Changes in meat color attribute as a function of aging method and time

	Aging time (d)	Outside				Inside			
		Wet aging	Dry aging	Packaged dry aging	Significance	Wet aging	Dry aging	Packaged dry aging	Significance
CIE L*	0	35.26^{C}±2.73	35.85^{A}±3.46	35.93^{A}±3.12	NS	37.13±2.45	36.01^{A}±2.77	36.06^{A}±2.66	NS
	15	37.90^{Ba}±3.70	26.00^{Bb}±1.93	30.43^{Bc}±3.71	***	38.97±4.53	36.59^{A}±2.19	37.98^{A}±2.95	NS
	30	37.87^{Ba}±3.08	26.82^{Bb}±2.28	25.77^{Cb}±2.88	***	38.53±3.26	36.90^{A}±6.56	36.91^{A}±2.68	NS
	45	37.94^{Ba}±1.53	25.99^{Bb}±4.06	23.49^{Dc}±3.48	***	38.94^{a}±4.49	34.75^{Ab}±3.04	33.56^{Bb}±2.04	***
	60	41.90^{Aa}±4.97	26.53^{Bb}±3.80	22.40^{Dc}±2.92	***	37.01^{a}±3.44	32.21^{Bb}±2.53	36.50^{Aa}±4.31	***
	Significance	***	***	***		NS	***	***	
CIE a*	0	18.80^{Cab}±1.74	19.48^{Aa}±2.17	17.53^{Ab}±2.35	*	22.86^{a}±2.27	20.54^{Bb}±2.72	18.84^{Bc}±2.47	***
	15	19.97^{a}±1.82	13.92^{Bb}±2.86	15.68^{Ab}±4.72	***	23.40^{a}±1.30	22.71^{Aa}±2.27	21.35^{Ab}±1.96	**
	30	19.74^{BCa}±1.53	7.27^{Cb}±3.09	8.18^{Bb}±2.25	***	23.91^{a}±2.29	20.74^{Bb}±2.27	20.90^{Ab}±2.34	***
	45	20.55^{Ba}±1.47	5.63^{CDc}±2.67	8.15^{Bb}±2.47	***	23.41^{a}±1.68	18.97^{Cb}±1.18	17.83^{Bc}±1.56	***
	60	21.61^{Aa}±1.50	5.48^{Db}±2.61	4.86^{Cb}±2.41	***	23.03^{a}±2.02	16.11^{Db}±2.60	16.43^{Cb}±2.70	***
	Significance	***	***	***		NS	***	***	
CIE b*	0	7.08±1.50	7.32^{A}±2.19	6.26^{B}±1.54	NS	11.19^{a}±1.51	9.99^{Bb}±1.74	9.38^{Bb}±1.09	***
	15	7.73^{a}±1.36	5.67^{Bb}±1.77	7.71^{Aa}±2.56	**	13.41^{a}±5.94	11.18^{Aab}±1.31	10.25^{Ab}±1.26	*
	30	7.06^{a}±0.99	3.39^{Cb}±1.46	3.52^{Cb}±1.73	***	11.99^{a}±1.57	10.20^{Bb}±1.46	10.06^{ABb}±1.58	***
	45	7.19^{a}±0.94	3.52^{Cb}±1.74	3.34^{CDb}±1.33	***	11.91^{a}±1.36	8.89^{Cb}±1.10	7.99^{Cc}±0.81	***
	60	7.71^{a}±1.79	3.00^{Cb}±1.43	2.29^{Db}±1.05	***	11.73^{a}±1.68	7.38^{Db}±1.42	7.09^{Cb}±1.11	***
	Significance	NS	***	***		NS	***	***	

Results are presented means±SD.
$^{A–D}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
^{a,b} Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
NS, not significant.

