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Fatty acid profile and lipid oxidation of dry-aged beef

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Abstract
The objective of this research was to determine the effect of the extended dry aging period (0, 20, 40, and 60 days) on lipid oxidation, fatty acid profile, and tenderness in selected dry-aged Hanwoo cow beef cut loins. Dry aging regimes were 4^0C and 85% relative humidity. Lipid oxidation was determined by using 2-thiobarbituric acid reactive substances. Shear force values of the highly marbled beef decreased first 20d aging and did not significantly change after that, until 60d. The hardness of texture profile analysis gradually decreased, but the chewiness value did not differ. A total of 16 fatty acids were identified in dry-aged beef. The most predominant FAs in the aged beef were C18:1, C16:0, and C18:0. The C18:2 was the predominant polyunsaturated fatty acid in the samples. Total PUFA gradually and C18:2n-6 decreased ($p<0.05$) during aging**.** C20:1, C18:3n-3, C20:4, and C22:1 acid significantly decreased ($p<0.05$) until 20d aging, and no changes were observed after 20d to 60d. 2-thiobarbituric acid reactive values of beef increased ($p<0.001$) during dry-aging days, dry aging for 40d and 60d resulted in excessive oxidation of lipids. Dry-aged meat is so popular because of its unique taste. Improvements in tenderness are associated with the 20d aging of beef subprimal, however, some lipid oxidation may be associated with the development of specific dry-aged beef flavor.

Keywords: Hanwoo, beef, postmortem aging, TBARS, Texture, fatty acid profile, lipid oxidation

Introduction
There appears to be a strong interest in Asian countries in beef dry-aging, especially since high-end restaurants are beginning to feature dry-aged beef on their menus. Dry aging is the process of hanging beef carcasses or placing unpackaged primal wholesale cuts in a refrigerated room and leaving them to age for several weeks. Processors and chefs also suggest that the aging time ranges for dry aging from 14 to 70 days. Dry aging parameters, including aging time, temperature, relative humidity, and air velocity need to be carefully balanced and monitored to inhibit microbial growth and minimize weight loss while producing excellent eating quality resulting from tenderisation and enhanced flavor [1, 2, 3, 4, 5]. Dry aging produces more flavorful beef with umami, butter-fried meat, and a nutty odor than wet aging. The longer it ages, the more intense and complex the flavors become. According to Lepper-Blilie et al., [6] days 42 and 49 had the highest aged flavor compared to days 14 and 21. The taste and tenderness of Holstein beef improved during postmortem aging for 32 to 56 days; the taste worsened with further aging [7].
Proteolysis, lipolysis, and oxidation are the major biochemical processes involved during the postmortem aging of meat that affect the tenderness, flavor, and juiciness of the meat, although it may additionally introduce certain undesirable traits [8]. Furthermore, oxidation of lipids during cooking also produces volatile compounds that contribute to beef flavor; however, undesirable flavors also can be developed due to the rancidity of the fat during longer aging, because the dry-aging process typically requires beef with ample marbling within the meat [9, 10].

To our knowledge, limited information about the fatty acid profile and lipid oxidation of lipolysis regarding the quality of dry-aged beef is available in the scientific literature. The objective of this research was to determine the extended aging period /0, 20, 40, 60 days/ effect on lipid oxidation, fatty acids content, and texture parameters in selected dry-aged Hanwoo cow beef cuts from the loin.

Materials and methods

Korean native cattle Hanwoo cow carcasses (n=3) with a marbling score of 3 were selected randomly according to the Korean grading standard [11]. Half carcasses were assigned to the dry-aged for 0d (control), 20d, 40d, and 60d respectively (n=3 for each aging day) by direct air exposure to the conditions in the cold room. Firstly, carcass sides were hung in a cold room for 20d at 2°C with an average relative humidity of 65%. After 20d aging, relative humidity was raised to 75%, and the sides aged until 40d. Then carcass sides aged continuously for 60d. At the end of each aging period, each carcass side is divided into the primal cuts. *Longissimus lumborum (LL,* striploin*)* muscles were dissected from carcass sides for subsequent analysis. Strip loins vacuum packaged and transported to the laboratory under refrigeration 2^0C. Dry-aged muscles were weighed before trimming to determine an initial weight.

