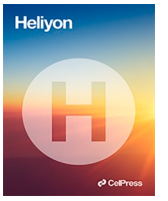
Heliyon 9 (2023) e17091

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Analysis of physicochemical properties of dry-cured beef made from Hanwoo and Holstein meat distributed in South Korea

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ARTICLE INFO

Keywords:
Dry-cured ham
Hanwoo (South Korean native cattle)
Holstein
Storage
Physicochemical properties

ABSTRACT

The purpose of the study is to check the possibility of developing dry-cured meat from Hanwoo (South Korean native cattle) and Holstein cattle considering the differences between breeds and use this data for the preparation and development of dry cured ham unique to South Korea. Same-grade *Semitendinosus* muscle from Hanwoo and Holstein was cured using a curing agent with 4.6% salt content at 4 °C for 7 days, and then aged for 70 days. Data was analyzed through physicochemical characterization, and the manufacturing period was established through weight loss, volatile basic nitrogen (VBN), thiobarbituric acid reactive substances (TBARS). Moisture content and weight loss of both samples significantly decreased during the manufacturing process ($P < 0.05$). TBARS was significantly higher in Hanwoo and VBN in Holstein ($P < 0.05$). According to the values of VBN (less than 20 mg/100 g) and TBARS (less than 2 mg MDA/kg), dry aging for 5 weeks is appropriate for both samples. The principal component analysis of 5 weeks-aged Holstein showed a dramatically changing trend due to myofibril fragmentation as indicated by Sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. In addition, 5 weeks-aged Holstein contains methanethiol (cheese), butan-2-one (butter), and 3-3-ethyl-2-methyl-1,3-hexadiene (fatty acid-derive) compounds that represent fermentation and aging flavors. Therefore, the possibility of product development was confirmed by the 5-week aging of Holstein dry-cured ham.

1. Introduction

Aging improves the sensory qualities of non-preferred meat, thereby increasing the consumption of a high value-added product [1]. Meat aging improves the flavor and tenderness by storing the meat at 4–10 °C for a specified period [2]. Aging can activate proteases such as calpain and cathepsin (depending on the pH), degrade muscle fiber tissues such as Z-lines, nebulin, and titin, and thereby promote muscle fragmentation and increase muscle tenderness [3].

Dry-curing activates enzymes, which actively degrade carbohydrates, proteins, and lipids [4]. These molecules enhance the flavor of meat by releasing phosphate compounds, free sugars, amino acids and lipids inside the meat [5,6]. These aromatic substances are known to have a favourable effect on taste and flavor, and the development of these aromatic substances increases proportionally with the aging period of the meat [7]. The various compositions of intramuscular fat may be significantly different depending on livestock breed and enzyme levels [8].

Various dry-cured ham products have been developed depending on the different processing methods and additives specific to each

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https://doi.org/10.1016/j.heliyon.2023.e17091
Received 15 December 2022; Received in revised form 31 May 2023; Accepted 7 June 2023
Available online 12 June 2023

country, such as *Cecina de Leon* of Spain, *Schwarzwälder Schinken* of Germany, *Pastirma* of Turkeym Egypt, *biltong* of South Africa and *prosciutto di Parma* of Italy [9,10]. However, only a few companies produce small amounts of dry ham in Korea, and most of the available ham in Korea is imported [11]. The temperature difference between seasons is extreme, so the climate is not suitable for natural drying of meat. In addition, as the preference for unprocessed fresh meat is very high, studies on dried meat products such as dried ham are insufficient in Korea [12]. However, the perception and preference of consumers for high-quality meat products are increasing, and dry-cured ham for the Korean market needs to be developed [13].

Hanwoo (Korean Brown cattle) was originally used as a draft cattle, but has been purebred for beef cattle since 1960s [14,15]. Holstein, another cattle distributed in Korea, has a dual-purpose use (i.e., female for dairy cattle and male for beef production). Hanwoo is renowned for its meat quality characteristics, such as a high degree of marbling, juiciness, and softness, compared with the traits of Holstein [16]. Hanwoo (beef native to Korea) has a high polyunsaturated fat (such as linolenic acids) content that affects palatability, these polyunsaturated fat are needed to prevent cardiovascular inflammation [17]. The meat quality of Holstein beef is inferior to Hanwoo beef; it gains its popularity in the Korean beef market because of its low price [18]. The genetic diversity of the two cattles can be clearly distinguished based on whole genome sequence analyses [19,20].

In this study, dry-cured hams of Hanwoo and Holstein breeds produced in South Korea were compared to test the hypothesis that the meat of the different breeds are affected differently during the curing process. In addition, these results and data may be used as basic research data for the development and commercialization of dry-cured *Hanwoo* and *Holstein* hams unique to South Korea.