Crust thickness and aging loss

Dry aging of beef generally results in aging loss by 30%–40%. It also causes a crust on the surface because of moisture evaporation during the aging period (Dashdorj et al., 2016). Higher aging loss and crust thickness would result in higher economical loss. Aging loss was increased gradually ($p<0.01$) with increasing aging time for all aging methods (Table 2). In addition, significantly ($p<0.001$) higher aging loss was observed for all dry aging methods during the entire aging time regardless of packaging compared with wet aging. After 15 d of aging, dry-aged beef lost ($p<0.001$) more moisture than packaged dry-aged beef. These results were consistent with results of a previous research (Lepper-Blilie et al., 2016) showing increased weight loss of aged beef with increasing aging period regardless of aging method (dry vs. wet) or beef type (bone-in vs. boneless). Dry-aged beef had a thicker ($p<0.05$) crust than packaged dry-aged beef after 15 d to 45 d of aging. However, there was no significant difference in crust thickness between dry-aged beef and packaged dry-aged beef after 60 d of aging. Moisture evaporation might be a reason for the difference in aging loss between wet and dry aging including packaged dry aging. Wet-aged beef is generally vacuum packaged with moisture impermeable materials. On the other hand, moisture of dry-aged beef and dry aging using commercial bag can evaporate (Dashdorj et al., 2016). Thus, aging loss could

Table 2. Crust thickness and aging loss during aging period in different aging methods and aging time

Aging time (d)	Crust thickness (mm)				Aging loss (%)			
	Wet aging	Dry aging	Packaged dry aging	Significance	Wet aging	Dry aging	Packaged dry aging	Significance
15	-	6.75^{ABa}±1.28	3.75^{Bb}±0.89	***	1.98^{Bb}±0.44	16.95^{Da}±1.85	15.06^{Da}±1.15	***
30	-	6.13^{Ba}±0.83	4.00^{Bb}±1.85	*	2.33^{Bc}±0.63	29.49^{Ca}±3.03	24.57^{Cb}±0.71	***
45	-	6.00^{Ba}±0.76	3.88^{Bb}±1.55	**	3.35^{Ac}±0.55	35.55^{Ba}±1.79	31.79^{Bb}±1.23	***
60	-	7.75^{A}±1.04	6.38^{A}±2.33	NS	3.39^{Ac}±0.62	43.66^{Aa}±2.59	39.82^{Ab}±0.25	***
Significance	-	**	*		**	***	***	

Results are presented means±SD.
$^{A–D}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
$^{a–c}$ Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
NS, not significant.

increase during a prolonged aging period.

Cooking loss, Warner-Bratzler shear force (WBSF), and texture profile analysis (TPA)

Cooking loss, one of water holding capacity measurements, is closely associated with texture property and eating quality (Lee et al., 2012) because moisture loss during cooking can lead to loss of flavor compounds dissolved in water and loss of assistant role of moisture during chewing. On 0 d of aging, there was no difference in cooking loss among beef aged with different aging methods (Table 3). However, after 15 d of aging wet-aged beef showed significantly ($p<0.05$) higher cooking loss than dry-aged and packaged dry-aged beef. Cooking loss was not significantly changed during wet aging. On the other hand, cooking losses of dry aged and packaged dry aged beef were significantly ($p<0.01$) decreased from 15 d to 60 d of aging.

Meat tenderness is an important factor affecting the eating quality (Becker, 2000) and the willingness to purchase tender meat repeatedly. Meat aging is a process that can make meat become more tender, juicy, and full of flavor owing to proteolysis, lipolysis, moisture loss, interaction with microbes, and so on (Khan et al., 2016; Kim et al., 2016; Koohmaraie, 1996; Lee et al., 2019a). WBSF and TPA are methods for measuring meat tenderness (Choe et al., 2016). The present study measured the tenderness and texture property of aged beef using WBSF and TPA. Beef samples after 15 d aging with all aging methods were more tender ($p<0.05$) than beef on 0 d except for dry-aged beef. However, there was no significant difference in hardness values after 15 d. Wet-aged beef had significantly ($p<0.05$) lower value of WBSF than beef aged with other methods. However, there was no difference in WBSF among beef with different aging methods after 45 d of aging. Packaged dry-aged beef showed significantly higher values of WBSF after 0 d and 15 of aging than dry-aged beef. Although packaged dry-aged beef was not as tender as dry-aged beef during the initial aging time, its tenderness showed an improvement similar to dry-aged beef after 30 d of aging. This result implies that aging can improve tenderness of meat regardless of the aging method, in is agreement with results of other studies (Smith et al., 2008) showing the important factors that could improve tenderness are aging period and raw material quality grade, not aging method.