The intramuscular fat

The crude fat content was extracted from 5g of the minced meat sample using the Soxhlet method with petroleum ether (ISO 1443:1973). Rinsed all glassware with petroleum spirit, drain, and dry in an oven at 102^0C for 30 min. and cool in a desiccator. 1 g of sand and sample put in a beaker and mix with a glass rod. The sample dried in an oven at 102ºC for 5 hours. Then cooled in a desiccator Samples were placed in a Soxhlet liquid/solid extractor. Accurately weigh a clean, dry 150 mL round bottom flash and put about 90 mL of petroleum spirit into the flask. Continue the extraction for 6 hours. Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent. Place the flask in an oven at 102ºC and dry the contents until a constant weight is reached 13. Cool the flask in a desiccator and weigh the flask. The crude fat content was quantified as the weight percentage of wet muscle tissue.

Fatty acid analysis

Direct transesterification of fatty acids followed the procedure developed by Rule [12]. Fatty acid methyl esters were analyzed by a gas chromatograph (Shimadzu GC-FID 2010 Shimadzu) fitted with a fused silica capillary column, and Famewax columns (30m×0.32 mm×0.25 μm). The split ratio used was 30:1. The N_2, H_2, and air were used as the carrier gas with a flow rate of 1.65ml/min. The injection temperature was at 250°C and the detector was maintained at 300°C**.** The oven temperature program used was as follows: 150ºC for 5 min, then an increase to 240ºC and hold at that temperature for 15 min. Fatty acid standards of 20 fatty acids were used for identification (Restek, Cat# 35066). Results were expressed as percentages of the total fatty acid detected based on the total peak area.

Oxidative stability (TBARS)

Oxidative stability was determined by measuring the thiobarbituric acid reactive substance following the procedure of Buege and Aust [13]. Briefly, a 2.5 g meat sample with 7.5 mL distilled water, 25 μL saturated butylated hydroxyanisole solution, and 10 mL thiobarbituric acid (trichloroacetic acid) solution was homogenized at 11,000 rpm for 15 sec. The volume of the homogenate was adjusted to 30 mL with a TBA/TCA solution and was then immediately placed in ice. The tube containing the homogenate was immersed in a 90°C water bath for 15 min. Thereafter, it was placed on ice to cool for 20 min. Centrifugation at 3000 rpm for 10 min was followed. About 1 to 1.5 mL supernatant was taken and the absorbance was measured at 531 nm in spectrophotometer. The TBARS value was calculated by multiplying the absorbance reading by 5.88. TBARS was expressed as mg malonaldehyde/kg meat sample

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Texture measurements (WBSF and TPA)

Muscle sampling for texture measurements was evaluated by using a modified method by Hwang et al. [14] (2004). The WBSF was evaluated on samples with a 0.5-inch diameter, using a V-shaped blade. Samples were sheared at a with a shallow end of 0.5mm, a deep end of 1.5mm, 70 mm long, and 60 mm wide per sample. Constant speed was held at 50 mm/min. The variables evaluated were hardness (maximum force required to compress the samples, in kg), and chewiness (the work required to masticate the sample before swallowing). crosshead speed of 400 mm/min, using a 40 kgf load cell. The texture profile analysis (TPA) was made by using a modified method by Herrero et al., [15]. It used a compression device on 3 cuts

Data analysis

All analyses were conducted using statistical procedures of SAS Version 9.3 (SAS Institute, Cary, NC, USA).

Results and Discussion

Texture parameters (WBSF) and texture profile analysis (TPA)

Results from the texture parameters including WBSF and TPA affected by postmortem aging are presented in Table 1. The dry-aging duration had a notable influence on WBSF (p<0.001) value of beef and the tenderness of the highly marbled LL muscle increased within the first 20d of aging but did not exhibit any additional changes until 60d. Numerous researchers have characterized improvements in tenderness associated with the aging of a variety of beef subprimals [16, 17]. According to Iida et al., [18] highly marbled beef was tender even if it was aged for only short periods of time; the increased tenderization after day 50 was likely caused by the additional action of Ca ions and proteases.

The chewiness of LL muscles did not differ during dry aging. The postmortem effect of aging on beef tenderness has been well documented [1, 2, 3, 10, 14]. However, data detailing the effects of prolonged dry-aging periods (40d and 60d) on TPA values are lacking.

Table 1.

