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#### \*Corresponding author

Hack-Youn Kim Department of Animal Resources Science, Kongju National University, Yesan 32439, Korea. Tel: +82-41-330-1241 E-mail: kimhy@kongju.ac.kr

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#### ORCID

Jeong-Ah Lee https://orcid.org/0000-0003-3019-8321 Hack-Youn Kim https://orcid.org/0000-0001-5303-4595

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#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

# Physicochemical properties of crust derived from dry-aged *Holstein* and *Hanwoo* loin

Jeong-Ah Lee and Hack-Youn Kim\*

Department of Animal Resources Science, Kongju National University, Yesan 32439, Korea

#### Abstract

This study evaluated the quality characteristics of crust derived from dry-aged Holstein and Hanwoo loins and their effects on food as additives. With respect to physicochemical properties, we examined the proximate composition, pH value, salinity, color, water and fat absorption, emulsifying capacity, and swelling yield. The protein and ash contents in the Holstein crust were significantly higher than those in the Hanwoo crust (p < 0.0001). The fat content in the Hanwoo crust was significantly higher than that in the Holstein crust (p < 0.01). The salinity, lightness, and yellowness of the Hanwoo crust were significantly lower than those of the Holstein crust (p < 0.001). Furthermore, the pH value and emulsifying capacity of the Hanwoo crust were significantly higher than those of the *Holstein* crust (p < 0.001). The fat absorption of the Holstein crust was significantly higher than that of the Hanwoo crust (p < 0.001). The swelling yield of the Holstein crust was significantly higher than that of the Hanwoo crust at pH 3 and 4 (p < 0.001), whereas the swelling yield of the Hanwoo crust was significantly higher than that of the *Holstein* crust at pH 7 (p < 0.001). Principal component analysis of dry-aged Hanwoo, Holstein, and non-aged Holstein showed different flavor patterns for each sample. Finally, the results showed that the crusts derived from dry-aged Hanwoo and Holstein loins were suitable flavor enhancers.

Keywords: Crust, Dry aging, Holstein, Hanwoo, Quality characteristics

## **INTRODUCTION**

The aging of meat involves myofibrillar fragmentation of protein, and the methods of aging can be categorized as wet- and dry-aging. During the aging process of the meat, a change in pH in accordance with a corresponding action activates calpain and cathepsin, which are proteolytic enzymes in muscles and increase free amino acids [1–5]. Additionally, triglycerides produce free fatty acids, which enhance tenderness and flavor [6]. Recent studies evaluated that dry-aging, an aging method that involves controlling humidity and wind speed in the air to maximize the flavor and taste of meat, improves and enriches the flavor, taste, and tenderness of meats more than wet-aging. [7–9]. Dashdorij et al. [10] reported that dry-aged meat with a unique flavor was highly preferred by consumers. Choe and Kim [11] reported that this aging technique was being used to add market value to low-grade cattle beef.

Crust is produced by surface hardening during the dry-aging of meat, and thus, the crust has low utility as meat because of its hard texture [10]. As the duration of dry-aging increases, the quantity of

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Conceptualization: Kim HY. Data curation: Kim HY. Formal analysis: Lee JA. Methodology: Kim HY. Software: Lee JA. Validation: Kim HY. Investigation: Lee JA. Writing - original draft: Lee JA. Writing - review & editing: Lee JA, Kim HY.

#### Ethics approval and consent to participate

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

crust increases, which decreases the yield of dry-aged meat. In addition, dry-aged meats are distributed at high market prices because of the duration of dry-aging [1,2,8,12]. DeGeer et al. [13] reported a 34% trimming loss after beef loins were dry-aged for 28 days. Several studies have been conducted to reduce this trimming loss. Lee et al. [14] reported that the microbial composition of crust changed depending on wind speed and direction in addition to further changes in the physicochemical properties and composition of the flavor compounds. Park et al. [15] improved some functionalities, including the flavor and antioxidant properties, by applying crust with antioxidant and antihypertensive properties to beef patties. However, only a few studies on crust depending on bovine species and breed have been conducted, and product development using crust is limited. In particular, dry-aging has been performed mainly on beef for industrialization; however, crust derived from *Holstein* and *Hanwoo* has not been researched.