Meat texture is determined by many factors such as breed, sex, meat structure, composition, water holding capacity, rigor state, and so on. TPA is a convenient and widely used method to evaluate food texture or rheology rapidly, although food texture can be measured only by humans (Nishinari et al., 2013). The current research examined texture properties using TPA. No difference in cohesiveness or springiness was detected among aging methods or aging time with each aging method

Table 3. Cooking loss, Warner-Bratzler shear force (WBSF), and texture profile analysis in different aging methods and aging time

	Aging time (d)	Aging method			Significance
		Wet aging	Dry aging	Packaged dry aging	
Cooking loss (%)	0	20.12±3.33	18.20^{A}±5.31	18.44^{A}±0.10	NS
	15	20.23^{a}±1.43	11.32^{ABb}±1.12	11.96^{Bb}±2.41	*
	30	23.72^{a}±0.18	5.07^{BCb}±0.81	6.52^{Cb}±1.46	***
	45	21.54^{a}±0.15	4.96^{BCb}±2.41	4.40^{CDb}±0.62	**
	60	20.90^{a}±3.45	1.54^{Cb}±0.34	2.12^{Db}±0.24	**
	Significance	NS	**	***	
WBSF (kg)	0	6.36^{Ab}±1.41	6.10^{Ab}±2.64	8.32^{Aa}±2.75	***
	15	4.01^{Bb}±1.09	4.06^{Cb}±0.94	4.70^{Ba}±1.40	*
	30	3.50^{Cb}±0.94	4.66^{BCa}±1.21	4.95^{Ba}±1.58	***
	45	3.36^{C}±1.06	3.84^{C}±1.50	4.04^{B}±1.40	NS
	60	3.10^{Cb}±0.98	5.51^{ABa}±1.99	4.87^{Ba}±1.35	***
	Significance	***	***	***	
Hardness (kg)	0	2.98^{Aa}±0.89	2.34^{ABb}±0.71	2.69^{Aab}±1.10	*
	15	2.17^{BCb}±0.63	2.60^{Aa}±0.72	2.40^{ABab}±0.68	*
	30	2.32^{BC}±0.52	2.31^{AB}±0.5	2.31^{AB}±0.42	NS
	45	2.47^{B}±0.55	2.12^{BC}±0.84	2.18^{B}±0.39	NS
	60	2.04^{C}±0.44	1.88^{C}±0.51	2.05^{B}±0.41	NS
	Significance	***	**	**	
Adhesiveness	0	1.60^{A}±1.31	1.21^{A}±1.01	1.52^{A}±1.40	NS
	15	0.95^{B}±0.67	1.24^{A}±1.01	1.17^{AB}±1.01	NS
	30	0.86^{BC}±0.59	0.77^{B}±0.38	0.98^{B}±0.55	NS
	45	0.85^{BCa}±0.57	0.42^{Bb}±0.31	0.52^{Bb}±0.22	***
	60	0.52^{C}±0.43	0.35^{B}±0.24	0.36^{B}±0.24	NS
	Significance	***	***	***	
Gumminess	0	1.24^{A}±0.50	1.05^{A}±0.38	1.13^{A}±0.43	NS
	15	1.01^{B}±0.44	1.06^{A}±0.46	1.13^{A}±0.39	NS
	30	0.87^{BC}±0.34	0.93^{A}±0.28	0.92^{A}±0.28	NS
	45	0.94^{BCa}±0.30	0.72^{Bb}±0.33	1.04^{Aa}±0.50	**
	60	0.76^{C}±0.23	0.67^{B}±0.47	0.71^{B}±0.25	NS
	Significance	***	***	***	
Chewiness	0	92.68^{Aa}±58.37	62.69^{b}±33.28	77.07^{ABab}±37.61	*
	15	74.15^{AB}±47.24	77.05±53.20	84.62^{A}±44.00	NS
	30	64.37^{B}±37.19	68.18±32.26	65.42^{AB}±33.30	NS
	45	65.32^{Bab}±29.24	51.87^{b}±29.47	85.61^{Aa}±63.29	*
	60	52.63^{B}±22.40	56.29±53.28	54.83^{B}±25.73	NS
	Significance	**	NS	*	

Results are presented means±SD.
$^{A–D}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
^{a,b} Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
NS, not significant.