2. Materials and methods

2.1. Preparation of dry-cured ham from Hanwoo and Holstein cattle

The raw meat used for dry-cured ham production was grade 1 (There are five grades available: 1++, 1+, 1, 2, and 3, in order from the highest quality to the lowest quality, as per the Korean male cattle quality grading system). *M. semitendinosus* was bought 24 h after slaughter from Tobau Korea native beef market (Tobau Co., Sejong, Korea) for Hanwoo male cattle and from Holstein beef market (Dr. Im Co., Seoul, Korea) for Holstein male cattle. The average weight of Hanwoo *M. Semitendinosus* meat was about 614.33 g (±100 g) and the average weight of Holstein *M. Semitendinosus* meat was about 609.67 g (±100 g). Excess fat and fascia were removed from specimens, the meat was cut in half, and the meat samples were randomly placed to reduce interindividual differences.

The curing agent (Total curing agent salinity: 4.6%) was comprised of sea salt (2%; salinity of about 90%), ice (2%), nitrite pickling salt (0.2%, ppm 0.006), onion powder (1%), garlic powder (1%), and ginger powder (1%). Seven times of the prepared curing agent (compared to the weight of raw meat) was weighed in proportion to the meat and massaged to spread evenly. Samples were cured for 7 days in a refrigerator at 4 °C. Two separate aging processes were conducted for 35 days each in the curing room at 4 °C and 75% relative humidity.

The dry-cured hams were marked at four time points (week 0 = raw treatment, week 1 = end point of curing process, week 5 = end point of 1st aging process, and week 10 = end point of 2nd aging process, total process from week 0–11 = manufacturing process). The processing time was set according to the 'raw ham manufacturing process' provided by the Rural Development Administration of Korea. For use in the experiments, dry-cured ham was individually vacuum packed using vacuum machine (Vacuum packaging machine S-460, Webo-matic, Hansastrasse, Germany) and stored in a refrigerator at 4 °C.

2.2. Proximate composition

The proximate composition of two treatments were measured using analysis methods from the AOAC. Moisture content was measured by drying for 16 h in a drying oven at 105 °C (SW-90D, Sang Woo Scientific, Bucheon, Korea) using the atmospheric drying method [21]. The protein and fat contents were determined by the Kjeldahl nitrogen analysis method [22] using Kjeltec equipment (Kjeltec® 2300 Analyzer Unit, Foss Tecator AB, Sweden), and Soxhlet extraction method [23] using Soxtec equipment (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Sweden) respectively. The ash content was analyzed by burning the sample at 550 °C for at least 4 h [24].

2.3. Weight loss

The weight loss of the dry-cured ham was determined after curing process and aging process; the percentage weight loss for each stage was calculated relative to raw meat (week 0) weight as shown in the equation below.

$$\text{Weight lose (\%)} = (\text{sample after curing or aging process (g)})/(\text{raw meat (g)}) \times 100$$

2.4. Salinity

The 4 g of the samples were homogenized with 16 mL of distilled water (DW) for 2 min at 8092×*g* using a homogenizer (AM-5, Nihon Seiki, Tokyo, Japan). Salinity was then measured using a salinity meter (SB-2000PRO, HM Digital, Seoul, Korea).

2.5. pH

The dry-cured ham was weighed and mixed with DW at a ratio of 1:4, followed by homogenization for 2 min at 6991×*g* using an ultra-turrax homogenizer (HMZ-20DN, Pooglim Tech, Seongnam, Korea). The pH was measured using a glass electrode pH meter (Model S220, Mettler-Twoldo, Schwitzer, Switzer-land).

2.6. Volatile basic nitrogen (VBN)

Volatile basic nitrogen was measured by modifying the Sujiwo method [25]. Ten grams of samples and 90 mL of DW were homogenized using the homogenizer (Ultra-Turex, HMZ-20DN, Poolim Tech., Seoul, Korea) at 22.4×*g*, and then filtered through Whatman No. 4 filter papers (GE Healthcare, Chicago, IL, USA). One milliliter of the filtrate and 50% K_2CO_3 were placed in the outer chamber of a Conway container, and 0.01 N H_3BO_3 and the Conway indicator were placed in the inner chamber. Volatile basic nitrogen was collected in the inner chamber of a Conway container in a drying oven maintained at 37 °C for 2 h. The nitrogen collected was titrated with a 0.02 N H_2SO_4 solution until the color returned to red-pink. The amount of VBN generated was calculated using equation,

$$\text{VBN (mg/100 g)} = ((a - b) \times F \times 0.02 \times 14.007)/(s) \times 100$$

where 'a' is the titration amount of the sample (mL), 'b' is the titration of blank (mL), 'F' is the standardization index of 0.02 N sulfuric acid, and 's' is the sample weight (g).