Hanwoo is a breed highly accepted by Koreans, unlike Holstein and imported meats, and it has a high degree of marbling, excellent flavor, and soft meat quality [16,17]. Hanwoo has a high tenderness sufficient for roasting, and it can be distributed as high-quality meat following dry-aging [18,19]. Furthermore, Hanwoo has significantly high distribution prices, which means that the development of meat products using crust derived from Hanwoo will promote the development of the Hanwoo industry [20]. Therefore, in this study we prepared loin (musculus longissimus dorsi) crusts from dry-aged Hanwoo and Holstein to compare their physicochemical quality characteristics as well as processing suitability.

## MATERIALS AND METHODS

#### Materials and preparation of loin crust

In total, 12 pieces of beef loin (*M. longissimus dorsi*) were obtained from 6 carcasses (*Holstein*, Korea quality grade 2; *Hanwoo*, Korea quality grade 2) that were two days postmortem, and were divided into three sections of equal length and width. *Holstein* loin (*M. longissimus dorsi*, I home meat, Seoul, Korea) and *Hanwoo* loin (*M. longissimus dorsi*, Dawoo hanwoo, Chungnam, Korea) were refrigerated for 24 h and used for the tests. Dry aging occurred in a dry-aging refrigerator at  $4^{\circ}$ C, 60%–70% relative humidity, and air velocity of  $5 \pm 3$  m/s for 4 weeks. After aging, *Holstein* and *Hanwoo* loins were trimmed off by 30–70 mm from outside. Subsequently, the loin crusts were stored at  $-18^{\circ}$ C for 24 h (CA-H17DZ, LG, Seoul, Korea) to freeze-dry, and lyophilization was performed at  $-80^{\circ}$ C for 15 h using a freeze dryer (FDU-1110, Eyela, Tokyo, Japan). The crust was pulverized to a size of 15 mesh using a mixer (MQ5135, Braun, kronberg im Taunus, Germany) and stored at  $4^{\circ}$ C until the analyses.

#### **Proximate composition**

In compliance with the AOAC method [21], the protein content was measured via the Kjeldahl method, the fat content was measured via the Soxhlet method, the moisture content was measured via the drying oven method at  $105 \,^{\circ}$ C, and the ash content was measured via dry ashing at  $550 \,^{\circ}$ C.

#### **Determination of salinity**

To measure the salinity, we mixed 1 g of sample with 20 mL of distilled water with an Ultra Turrax homogenizer (HMZ-20DN, Pooglim Tech., Seoul, Korea) at 6,991×g for 1 min. The salinity was recorded using a salinity meter (SB-2000PRO, HMdigital, Seoul, Korea), and the measured value was calculated as a percentage by multiplying dilution (× 20).

#### **Determination of pH values**

A mixture of loin crust and distilled water (1:4) was homogenized with an Ultra Turrax homogenizer (HMZ-20DN, Pooglim Tech) at 6,991×g for 1 min. The pH was determined using a pH-meter (Model S220, Mettler-Toledo, Greifensee, Switzerland).

#### **Determination of instrumental color**

We used a colorimeter (CR-10, Minolta, Tokyo, Japan) to measure the lightness (CIE L\*), redness (CIE a\*), and yellowness (CIE b\*) of the samples. The colorimeter was calibrated with a white standard plate  $L^* = 97.83$ ,  $a^* = -0.43$ , and  $b^* = +1.98$ .

#### **Determination of water absorption**

The water absorption was measured via the method of Lin et al. [22], with some modifications. First, 1 g of sample and 10 mL distilled water were mixed using a vortex mixer (SVM-10, SciLab, Seoul, Korea) for 2 min, and the mixture was centrifuged at  $15^{\circ}$ C,  $983 \times g$  for 20 min (Supra R22, Hanil, Gimpo, Korea). The supernatant was carefully decanted, and the weight of the precipitate was measured before drying (W<sub>1</sub>). Then, the precipitate was dried for 24 h at  $105^{\circ}$ C in a drying oven (C-F03, Cheil, Gyeonggi-do, Korea), and the weight was measured after drying (W<sub>2</sub>). The water absorption was calculated using the following equation:

Water absorption (%) = 
$$\frac{W_1 - W_2}{W_1} \times 100$$

#### **Determination of fat absorption**

The fat absorption was determined via the method of Lin et al. [22], with some modifications. Based on the protein content in the sample, the total protein amount was calculated to be 1 g and the sample was used (W<sub>1</sub>). The sample was mixed with 10 mL of soybean oil (V<sub>1</sub>) using a vortex mixer (SVM-10, SciLab) for 30 s, and the mixture rested at room temperature (25 °C) for 30 min. Then, the mixture was centrifuged at 25 °C, 983 ×g for 20 min (Supra R22, Hanil), and the volume of the supernatant was measured (V<sub>2</sub>). The fat absorption was calculated using the following equation.