(data not shown). Hardness of aged beef was significantly ($p<0.01$) decreased after 60 d aging with all aging methods compared with that on 0 d. Difference in hardness among aging methods was not clearly observed except during the early aging period. Adhesiveness and gumminess of aged beef showed similar tendencies to hardness. They were significantly ($p<0.001$) decreased after 60 d of aging with all aging methods. They showed no clear difference among aging methods. However, differences of chewiness according to aging time were observed for the wet aging group ($p<0.01$) and the packaged dry aging group ($p<0.05$), but not for the dry aging group. Changes of hardness during aging period were acceptable considering results of a previous study (Choe et al., 2016), indicating that WBSF and hardness of TPA could be used as indicators of meat tenderness. In addition, taking parameters measured in this study together, texture properties of aged beef showed changes regardless of aging method.

Meat pH and water activity (a_w)

Meat pH is one of the most important quality factors closely associated with water holding capacity, tenderness, and microbial growth (Lee and Yoon, 2015; Lee et al., 2012). It is affected by a process of converting muscles to meat through muscle protein proteolysis by enzymes secreted from microbes (Obuz et al., 2014; Terjung et al., 2021). Water activity (a_w) is defined as available moisture in food stuff. It affects chemical reactions and microbial growth. Both meat pH and a_w are important indicators of technological quality, eating quality, and microbial growth.

Meat pH was significantly ($p<0.001$) increased with prolonged aging time regardless of the aging method (Table 4). However, regardless of aging method or aging time, all meat pH values were lower than 6.2, the cutoff value for freshness of fresh meat and packaged meat established by Korean Ministry of Food and Drug Safety. Wet-aged beef showed significantly ($p<0.05$) lower pH than dry-aged and packaged dry-aged beef at all aging time except for 15 d of aging. Regarding a_w, it showed no statistically significant difference according to aging method or aging time except for after 45 d of aging. Beef aged by wet aging showed significantly ($p<0.05$) different a_w than beef aged by dry aging. Previous research has observed that round bottom of Hanwoo shows significantly different a_w only after 90 d aging, without showing statistically significant difference after 60 d of aging under the same dry aging conditions (Cho et al., 2018).

Table 4. Meat pH and water activity in different aging methods and aging time

Aging time (d)	Meat pH				Water activity (a_w)			
	Wet aging	Dry aging	Packaged dry aging	Significance	Wet aging	Dry aging	Packaged dry aging	Significance
0	$5.64^{Cb}\pm0.07$	$5.70^{Ca}\pm0.05$	$5.71^{Da}\pm0.08$	*	0.95±0.02	0.95±0.01	0.95±0.01	NS
15	$5.77^{B}\pm0.10$	$5.81^{B}\pm0.07$	$5.80^{C}\pm0.13$	NS	0.95±0.02	0.94±0.01	0.95±0.01	NS
30	$5.75^{Bb}\pm0.03$	$5.82^{Ba}\pm0.07$	$5.85^{BCa}\pm0.08$	***	0.95±0.02	0.94±0.01	0.95±0.01	NS
45	$5.75^{Bc}\pm0.06$	$5.96^{Aa}\pm0.07$	$5.91^{ABb}\pm0.05$	***	$0.95^{a}\pm0.01$	$0.93^{b}\pm0.01$	$0.94^{ab}\pm0.01$	*
60	$5.85^{Ab}\pm0.11$	$6.02^{Aa}\pm0.12$	$5.96^{Aa}\pm0.06$	***	0.94±0.01	0.94±0.01	0.93±0.02	NS
Significance	***	***	***		NS	NS	NS	

Results are presented means±SD.
$^{A–D}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
^{a,b} Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, *** $p<0.001$.
NS, not significant.