2.7. Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances were evaluated by modifying the distillation method [26]. Ten grams of the samples were mixed with 50 mL of DW and 0.2 mL of 0.3% butylated hydroxytoluene. The mixed liquid was homogenized using the AM-5 homogenizer (Nihonseiki) at 22.4×*g*, and then added to 47.5 mL of DW, 2.5 mL of HCl (4 N), a boiling stone, and 1 mL of antifoam reagent. The distilled sample and thiobarbituric acid (TBA, 0.02 M) were mixed in a 1:1 ratio and heated using a water bath (JSWB-30T, JSR, Gongju, Korea) at 100 °C for 30 min. The absorbance was measured at 538 nm using a Spectra Max iD3 microplate reader (Molecular devices, CA, USA). The blank used was a mixture of DW and 0.02 M TBA at 1:1 ratio. TBARS values were calculated from a linear standard curve of malondialdehyde (MDA) standard solution (1,1,3,3-tetra ethoxy propane), and expressed as an MDA mg per kg sample.

2.8. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Relative protein molecular weight was measured via sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, using a 12% separating gel and 4% stacking gel [27]. The sample homogenized with 3 mM phosphate buffer using homogenizer at 8600×*g*. The supernatants were mixed with 3 mM phosphate buffer and 5 × Sample Buffer (125 mM Tris, 10% SDS, 0.25% BPB, 10% 2-Mercaptoethanol, 50% Glycerol). Fifteen microliters of each sample were added to each well of the gel, and electrophoresis was performed for 80 min. The gel was immobilized to using a fixing solution [The component methanol (50%), glacial acetic acid (10%), DW (40%)], kept overnight on a rocker, and stained with Coomassie brilliant blue for 20 min with gentle agitation. The dyed gel was placed in a destaining solution [The component: methanol (40%), glacial acetic acid (10%), DW (50%)] for 1 h, and then the gel was stored in a storage solution (The component: 5% glacial acetic acid and 95% DW). The relative protein composition was presume to the appeared as band intensity (pixel intensity × band area).

2.9. Histological analysis of cross-sectional area

Cross-sectional area was observed by Lee and Kim [28] method. Each dry-cured ham sample was cut (1 × 1 cm) and stored in a deep freezer (Freezer, Thermo Fisher Scientific, USA) at −80 °C. The frozen samples were then cut into 16 μm cubes at −26 °C using a cryostat cryocut microtome (CM3050S, LEICA, Paris, France). The cross-sectional area of the dry-cured ham samples was observed using a photo-activation imaging microscope at 40 × magnification, and images were observed using the microscope (NIS-Elements imaging software, Nikon, Tokyo, Japan).

2.10. Electric nose analysis

The samples were chopped, and 5 g of the samples was placed in a 20 mL vial. The prepared vials were closed with leak proof caps and incubated for 20 min at 80 °C in a controlled thermostatic agitator. After incubation, a vial test was performed as follow: the total acquisition time was 190s, and the temperature increased from to 80–260 °C at the rate of 1 °C/s. The head space with a 2.0 mL sample at a speed of 200 μL/s, temperature of 200 °C, and detector temperature of 260 °C. The volatile compounds of dry-cured ham were identified using the Heracles II electronic nose (Alpha MOS, Toulouse, France) with different two columns, MTX-5 non-polar column (10 m × 0.18 mm × 0.4 μm) and MXT-1701 medium polar column (10 m × 0.18 mm × 0.4 μm). The sample aroma pattern revealed the principal components analysis (PCA).

2.11. Statistical analysis

The proximate compositions, weight loss, salinity, pH, VBN, and TBARS analyses (2 treatments × 4 storage periods × 6 replication) were analyzed by two-way ANOVA (2 types dry-cured hams and 4 times storage conditions). The fixed effects for analysis of properties and storage properties included the treatment types (Hanwoo and Holstein), and manufacturing periods (0, 1, 5, 10 weeks) with an interaction for treatments. The data are expressed as the mean values with the standard error (SE). Significant differences ($P < 0.05$) among the mean values were determined using and the Duncan's multiple range test by ANOVA.

The electric nose of the main volatile compound were used in the Alpha soft program (Alpha MOS, Toulouse, France). The significant variable for the application was selected using automatic data reduction.