Tenderness, TPA characteristics, and lipid oxidation of Hanwoo beef subjected to dry aging days

Quality traits	Aging				SEM	F value
	0d	20d	40d	60d		
WBSF[1], kgf	4.02^{a}	2.94^{b}	2.28^{b}	1.73^{b}	0.3	20***
Hardness, kgf	5.13^{a}	4.07^{ab}	3.74^{b}	3.96^{b}	0.4	3.9*
Chewiness, N*mm	0.11	0.15	0.08	0.09	0.06	0.2
IMF[2], %	18.3					
TBARS[3], mg MA/kg	0.18^{c}	0.30^{bc}	0.81^{b}	1.73^{a}	0.02	16***

$^{a-c,}$ means within a row with different superscripts are significantly different, *** $p<0.001$, ** $p<0.01$, * $p<0.05$

[1)] WBSF; Warner-Bratzler shear force, [2)] IMF: Intramuscular fat, [3)] TBARS; Thiobarbituric acid reactive substance

Lipid oxidation (TBARS)

The intramuscular fat (IMF) percentage was 18.3 in Hanwoo beef (Table 1). According to Cho et al., [19] Korean carcasses had higher IMF content (11.29%) than those of the Australian carcasses (5.72%) Adipocyte diameter in subcutaneous, intermuscular, and perirenal fat tissues of Hanwoo cattle continuously increased from 15 to 30 months of age [19]. Beef quality depends not only on the degree of marbling but also on fatty acid composition. Present results indicated that TBARS values of muscle increased (p<0.001) during dry-aging days (Table 1). Numerous workers have reported that lipid oxidation can adversely affect product flavor and there is a large range of reported thresholds for TBARS value.

It has been reported that the TBARS concentrations >0.5 mg malondialdehyde/kg indicate a concentration of lipid oxidation products that impart an undesirable rancid flavor which can be detected by consumers [20]. Moreover, McKenna et al. [21] and Campo et al., [22] used a threshold value of 1.0 mg MDA/kg or 2.3 mg MDA/kg of meat as the point at which off-flavors can be detected in beef. Kim et al. [1] observed a gradual increase in the TBARS values of Hanwoo *longissimus dorsi* muscle during the first 14 d of postmortem aging and a sharp increase in TBARS was observed after 14 d. Campo et al., [22] concluded that the 21d aging time yielded higher TBARS values in dry-aged beef. However, some researchers have demonstrated that as meat ages longer, the activity of the various enzymes decreases, and protective action against oxidation declines, thus increasing susceptibility to oxidation [20].

Therefore, data presented here indicate that dry aging for 40d and 60d resulted in excessive oxidation of lipids. However, DeGeer et al., [23] reported that the higher value; 1.35 mg MDA/kg did not seem to negatively affect sensory flavor. It seems that some lipid oxidation may be associated with the development of dry-aged beef flavor.

Fatty acid content

A total of 16 fatty acids (FA) were identified in dry-aged beef subjected to aging days. The most predominant FAs in the aged beef muscles were oleic acid (C18:1), palmitic acid (C16:0), and stearic acid (C18:0). The linoleic acid (C18:2) was the predominant polyunsaturated fatty acid in the samples. This fatty acid plays an important role in generating volatile oxidation products in cooked beef.

Table 2.

The fatty acid content of dry-aged Hanwoo beef subjected to an extended dry aging period

Fatty acid	Aging				SEM	F value
	0d	20d	40d	60d		Aging
C14:0 (Myristate)	3.47	3.14	2.99	3.95	1.3	1.8
C14:1 (Myristoleate)	0.21	0.17	0.16	0.16	0.04	0.4
C15:0 (Pentadecanoic)	0.35	0.45	0.42	0.46	0.05	1.6
C16:0 (Palmitate)	24.0	22.9	25.2	28.6	1.2	0.6
C16:1 (Palmitoleate)	2.81	2.67	1.8	1.5	0.8	1.8
C18:0 (Stearate)	12.5	17.8	18.1	16.6	0.6	0.7
C18:1 (Oleate)	48.8	46.7	45.7	43.9	1.1	0.9
C18:2n-6 (Linoleate)	4.1^{a}	3.3^{ab}	2.8^{ab}	2.2^{b}	0.5	3.1*
C18:3n-3 (Linolenate)	0.55^{a}	0.68^{a}	0.47^{b}	0.37^{b}	0.07	2.4*
C20:0 (Arachidate)	1.61^{a}	1.05^{a}	1.07^{a}	1.13^{a}	0.4^{b}	1.1*
C20:1 (Eicosenoate)	0.35^{a}	0.15^{b}	0.11^{b}	0.13^{b}	1.8	1.8
C20:4 (Arachidonic)	0.68^{a}	0.44^{b}	0.17^{b}	0.31^{b}	0.5	2.9*
C22:0 (Behenate)	0.12	0.15	0.13	0.19	0.04	2.4
C22:5n-3 (Docosahexaenoic)	0.14	0.07	0.06	0.10	0.61	0.7
C22:1 (Erucate)	0.15^{a}	0.11^{a}	0.06^{b}	0.02^{b}	0.10	3.8*
C24:0 (Lignocerate)	0.17	0.18	0.10	0.25	0.08	1.2
SFA[1)]	42.21	45.67	47.91	51.18	0.6	1.7
MUFA[2)]	52.32	49.80	47.83	45.71	1.1	1.9
PUFA[3)]	4.79^{a}	4.05^{b}	3.33^{b}	2.67^{b}	0.5	4.6*
PUFA n-3	0.69	0.75	0.53	0.47	0.07	2.4
PUFA n-6	4.10^{a}	3.30^{ab}	2.80^{ab}	2.20^{b}	0.5	3.8*
n-6/n-3	5.94	4.40	5.28	4.68	0.2	1.1