Fat absorption (%) = 
$$\frac{V_1 - V_2}{W_1} \times 100$$

#### Determination of emulsifying capacity

The emulsifying capacity was determined using the method of Yasumatsu et al. [23]. First, 7 g of the sample, 50 mL of distilled water, and 100 mL of soybean oil were mixed using the Ultra Turrax homogenizer (HMZ-20DN, Pooglin Tech.) at 983×g for 1 min. The prepared emulsion was allowed to stand in a graduated cylinder at room temperature (25 °C) for approximately 1 h or more. The emulsifying capacity was measured based on the total amount of solution (B) and amount of mixture (A) using the following equation.

Emulsifying capacity (%) = 
$$\frac{A}{B} \times 100$$

#### **Determination of swelling yield**

First, 10 g of the sample was mixed with 100 mL of Tris-HCl solution at pH 3, 4, and 7 using

a vortex mixer (SVM-10, SciLab) for 1 min. Then, the prepared mixture was allowed to stand at room temperature ( $25^{\circ}$ C) for 1 h. The supernatant was carefully decanted, and the weight of the precipitate was measured. The swelling yield was calculated using the following equation.

Swelling yield(%) = 
$$\frac{\text{After swelling}(g)}{\text{Before swelling}(g)} \times 100$$

#### **Electronic nose analysis**

The loin crusts were individually placed in a 20 mL vial on a sample holder heated at 80 °C for 20 min. The headspace volatile compounds were injected into a gas chromatography-type electronic nose (HERACLES-2-E-NOSE, alpha-mos, Toulouse, France) equipped with dual columns (MXT-5 and 1701, Restek, Bellefonte, PA, USA) (length 10 m, inner diameter 180  $\mu$ m, MXT-5: non-polarity, MXT-1701: slight polarity). The analysis conditions were set as follows: injection time of 20 min, volume of 2 mL, rate of 250  $\mu$ L/s, temperature of 200 °C, and detector temperature of 260 °C. The principal component analysis (PCA) was integrated using the Alpha Soft program (Alphasoft, Alpha MOS, Toulouse, France).

#### **Statistical analysis**

The experimental results were assessed after a minimum of three repeated trials. Statistical analyses were performed using the General Linear Model procedure of the SAS program (2015, SAS Software for Window, Version 9.3, SAS Institute, Cary, NC, USA). *T*-tests were performed to compare each average of the treatments, and the significance was expressed as p < 0.05, p < 0.01, p < 0.001.

### **RESULTS AND DISCUSSION**

#### Proximate composition, pH, salinity, and color

Table 1 shows the general components of the loin crusts of *Holstein* and *Hanwoo*. The moisture contents in *Hanwoo* and *Holstein* crusts showed no significant difference. The fat content in the *Hanwoo* crust was significantly higher than that in the *Holstein* crust (p < 0.01), whereas the protein and ash contents in the *Holstein* crust were significantly higher than those in the *Hanwoo* crust (p < 0.001). The moisture content in the crust during drying showed no significant difference because most of the free water was dried. Choe and Kim [11] reported that the 2nd grade *Holstein* loin contained 19.95% protein, 1.10% ash, and 5.97% fat, whereas the 2nd class *Hanwoo* loin contained 17% protein, 0.51% ash, and 23.71% fat [3]. The results suggested that the crust after dry-aging showed a similar tendency because the fat content in the *Holstein* loin was lower but the protein

#### Table 1. Proximate composition of crust derived from dry-aged Holstein and Hanwoo loin

Traits	Dry aging crust		SEM <sup>1)</sup>	Statistical analysis	
	Holstein	Hanwoo	SEIVI	t-value	<i>p</i> -value
Moisture content (%)	1.15	1.20	6.92	-0.25 <sup>ns</sup>	0.8123
Protein content (%)	62.82	44.06	3.99	12.05***	0.0003
Fat content (%)	23.90	49.33	12.09	-6.34**	0.0032
Ash content (%)	3.90	1.95	0.75	15.45***	0.0001

All values are mean.

<sup>1)</sup>n = 2.

<sup>••</sup>*p* < 0.01, <sup>•••</sup>*p* < 0.001.

ns, non-significant.

and ash contents were higher than those in Hanwoo loin of the same grade.