3. Results and discussion

3.1. Proximate compositions, weight loss, salinity

Table 1 shows the moisture, protein, fat, and ash contents of Hanwoo and Holstein dry cured ham during each of the manufacturing process. Samples at week 0 can be used to indicate the proximate composition of the raw meat, data such as moisture, protein, fat, and ash contents were similar to the results of [29]. Moisture content in both Hanwoo and Holstein dry cured ham significantly decreased with the manufacturing periods ($P < 0.05$). The moisture content in Hanwoo decreased by more than half, from 65.25% at week 0–27.23% at week 10. The moisture content in Holstein had even greater weight loss, decreasing from 67.99% at week 0–26.82% at week 10 (Table 1). During week 1, Holstein showed significantly higher moisture values than Hanwoo ($P < 0.05$). Moisture in meat is divided into bound, immobilized, and free water. Among them, free water is reduced in the initial drying process, and fixed water is reduced in the subsequent dry aging process [30]. Furthermore, the protein content significantly increased with the manufacturing periods in both Hanwoo and Holstein. This followed the findings by Lee et al. [31], who reported that the increase of protein is a consequence of moisture decrease. Fat content of Hanwoo and Holstein showed increase tendency at all the manufacturing periods ($P < 0.05$). At weeks 5 and 10, Hanwoo exhibited significantly higher fat content than Holstein. Cannate et al. [32] reported that moisture content and fat content were negatively correlated. Ash content showed significantly increase for both Hanwoo and Holstein as the manufacturing periods increased ($P < 0.05$). Holstein only showed a significantly higher ash content than Hanwoo at week 0.

Both Hanwoo and Holstein showed significant decrease in weight as the manufacturing process progresses (Table 1). At week 5, 54.03% weight loss was observed in Hanwoo, which was significantly higher than the 47.83% weight loss observed in Holstein ($P < 0.05$). During the aging process, myofibril proteins are fragmented, and Z-line structures are weakened. This results in a decreased water holding capacity, and therefore loss of moisture during aging process [33]. Although dry aging showed a high weight loss rate early on because of evaporation of moisture from the outer portion of the meat [34], hardening of the outer surface may have prevented the evaporation of internal moisture, resulting in lower weight loss as curing progressed.

As the manufacturing periods passed, salinity significantly increased in both Hanwoo and Holstein ($P < 0.05$). Salinity increased seven-fold from 0.36 to 0.37% in both samples at week 0–2.89% and 2.81% at week 10 in Hanwoo and Holstein, respectively. The curing agent applied on the surface probably diffused into the muscle due to osmotic pressure, increasing salinity [35].

Table 1
Proximate composition, weight loss (%), and salinity of dry-cured ham manufactured from Hanwoo and Holstein during the manufacturing period.

Traits (%)		Treatments	Manufacturing periods (week)				ANOVA
			0	1	5	10	
Proximate composition	Moisture	Hanwoo	65.43^{a} (0.82)	56.88^{Bb} (0.70)	38.49^{c} (0.50)	25.84^{d} (1.73)	T-/S***
		Holstein	68.96^{a} (1.20)	60.43^{Ab} (0.84)	38.96^{c} (0.49)	27.43^{d} (0.20)	T × S-
	Protein	Hanwoo	25.18^{d} (0.32)	28.68^{c} (0.15)	40.04^{b} (1.30)	46.69^{a} (0.66)	T***/S***
		Holstein	22.33^{d} (0.11)	25.52^{c} (0.31)	48.77^{b} (0.57)	50.72^{a} (0.75)	T × S-
	Fat	Hanwoo	6.67^{b} (0.33)	7.67^{b} (0.84)	15.33^{Aab} (0.88)	20.01^{Aa} (0.58)	T**/S***
		Holstein	3.00^{c} (1.00)	4.02^{bc} (1.00)	6.67^{Bab} (0.88)	8.50^{Ba} (0.50)	T × S***
	Ash	Hanwoo	1.57^{Ad} (0.49)	3.54^{c} (0.06)	5.19^{b} (0.09)	6.10^{a} (0.04)	T-/S***
		Holstein	1.34^{Bd} (0.06)	3.33^{c} (0.12)	5.49^{b} (0.08)	6.53^{a} (0.54)	T × S-
Weight loss		Hanwoo	100	84.89^{a} (1.49)	54.03^{Ab} (2.65)	47.51^{c} (2.89)	T-/S***
		Holstein	100	82.95^{a} (1.74)	47.83^{Bb} (2.84)	43.87^{c} (1.94)	T × S-
Salinity		Hanwoo	0.37^{d} (0.01)	1.85^{c} (0.05)	2.55^{b} (0.04)	2.89^{a} (0.08)	T-/S***
		Holstein	0.36^{d} (0.02)	1.79^{c} (0.13)	2.45^{b} (0.05)	2.81^{a} (0.19)	T × S-

All values are mean and (±SE).
$^{A-B}$ Mean in the same column with different letters are significantly different ($P < 0.05$).
$^{a-d}$ Mean in the same row with different letters are significantly different ($P < 0.05$).
ANOVA, two-way ANOVA analysis among the treatments.
T, treatment; S, storage; T × S, treatment × storage.
– no significantly, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3.2. pH, VBN, TBARS

The pH of dry-cured Hanwoo and Holstein samples was shown in Table 2. The pH of meat is an important factor in evaluating meat quality as it affects water-holding capacity, color, and cooking yield [36]. The pH values of Hanwoo ranged from 5.57 to 5.87, whereas the Holstein samples ranged from 5.45 to 5.77. During all manufacturing periods, the pH of Hanwoo sample was significantly higher than that of Holstein sample ($P < 0.05$), which is consistent with the findings of Li et al. [37], who reported that the pH depended on the fat content. There was no significant changes in pH for Hanwoo sample from week 5 and Holstein sample from week 1 up to 10 weeks. The pH has high positive correlation with fat content that affect palatability [38], therefore, it is expected that there will be differences in some flavor components between Hanwoo and Holstein.