Volatile basic nitrogen (VBN), thiobarbituric acid reactive substance (TBARS), and Torrymeter value

VBN and TBARS are frequently used as spoilage indicators of fresh meat. VBN is an indicate for meat protein spoilage from production of amines/ammonia by microbes. TBARS is an indicator for lipid oxidation from production of malondialdehyde (MDA; Lee et al., 2018). Lipid peroxidation in meat and meat products can produce MDA, one of the main products from rancidity and reaction with TBA. Secondary product resulting from reaction of MDA with TBA can be detected by color change, a relevant method in many studies to examine rancidity (Grotta et al., 2017). Torrymeter has been used originally in fish to examine fish freshness. It has been recently used to determine the degree of chicken freshness (Bae et al., 2014). Principle of Torrymeter is to measure modification of intra- and extra-cellular electrolytes caused by muscle spoilage due to microbes' activities. A higher value of Torrymeter means a higher degree of freshness (Bae et al., 2014). During aging period, the outside of dry-aged beef and packaged dry-aged beef are exposed to various circumstances. Thus, we measured VBN, TBARS, and Torrymeter for both the outside and inside of aged beef to examine the degree of freshness.

Results showed that VBN, TBARS, and Torrymeter values for the outside and inside of aged beef were not different (statistical analysis was not performed; Table 5). All values of VBN and TBARS with all aging methods showed an increased tendency with increasing aging time, whereas Torrymeter values were decreased with prolonged aging time. Differences of VBN among aging methods were detected for both the outside and inside of beef. Dry-aged beef showed the highest ($p<0.001$) values of VBN and TBARS regardless of the outside or the inside after 30 d of aging. Packaged dry-aged beef had similar or higher values of VBN and TBARS for both outside and inside of beef compared with wet-aged beef. These results might be because in the absence of packaging, dry-aged beef was exposed to air directly, providing a favorable environment to grow microorganisms known to cause beef spoilage.

In Korea, less than 20 mg% of VBN is a reference value for meat spoilage. The current study showed that VBN values of dry-aged beef and packaged dry-aged beef after 30 d of aging were higher than 20 mg%. However, one study has stated that the present recommendation for meat spoilage (20 mg% of VBN) should be re-considered and that below 89.31% is an acceptable value for dry-aged beef based on data of 7 d aged beef (Lee et al., 2018). It is known that TBARS value of more than 2 mg MDA/kg meat can negatively affect eating quality due to off-flavor and taste of lipids oxidation (Campo et al., 2006; Grotta et al., 2017). The current study showed that the inside of dry-aged beef after 45 d and 60 d of aging, the outside of dry-aged beef after 30 d of aging, and the outside of packaged dry-aged beef after 60 d of aging had TBARS values higher than 2 mg MDA/g. One study has reported that there is no significant change of TBARS during beef dry aging for 7 d (Lee et al., 2018). However, it also reported that TBARS might not be an appropriate indicator of dry-aged beef deterioration because TBARS value has no correlation with other quality parameters (Lee et al., 2018).

Torrymeter values of the outside and inside of aged beef showed a decreased tendency regardless of the aging method. They were significantly different between beef on 0 d of aging and beef on 60 d of aging for both the outside and inside of all aged beef. However, such decreasing pattern was not gradual.