Table 2 shows the measurements of pH, salinity, and instrumental color of the Holstein and Hanwoo crusts. The pH of the Hanwoo crust was significantly higher than that of the Holstein crust (p < 0.001). Li et al. [7] reported that intramuscular fat content and pH showed a positive correlation in the same bovine species, which suggested that the pH of the Hanwoo crust with a higher fat content was higher than that of Holstein. The salinity of the Holstein crust was significantly higher than that of the Hanwoo crust (p < 0.001), this was related to free amino acids because they affect the salinity measurements. These results suggested that numerous free amino acids were released from Holstein with a higher protein content than that of Hanwoo [24]. Free amino acids hydrolyzed from proteins contain taste components such as inosine monophosphate (IMP) and guanosine-5'-monophosphate [25] and that the total amount of free amino acids increases as the aging is prolonged [26]. Among inorganic ionic components, Na, K, and P significantly enhance the umami taste along with free amino acids and IMPs [27]. Thus, free amino acids are more accumulated in the Holstein crust with high protein content than in the Hanwoo crust, and the salinity of the Holstein crust is high because it is typically measured based on the content of the total inorganic substance [28]. In addition, both Hanwoo and Holstein crust have a salinity of 0.8%-1.2%, which will not have a significant impact on salinity as food additives. With respect to the instrumental color of the Hanwoo and Holstein crusts, the lightness and yellowness of Holstein were significantly higher than those of Hanwoo (p < 0.001), and there was no significant difference in redness between the two treatment groups. Lee et al. [29] reported that the lightness, redness, and yellowness of Hanwoo were significantly low during the analysis of the physicochemical quality characteristics of Holstein and Hanwoo. Difference color between Hanwoo and Holstein is related to the moisture contents. In general, it is known to *Holstein* beef loin is higher moisture content than *Hanwoo* beef loin [11]. And high moisture content of beef loin related to the increase the lightness, redness, and yellowness [30]. Therefore, the moisture content of the Holstein crust was higher than Hanwoo crust, it was related to the higher lightness and yellowness than Hanwoo crust. The lrightness and yellowness of the Hanwoo crust were significantly lower in this study, and no significant difference was found in the increase in redness due to dried moisture.

#### Water absorption, fat absorption, emulsifying capacity, and swelling yield

Table 3 shows the analytical results of water absorption, fat absorption, emulsifying capacity, and swelling yield of the *Hanwoo* and *Holstein* crusts. There was no significant difference in water absorption between the *Hanwoo* and *Holstein* treatment groups, whereas the fat absorption of the *Hol-*

Traits	Dry aging crust		SEM <sup>1)</sup>	Statistical analysis	
	Holstein	Hanwoo	SEIVI	t-value	<i>p</i> -value
pН	5.44	5.67	0.08	-22.30***	<.0001
Salinity (%)	1.20	0.80	0.15	Infty <sup>***</sup>	<.0001
Color					
CIE L*	54.26	37.82	6.13	77.50***	<.0001
CIE a*	15.90	12.87	2.00	1.63 <sup>ns</sup>	0.1534
CIE b*	12.38	2.08	3.86	26.70***	<.0001

#### Table 2. pH, salinity and color of crust derived from dry-aged Holstein and Hanwoo loin

All values are mean

<sup>1)</sup>n = 2.

<sup>....</sup>p < 0.001.

ns, non-significant.

Traits -	Dry aging crust		SEM <sup>1)</sup>	Statistical analysis	
	Holstein	Hanwoo	SEIVI	t-value	<i>p</i> -value
Water absorption (%)	2.96	2.26	0.39	1.83 <sup>ns</sup>	0.1420
Fat absorption (%)	2.85	0.62	0.86	40.49***	<.0001
Emulsifying capacity (%)	17.20	54.10	14.46	-12.72***	0.0002
Swelling yield					
рН 3	306.20	168.31	53.61	22.16***	<.0001
pH 4	317.43	164.78	59.58	15.97***	<.0001
pH 7	120.57	168.55	18.86	-11.55***	0.0003

Table 3. Water absorption, fat absorption, emulsifying capacity and swelling yield of crust derived from dry-aged *Holstein* and *Hanwoo* loin

All values are mean.

<sup>1)</sup>n = 2.

<sup>•••</sup>p < 0.001.

ns, non-significant.