The VBN did not change significantly in Hanwoo sample between week 5 and week 10, and was significantly higher than that of weeks 0 and 1 (Table 2), but the VBN of Holtein sample significantly increased as the manufacturing period passed ($P < 0.05$). Holstein sample values were significantly higher than those Hanwoo sample in the weeks 1 and 10 ($P < 0.05$). The VBN was measured for protein degradation during the aging period, and as a result, it is analyzed that aging degraded more protein in Holstein sample than in Hanwoo sample [39]. On a microbiological basis, considering that the VBN standard for packaged meat is 20 mg/100 g [40], it seems appropriate for both samples to be dry aged for 5 weeks (where 18.68 and 19.61 mg/100 g VBN were observed for Hanwoo and Holstein, respectively).

The rancidity of meat or meat products is an important quality parameter because acidity not only degrades the nutritional value of food, but also affects flavor, color, and texture [41]. Hanwoo had a significant increase in TBARS levels during the entire manufacturing period ($P < 0.05$). Holstein showed no significant changes at week 0 and week 1, but a significant increase as the aging period increased ($P < 0.05$). In contrast to VBN, TBARS was significantly higher in Hanwoo sample than in Holstein sample at other manufacturing periods except week 0. The fat content of Hanwoo sample was observed to be significantly higher than that of Holstein sample at all manufacturing periods. Proteins are known to interact with flavor compounds to form flavor compound-protein interactions, and the formed flavor compound-protein results in dramatic changes in the flavor profiles [42].

3.3. Histological properties

Fig. 1 shows the cross-sections of the dry-cured Hanwoo and Holstein samples under a microscope during the processing. No evident changes were observed between Hanwoo and Holstein at week 0, although a greater portion of the cross-sectional area of Hanwoo was covered in the cavity areas. The microscopic images for both Hanwoo sample and Holstein sample at week 1 are not presented (data not shown). However, Hanwoo sample still showed a greater proportion of the cross-sectional area covered in cavity areas compared to Holstein at week 5. A clear reduction in muscle fibers was observed in Hanwoo and Holstein samples at week 10. Aged meat shows myofibrillar structural changes, and the major causes of these changes include the removal of rigor bonds between actin and myosin, weakening of the connectin and nebulin filaments, and structural weakening of the Z-lines [43,44]. It is speculated that through the abovementioned mechanism, the myofibrillar protein weakens and therefore, the water in the myofibril protein is released and the size of the muscle fiber becomes smaller.

Fig. 2 shows the SDS-PAGE results of Hanwoo and Holstein sample during the manufacturing periods. At this time, the bands were identified against a protein standard (kD). Actin, which has a molecular weight of 45 kD, increases following the degradation of actomyosin during the manufacturing process [45]. The band thickness of this 32 kD protein increased during the manufacturing process. The observed 32 kD protein is predicted to be a degradation product of the high molecular weight compounds desmin and troponin T [46]. Troponin-T is a 30 kD protein important for assessing the extent of curing in meat, as it is strongly associated with tenderness during the manufacturing period [47,48]. A 28–32 kD protein molecule, such as myosin light chains, is a by-product of protein degradation and is known to increase the tenderness [49,50]. Hwang et al. [51] reported that myosin light chain 1 (MLC1), myosin light chain 2 (MLC2), and desmin are produced by proteolysis. In addition, proteins of less than 20 kD appear to be produced by bacterial proteases such as calpain and cathepsin during curing and aging as low molecular weight compounds [52]. Clearly, the heavy

Table 2
pH, VBN, TBARS of dry-cured ham manufactured from Hanwoo and Holstein during the manufacturing period.

Traits	Treatments	Manufacturing periods (week)				ANOVA
		0	1	5	10	
pH	Hanwoo	5.57^{Ac} (0.02)	5.87^{Ab} (0.01)	5.83^{Aa} (0.01)	5.87^{Aa} (0.01)	T***/S***
	Holstein	5.45^{Bb} (0.01)	5.77^{Ba} (0.01)	5.79^{Ba} (0.01)	5.77^{Ba} (0.02)	T × S**
VBN (mg/100 g)	Hanwoo	9.34^{c} (0.93)	13.07^{Bb} (0.93)	18.68^{a} (0.98)	21.48^{Ba} (0.93)	T*/S***
	Holstein	10.27^{d} (0.97)	14.93^{Ac} (0.93)	19.61^{b} (1.14)	23.81^{Aa} (1.40)	T × S-
TBARS (mg MDA/kg meat)	Hanwoo	0.78^{Bd} (0.01)	1.39^{Ac} (0.03)	1.84^{Ab} (0.15)	2.68^{Aa} (0.01)	T*/S***
	Holstein	0.90^{Ac} (0.04)	1.09^{Bc} (0.09)	1.48^{Bb} (0.17)	2.48^{Ba} (0.02)	T × S-