Microbial analysis

Although a lot of factors (temperature, oxygen, enzymes, moisture, light, etc.) are inter-related with meat spoilage, the type and number of microorganisms are considered as the most important factors (Lee et al., 2018). The current research performed microbial analysis for both the outside and inside of aged beef. The outside of aged beef showed higher microbial counts than the inside of aged beef (statistical analysis was not performed) regardless of aging method (Table 6). Only wet-aged beef showed significant increased AC during aging. No difference in AC during aging time was observed among aging

Table 5. Volatile basic nitrogen (VBN), thiobarbituric acid reactant substances (TBARS), and Torry meter values in different aging methods and aging time

	Aging time (d)	Outside				Inside			
		Wet aging	Dry aging	Packaged dry aging	Significance	Wet aging	Dry aging	Packaged dry aging	Significance
VBN (mg%)	0	8.43^{Db}±0.69	9.76^{Ea}±0.41	9.22^{Eab}±0.84	*	8.43^{Db}±0.69	9.76^{Ea}±0.41	9.22^{Eab}±0.84	*
	15	9.48^{CDc}±0.95	13.28^{Da}±1.40	11.44^{Db}±0.62	***	9.48^{CDc}±0.95	13.63^{Da}±2.25	11.30^{Db}±0.65	***
	30	11.25^{Cc}±0.26	21.90^{Ca}±2.03	14.89^{Cb}±0.82	***	11.25^{Cc}±0.26	19.73^{Ca}±3.22	14.31^{Cb}±0.21	***
	45	14.40^{Bc}±3.13	26.99^{Ba}±1.88	20.22^{Bb}±2.09	***	14.40^{Bc}±3.13	24.68^{Ba}±3.10	18.33^{Bb}±0.37	***
	60	17.67^{Ac}±1.63	32.19^{Aa}±2.86	24.77^{Ab}±1.73	***	17.67^{Ac}±1.63	32.08^{Aa}±3.33	24.72^{Ab}±1.71	***
	Significance	***	***	***		***	***	***	
TBARS (mg MDA/kg)	0	1.15^{B}±0.06	1.20^{D}±0.05	1.19^{B}±0.08	NS	1.15^{B}±0.06	1.20^{D}±0.05	1.19^{B}±0.08	NS
	15	1.21^{Ab}±0.06	1.57^{Da}±0.40	1.20^{Bb}±0.08	***	1.21^{Ab}±0.06	1.33^{Da}±0.08	1.20^{Bb}±0.04	***
	30	1.18^{ABc}±0.05	2.50^{Ca}±0.36	1.42^{Bb}±0.14	***	1.18^{ABb}±0.05	1.55^{Ca}±0.31	1.22^{Bb}±0.08	***
	45	1.19^{Ab}±0.05	5.38^{Ba}±1.46	1.41^{Bb}±0.15	***	1.19^{Ab}±0.05	2.10^{Ba}±0.34	1.13^{Cb}±0.09	***
	60	1.20^{Ac}±0.06	3.92^{Aa}±0.74	2.08^{Ab}±0.85	***	1.20^{Ac}±0.06	2.25^{Aa}±0.12	1.29^{Ab}±0.11	***
	Significance	*	***	***		*	***	***	
Torrymeter	0	15.03^{A}±2.80	13.88^{A}±3.29	13.47^{A}±2.77	NS	15.03^{A}±2.80	13.88^{A}±3.29	13.47^{A}±2.77	NS
	15	11.29^{B}±5.07	11.35^{B}±4.69	12.60^{A}±4.37	NS	12.11^{B}±5.16	10.17^{B}±5.73	9.24^{B}±4.64	NS
	30	6.04^{Db}±1.90	8.53^{Ca}±3.24	7.92^{Ba}±6.12	**	5.57^{Cb}±0.78	5.74^{Cb}±0.95	6.93^{Ca}±2.55	**
	45	8.14^{C}±4.39	10.86^{B}±3.73	8.70^{B}±4.35	NS	6.53^{C}±3.90	7.18^{C}±1.60	5.68^{C}±2.74	NS
	60	5.64^{Db}±1.39	7.21^{Cab}±4.31	8.59^{Ba}±3.67	*	5.97^{Cab}±1.99	7.52^{Ca}±4.00	5.62^{Cb}±1.50	*
	Significance	***	***	***		***	***	***	

Results are presented means±SD.
$^{A–E}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
$^{a–c}$ Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
MDA, malondialdehyde; NS, not significant.

methods. Inside AC of dry-aged beef was changed without showing a pattern. The inside of wet-aged beef and packaged dry-aged beef showed similar AC counts. The inside of dry-aged beef had significantly ($p<0.05$) lower AC counts than the inside of wet-aged and packaged dry-aged beef after 45 d and 60 d of aging.