$^{a-b,}$ means within each row with different superscripts are significantly different, *** p<0.001, ** p<0.01, *p<0.05

[1)] SFA = C14:0 + C16:0 + C18:0 + C20:0+ C22:0+ C24:0, [2)] MUFA = C14:1+ C16:1+ C18:1+ C20:1+ C22:1+ C24:1, [3)] PUFA = C18:2n-6 + C18:3n-3 + C20:4 + C22:5n-3

Present data showed that C18:2n-6 gradually decreased ($p<0.05$) during aging. C20:1, C18:3n-3, C20:4 and C22:1 acid significantly decreased ($p<0.05$) until 20d aging and no changes were observed after 20d to 60d. Total SFA slightly increased, while MUFA slightly increased during aging. Total PUFA decreased ($p<0.05$) until 20d aging while Total PUFA n-6 gradually decreased ($p<0.05$) until 60d aging.

Total PUFA decreased ($p<0.05$) during longer aging. C16:1 and C18:1, C18:3n-3 acids also decreased slightly during aging. Similar results were presented in studies by Wood et al., [20] (1994) who noted that PUFA is more susceptible to oxidation than MUFA or SFAs. Korean Hanwoo cattle consistently produce well-marbled beef. This marbled beef was dry-aged in the open air throughout the aging period, therefore, PUFA and MUFA may be playing a large role in the extent to which oxidation occurs.

Wood et al., [20] reported that the unsaturated fatty acids, especially with more than two double bonds that oxidize rapidly are important in regulating the shelf life of meat (rancidity and color deterioration). The rate of autoxidation increases with the number of double bonds. Unsaturated fat containing a number of double bonds is easily oxidized, either by direct chemical action or through the intermediary activity of lipolytic enzymes. However, many compounds were derived from the autoxidation of the mono- and di-unsaturated fatty acids during cooking, and autoxidation appeared to be promoted by PUFA. However, this propensity to oxidize unsaturated phospholipid fatty acids is important in flavor development during cooking [20].

Numerous researchers demonstrated that the fat tissues in meat were the source of the characteristic species flavor [24]. The effect of fatty acid on meat flavor is due to the production of volatile lipid oxidation products during cooking and the involvement of Maillard reaction products to form other volatiles that contribute to odor and flavor [24]. Some volatile lipid oxidation products such as hexanal, heptanal, and octane could be formed from lipid autoxidation and generated by cooking via thermal oxidation. Lipid autoxidation and oxidation would form volatile compounds, but time-dependent changes could be mainly explained by autoxidation during storage [24]. Wood et al., [20] showed that the samples with high n-3 PUFA concentrations produced higher concentrations of lipid degradation products, particularly saturated and unsaturated aldehydes, alcohols, and ketones.

The cooked meat palatability is positively correlated with the concentration of oleic acid (18:1n9) [5, 10] and with C18:2, and total unsaturated fatty acid content [10]. However, as in other studies, undesirable flavor notes including rancid and sour flavors also increased in sirloin and tenderloin steaks as age time was increased from 7 to 35 days [3, 23].

Conclusion

There seems to be a heightened interest in the international market for dry-aged beef. Days of dry aging-dependent increases were observed for tenderness, and decreases were observed for hardness in beef. This was the first study evaluating the lipid oxidation and changes in the fatty acid profile of dry-aged Hanwoo beef. Total PUFA decreased during longer aging. The longer aging time (40d and 60d) yielded higher TBARS values, however, some lipid oxidation may be associated with the development of specific dry-aged beef flavor. Future research in the field of dry-aging should be focused on the sensorial characteristics of dry-aged beef and identifying volatile compounds governing the unique flavor of dry-aged beef.

Conflict of Interests

The authors declare no conflict of interests.

Authors' contribution

ZG drafted and wrote this manuscript, NC contributed supplementary materials and DD did English corrections and revised the manuscript. All authors read and approved the final manuscript.

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