All values are mean and (±SE).
A-B Mean in the same column with different letters are significantly different ($P < 0.05$).
a-d Mean in the same row with different letters are significantly different ($P < 0.05$).
ANOVA, two-way ANOVA analysis among the treatments.
T, treatment; S, storage; T × S, treatment × storage.
– no significantly, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

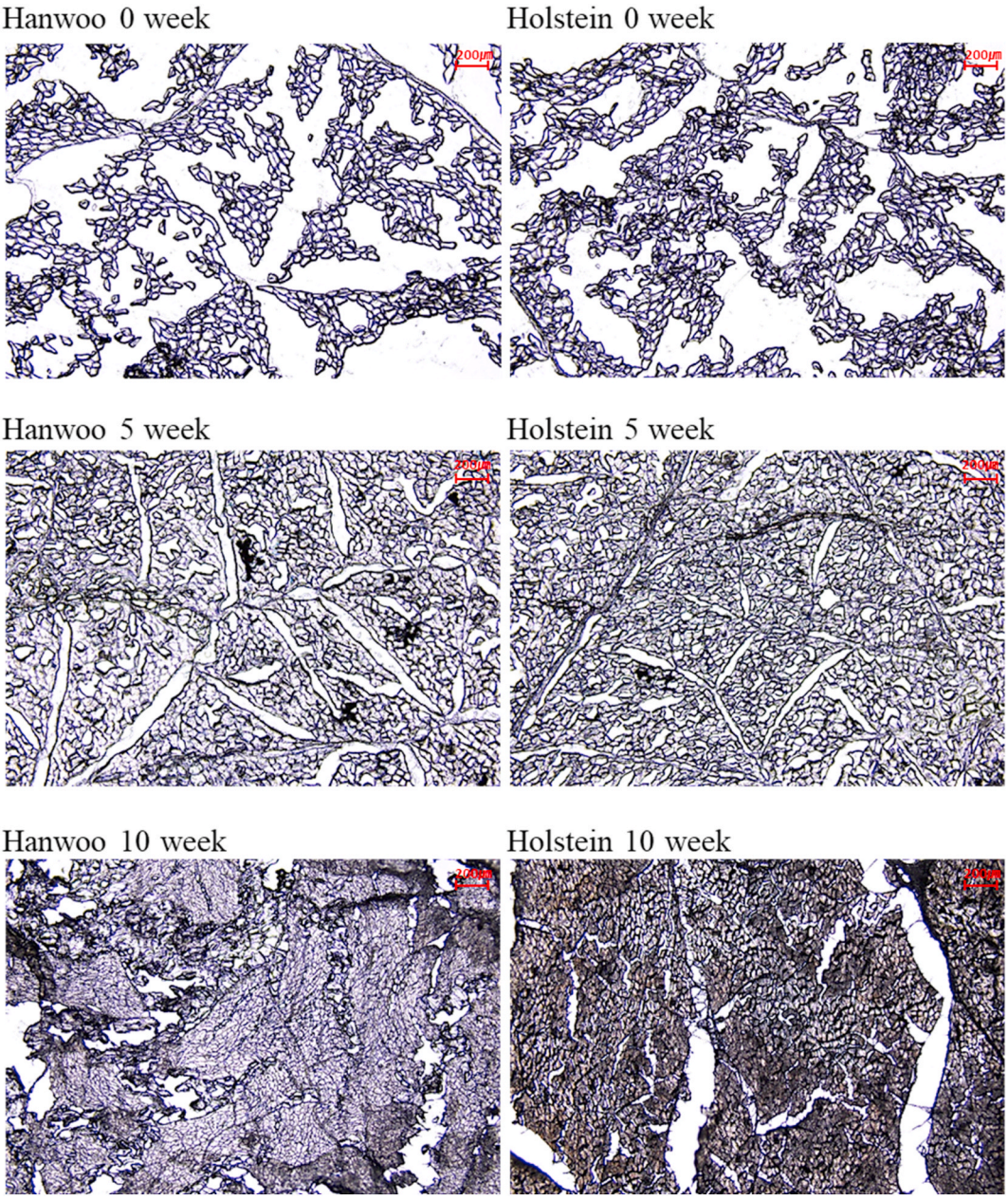

Fig. 1. Cross sectional area of dry-cured ham manufactured from Hanwoo and Holstein cattle during the manufacturing period. The magnification of all the microphotographs is ×40.

chain was dissociated to light chain in both Hanwoo and Holstein samples, therefore, flavor analysis was needed to see how it affects taste.