Both the outside and inside of aged beef showed no differences in EC count regardless of aging method or aging time. EC was not detected for the inside of aged beef after more than 30 d of aging regardless of aging method. However, one study has reported that EC is not detected from dry aged beef from the beginning of aging (Cho et al., 2018).

Microorganisms such as bacteria, yeasts, and molds can grow both on surface and inside of beef during long-term aging. They can metabolize ingredients of beef and produce various metabolites, resulting in unique flavors of beef (Kim et al., 2021). On the other hand, they can also cause lipid peroxidation and off-flavor (Kim et al., 2021). Numerous microbes can colonize on the surface of dry aged beef (crust). The microbial community on the crust can change continuously (Ryu et al., 2020). Lactic acid bacteria can produce antimicrobial agent to inhibit the growth of other species, causing greening due to H_2O_2 generation (Kim et al., 2021). *Pseudomonas* spp. is one of the main spoilage bacteria, resulting in slime formation and off-flavor generation by metabolizing glucose, lactate, and amino acids (Kim et al., 2021). Lactobacilli dominate under

Table 6. **Microbial analysis in different aging methods and aging time**

	Aging time (d)	Outside				Inside			
		Wet aging	Dry aging	Packaged dry aging	Significance	Wet aging	Dry aging	Packaged dry aging	Significance
AC	0	3.87^{C}±0.49	2.99±2.81	3.92±1.05	NS	2.76^{b}±0.03	1.94^{Bc}±0.04	2.96^{a}±0.02	***
	15	5.67^{B}±0.56	5.37±1.26	4.80±1.86	NS	2.51±3.55	3.11^{A}±0.89	2.02±2.86	NS
	30	6.87^{AB}±0.33	5.45±0.70	5.59±1.57	NS	4.83±2.04	ND	4.74±2.88	.
	45	7.08^{AB}±0.63	5.83±1.23	5.18±2.01	NS	5.50^{a}±0.64	3.59^{Ab}±1.06	5.70^{a}±1.47	*
	60	7.25^{A}±0.69	5.90±0.72	5.95±0.41	NS	5.98^{a}±0.77	0.24^{Bc}±0.34	3.57^{b}±0.86	**
	Significance	**	NS	NS		NS	*	NS	
Escherichia coli	0	2.90±0.43	0.95±1.34	2.18±1.55	NS	1.46±0.96	0.39±0.55	0.99±1.40	NS
	15	3.00±2.15	ND	ND	NS	1.54±2.17	ND	ND	NS
	30	ND	ND	1.26±1.78	NS	ND	ND	ND	.
	45	0.98±1.39	3.98±1.14	ND	NS	ND	ND	ND	.
	60	2.11±2.98	2.24±3.16	0.48±0.68	NS	ND	ND	ND	.
	Significance	NS	NS	NS		NS	NS	NS	
LAB	15	5.60^{B}±0.27	4.74±1.08	3.47±2.38	NS	3.02±2.24	2.41±0.00	1.77±2.50	NS
	30	7.12^{A}±0.41	5.92±1.88	5.22±1.32	NS	4.37±1.27	2.54±0.65	2.71±1.87	NS
	45	7.14^{A}±0.05	6.44±0.27	4.71±2.47	NS	4.64±0.37	1.76±1.13	3.14±3.50	NS
	60	6.83^{A}±0.13	5.77±0.30	4.83±1.83	NS	5.67±1.10	1.72±2.43	4.17±0.75	NS
	Significance	*	NS	NS		NS	NS	NS	
Yeast	15	1.99^{b}±0.29	6.02^{a}±0.64	5.25^{a}±0.39	**	0.00^{b}±0.00	4.10^{a}±0.55	3.64^{a}±0.52	**
	30	2.67^{b}±1.01	7.09^{a}±0.04	6.43^{a}±0.36	*	0.00^{a}±0.00	5.13^{b}±0.61	4.71^{c}±0.49	***
	45	1.79±2.53	5.98±0.03	5.52±0.80	NS	1.80±1.13	3.48±1.14	2.96±1.41	NS
	60	1.82±2.57	6.90±0.38	6.45±0.06	NS	0.00^{b}±0.00	4.88^{a}±0.84	3.62^{a}±0.13	**
	Significance	NS	NS	NS		NS	NS	NS	
Mold	15	2.76^{b}±0.13	5.38^{Ca}±0.07	5.35^{Ba}±0.03	***	0.24^{b}±0.34	4.25^{a}±0.47	3.92^{a}±0.09	**
	30	3.81^{b}±0.51	8.04^{Aa}±0.20	7.21^{Aa}±0.15	**	0.99^{b}±1.40	5.70^{a}±0.03	4.94^{a}±0.53	*
	45	3.79^{b}±0.81	7.69^{Ba}±0.05	7.00^{Aa}±0.23	**	1.29±1.82	4.78±0.09	4.66±0.65	NS
	60	3.98^{b}±0.70	7.57^{Ba}±0.01	7.23^{Aa}±0.24	**	1.37±1.94	5.50±1.37	4.57±0.02	NS
	Significance	NS	***	**		NS	NS	NS	
Pseudomonas	15	5.66±1.64	6.41±1.87	5.06±1.87	NS	4.50±1.87	4.61±1.02	4.96±1.54	NS
	30	ND	8.02±1.84	6.66±1.19	NS	2.98±0.71	ND	6.29±2.86	NS
	45	5.73±1.27	6.26±1.67	6.14±2.91	NS	4.49±0.29	2.01±0.09	4.44±2.78	NS
	60	5.31±1.36	6.99±1.92	5.78±0.99	NS	4.47±0.07	1.66±2.35	3.60±1.97	NS
	Significance	NS	NS	NS		NS	NS	NS	