*stein* crust was significantly higher than that of the *Hanwoo* crust (p < 0.001). Lee et al. [31] showed that the fat absorption of rice protein concentrate with low fat content was high when testing the dry frozen rice protein concentrate and isolated soy protein, which suggested that the fat absorption decreased as the fat content increased. Thus, it would be efficient to apply the *Holstein* crust with the high fat absorption capacity to products with high fat content and the *Hanwoo* crust with low fat absorption to low-fat food ingredients.

The emulsifying capacity of the *Hanwoo* crust was significantly higher than that of the *Holstein* crust (p < 0.001). Beef fat can be divided into neutral lipids and phospholipids. Phospholipids possess an emulsifying capacity to combine water and oil [32,33]. *Hanwoo* has a higher emulsifying capacity than *Holstein* because of its higher content of palmitic acid and stearic acid in phosphoglycerides and sphingolipids [34]. Thus, the *Hanwoo* crust can be utilized as a food additive for enhancing the binding power between moisture and oil [35].

The measurement of the swelling yield based on the pH must be preceded to increase the applicability of the powder crust that enhances flavor [36]. The larger the swelling yield of the crust, the less physicochemical change in the food processing, such as sauces, and the less drying loss [37,38]. The swelling yields of the *Hanwoo* and *Holstein* crusts were measured at pH values of 3, 4, and 7 for sauces. At pH 3 and 4, the *Holstein* crust showed significantly higher swelling yield than the *Hanwoo* crust (p < 0.001), whereas at pH 7, the *Hanwoo* crust showed a significantly higher swelling yield than the *Holstein* crust (p < 0.001). The swelling yield is a factor that highly affects the yield of liquid or semi-solid products [39], and it would be ideal to prepare food at a high pH corresponding to high swelling yield. The *Holstein* crust showed excellent swelling yield under pH 3 and 4; thus, it can be effectively applied to the sauce and vinegar product groups [40,41]. Conversely, the *Hanwoo* crust showed a high swelling yield under pH 7 (neutral); thus, it can be suitably applied to sauces and processed meat products [42].

#### Principal component analysis in electric nose

An electric nose analysis can be widely used to evaluate food quality and aging by classifying the characteristics that determine the overall flavor of food [43]. Fig. 1 shows the PCA for the *Holstein* and *Hanwoo* crusts using the electric nose technique. According to the PCA analysis, the contribution rate of the first principal component (PC1) value was 96.484%, and that of the second principal component (PC2) value was 3.441%. Because the contribution rate of PC1 was 90% or higher, the flavor was more discriminated by PC1 than PC2 [43]. The PC1 range of the non-aged *Holstein*,

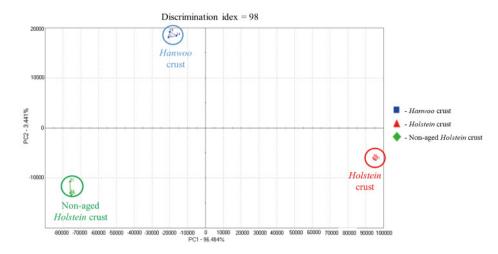


Fig. 1. Principal component analysis of crust derived from dry-aged Holstein and Hanwoo loin.

*Hanwoo*, and *Holstein* crusts were approximately -75,000, -20,000, 95,000, respectively. The PC2 range of the *Hanwoo* crust was approximately 19,500, whereas those of the non-aged *Holstein* and *Holstein* crusts were between -5,000 and -15,000. Thus, in terms of PC1, which greatly contributed to flavor discrimination, the discrimination in flavor could be determined based on the difference between the treatment groups. The difference in the flavor of the *Hanwoo* and *Holstein* crusts depends on the breed in the same bovine species and site. Kang et al. [44] reported similar results on the difference between breeds at the same site of pork through electric nose analysis of flavor because various compositions of fatty acids and amino acids that depend on breed resulted in various aromatic patterns.

## CONCLUSION

The present study analyzed the physicochemical properties of crust derived from dry-aged *Holstein* and *Hanwoo* loins as flavor enhancers. The emulsifying capacity of the *Hanwoo* crust was significantly higher than that of the *Holstein* crust. The fat absorption of the *Holstein* crust was higher than that of the *Holstein* crust. The dry-aged *Hanwoo* and *Holstein* samples showed notably different flavor patterns via PCA. Therefore, the addition of crust derived from dry-aged *Hanwoo* and *Holstein* loins would lead to enhanced flavor in food. And choose the type of beef crust consider to purpose of food flavor and characteristics.

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