3.4. Electronic nose

The electronic nose analyzes the difference between samples through principal component analysis (PCA) simplifying data for dimensionality reduction [53]. PCA demonstrates the ability to apply an electronic nose to dry-cured hams made from Hanwoo and Holstein during the manufacturing process (Fig. 3). The PC1 and PC2 accounted for the majority of the information, with 90.689% and 6.521%, respectively. Accordingly, it can be confirmed that the difference between the x-axis represents a significant difference in the treatment group [54]. Based on the x-axis, it was confirmed that there was difference between 0 (raw meat), 1 (cured meat) weeks and aged meat at 5 and 10 weeks. Also, Holstein sample aged for 5 weeks is thought to have a distinctly distinct flavor from other

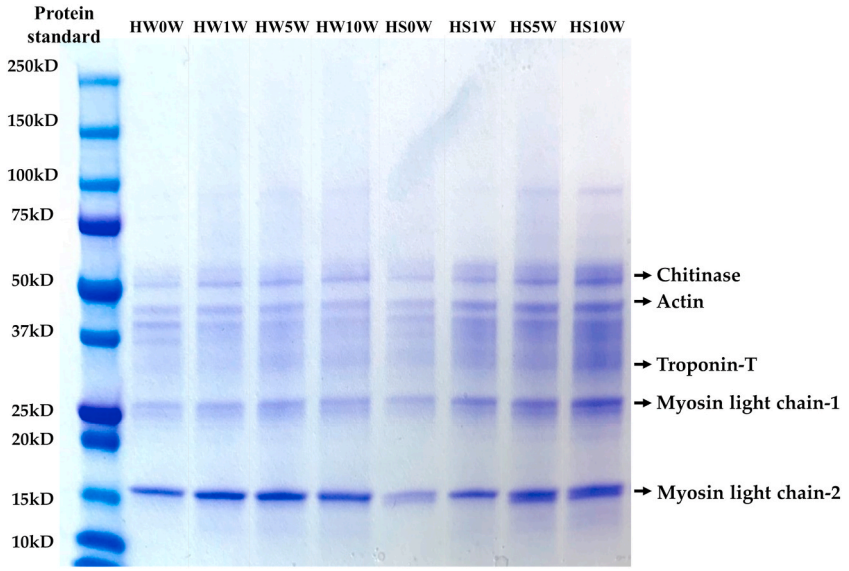

Fig. 2. SDS-PAGE analysis of dry-cured ham manufactured from Hanwoo and Holstein cattle during the manufacturing period. HW0W: Hanwoo 0 week, HW1W: Hanwoo 1 week; HW5W: Hanwoo 5 week, HW10W: Hanwoo 10 week; HS0W: Holstein 0 week; HS1W: Holstein 1 week; HS5W: Holstein 5 week; HS10W: Holstein 10 week.

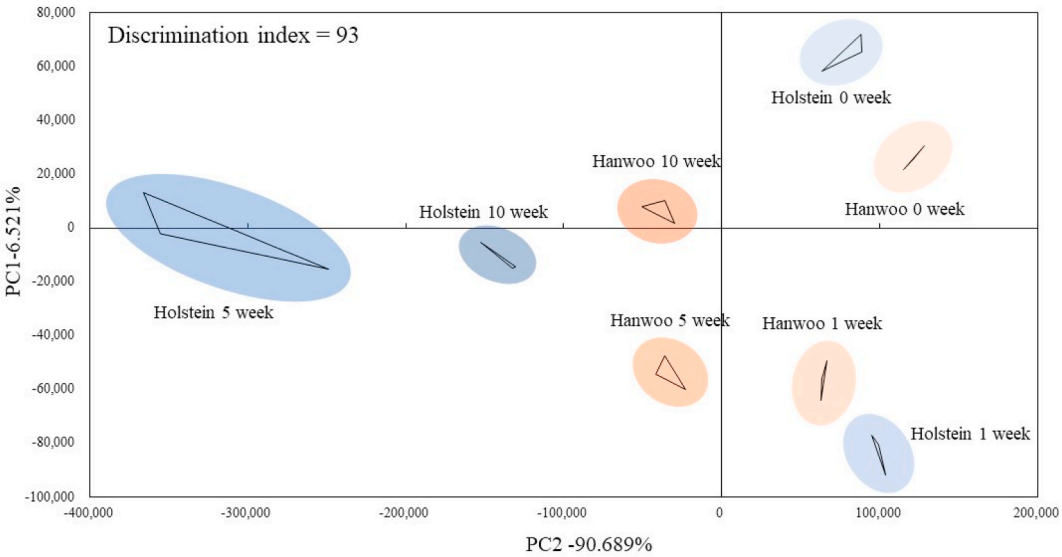

Fig. 3. Principal components analysis for dry-cured ham manufactured from Hanwoo and Holstein cattle during the manufacturing period.

treatments.