Results are presented means±SD (Log CFU/g).
$^{A–C}$ Means with different superscripts in the same column significantly differ ($p<0.05$).
$^{a–c}$ Means with different superscripts in the same row significantly differ ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
AC, aerobic bacteria count; LAB, lactic acid bacteria; NS, not significant; ND, not detected.

anaerobic conditions and *Pseudomonas* species dominate under aerobic conditions (Kim et al., 2021). In the present study, LAB counts showed no differences regardless of aging method or aging time except for wet aging. Wet-aged beef after more than 30 d of aging showed significant higher LAB count than that those aged for less than 30 d. There were no significant differences in *Pseudomonas* counts regardless of aging method or aging time.

Yeast counts on the outside and inside of aged beef showed no difference with increasing aging time. At the initial aging time (15 d and 30 d aging), yeast counts were significantly ($p<0.05$) higher in both the outside and inside of dry-aged and packaged dry-aged beef compared with those of wet aged beef. Especially, the inside of wet-aged beef had extremely lower yeast counts during all aging time compared with the inside of dry-aged and packaged dry-aged beef.

Mold showed no difference in the inside of aged beef during aging time. However, mold counts on the crust dry-aged or packaged dry-aged beef after more than 30 d of aging were statistically ($p<0.01$) higher than those after 15 d of aging. In addition, mold counts showed significant differences on both the outside and inside among aging methods. Outside mild count of wet-aged beef was statistically ($p<0.01$) lower than that of dry-aged and packaged dry-aged beef during aging. Inside mold counts of wet-aged beef were significantly ($p<0.05$) lower than those of dry-aged and packaged dry-aged beef during the initial aging period (15 d and 30 d of aging).

Conclusion

As a result of the current research, significant changes are occurred during aging time in all aging methods. Aging losses of dry-aged and packaged dry-aged samples were higher than those of wet-aged samples. Wet-aged beef showed higher cooking loss, lower WBSF than dry-aged and packaged dry-aged beef. Dry-aged beef showed the highest values of VBN and TBARS. Packaged dry aging could result in similar quality traits and microbial characteristics with dry aging.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Ethics Approval

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

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