Fig. 4 is the chromatogram result of analyzing the difference in volatile compounds between weeks 5 and 10 of Hanwoo and Holstein samples. Volatile compounds of electronic nose shows the intensity peaks of substances that contribute to flavor, along with their predicted value. All results of the analysis of volatile compounds were not shown, but 6 peaks among them are predicted to be the main compounds that classify the treatment group. Areas under the peaks (6 peaks) were expressed as counts, and they are correlated with the compound concentration. The strong intensity value at peak A (methanethiol) corresponds to a flavor similar to that of cheese and cooked vegetables, and that at peak C (butan-2-one) corresponds to a flavor similar to that of cheese and butter. Substances with these predicted compounds and flavors belong to the category of dry-cured meat products, according to the figure classified by Flores [55]. More experiments are needed to determine whether these compounds produce these flavors, but in this study, dry-curing flavors are expected to be stronger with higher concentrations of these compounds. Holstein samples in week 5 showed a higher intensity at peak E; 3-3-ethyl-2-methyl-1,3-hexadiene is known to be a fatty acid-derived predictive flavor compound [56], and it is presumed that this peak affected the PCA plot (Fig. 3).

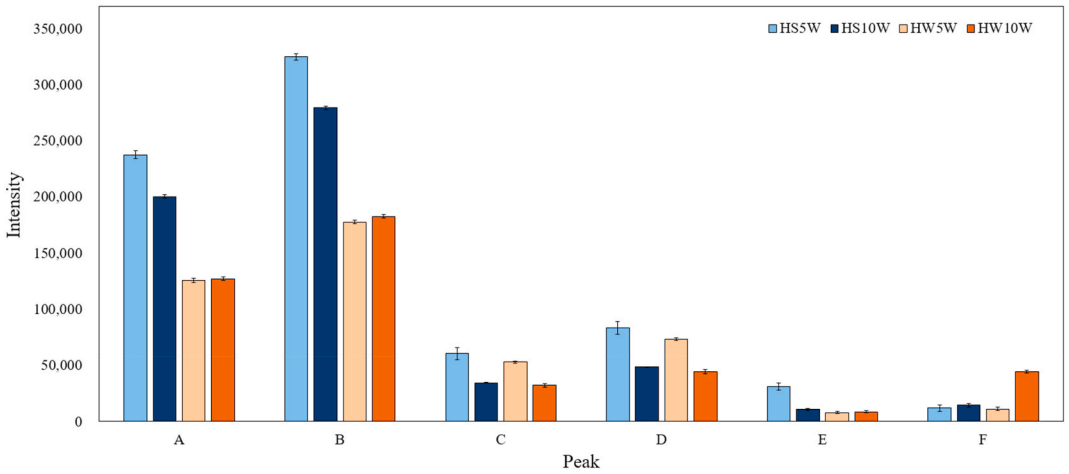

Fig. 4. The main volatile compounds in dry-cured ham manufactured from Hanwoo and Holstein cattle during the curing period. HW5W: Hanwoo 5 week, HW10W: Hanwoo 10 week; HS5W: Holstein 5 week; HS10W: Holstein 10 week. Peaks are presented in order of elution: A: Methanethiol; B: 2-propanol; C: butan-2-one; D: n-butanol; E: 3-3-ethyl-2-methyl-1,3-hexadiene; F: 3-Furanthiol.

4. Conclusions

The present study investigated the development of dry-cured ham distributed in South Korea via analyses of physicochemical during the entire manufacturing process. As the moisture content decreases, protein, fat, ash contents increased during the manufacturing process in both samples. Also, the Hanwoo and Holstein dry-cured ham were presented the weight loss about 50% at week 10. Based on VBN and TBARS, which are indicators of shelf life, it is estimated that it is safe to age meat for up to 5 weeks. Hanwoo sample showed a significantly higher TBARS value than Holstein sample, thus it is necessary to pay attention to fat oxidation during the aging process of Hanwoo sample. The principal component analysis readings of Holstein samples showed a dramatical changing trend at week 5 or 10, the other side, Hanwoo samples showed an insignificant difference. In particular, 3-3-ethyl-2-methyl-1,3-hexadiene, which corresponds to the flavor of fermentation and aging, was highly expressed at week 5 in Holstein samples. This data may be used to achieve better quality and flavor during the production of dry-cured ham from cattle distributed in Korea. Based on the results of this study, it is necessary to analyze the possibility of industrialization through detailed organoleptic characteristics analysis.

Funding

This work was supported by the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0155902022) Rural Development Administration, Korea.

Author contribution statement

Sol-Hee Lee: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hack-Youn Kim: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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