Food Science of Animal Resources

ARTICLE

Distinguishing Aroma Profile of Highly-Marbled Beef according to Quality Grade using Electronic Nose Sensors Data and Chemometrics Approach

Dicky Tri Utama[1,2], Aera Jang[1], Gur Yoo Kim[1], Sun-Moon Kang[3], and Sung Ki Lee[1,*]

[1]Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea
[2]Department of Animal Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Sumedang 45363, Indonesia
[3]Department of Animal Products Development and Utilization, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

OPEN ACCESS

*Corresponding author : Sung Ki Lee
Department of Applied Animal Science,
College of Animal Life Sciences,
Kangwon National University,
Chuncheon 24341, Korea
Tel: +82-33-250-8646
Fax: +82-33-259-5574
E-mail: skilee@kangwon.ac.kr

*ORCID
Dicky Tri Utama
https://orcid.org/0000-0003-2344-8548
Aera Jang
https://orcid.org/0000-0003-1789-8956
Gur Yoo Kim
https://orcid.org/0000-0001-9973-3367
Sun-Moon Kang
https://orcid.org/0000-0003-3947-4337
Sung Ki Lee
https://orcid.org/0000-0002-2989-4787

Abstract   Fat deposition in animal muscles differs according to the genetics and muscle anatomical locations. Moreover, different fat to lean muscle ratios (quality grade, QG) might contribute to aroma development in highly marbled beef. Scientific evidence is required to determine whether the abundance of aroma volatiles is positively correlated with the amount of fat in highly marbled beef. Therefore, this study aims to investigate the effect of QG on beef aroma profile using electronic nose data and a chemometric approach. An electronic nose with metal oxide semiconductors was used, and discrimination was performed using multivariate analysis, including principal component analysis and hierarchical clustering. The *M. longissimus lumborum* (striploin) of QG 1++, 1+, 1, and 2 of Hanwoo steers (n=6), finished under identical feeding systems on similar farms, were used. In contrast to the proportion of monounsaturated fatty acids (MUFAs), the abundance of volatile compounds and the proportion of polyunsaturated fatty acids (PUFAs) decreased as the QG increased. The aroma profile of striploin from carcasses of different QGs was well-discriminated. QG1++ was close to QG1+, while QG1 and QG2 were within a cluster. In conclusion, aroma development in beef is strongly influenced by fat deposition, particularly the fat-to-lean muscle ratio with regard to the proportion of PUFA. As MUFA slows down the oxidation and release of volatile compounds, leaner beef containing a higher proportion of PUFA produces more volatile compounds than beef with a higher amount of intramuscular fat.

Keywords   hierarchical clustering, lipid oxidation, marbling, multivariate analysis, principal component analysis, volatile compounds

Introduction

Studies on the effect of fat content on the volatile composition of meat have focused on processed meat products, such as meat batter, frankfurter, and ham (Domínguez et al., 2017; Jo et al., 1999; Sirtori et al., 2021). Meanwhile, studies on the effect of

carcass quality grade (QG) or the intramuscular fat (IMF) level on the volatile profile of beef are still limited. Fat content in beef is positively associated with taste preference (Frank et al., 2016). Further study is necessary in order to provide more scientific evidence to clarify whether the abundance of aroma volatiles is positively correlated with the fat content in highly marbled beef.

In Korea, Hanwoo steers are finished on a high-energy diet and slaughtered at the age of 30–32 months, so that the marbling score and fat content of the highest QG loin can reach above 7% and 20%, respectively (Koh et al., 2019). The QG, which is determined by the marbling score, influences the generation of beef volatile compounds. Piao et al. (2017) reported that the release of some volatile compounds is affected by the QG of Hanwoo beef. The deposition of fat to muscle is affected by genetic factors; even though the fat amount is similar, beef from different breeds have different aroma profiles (Utama et al., 2018). Moreover, IMF content could influence the generation of volatile compounds and the release of such compounds from the matrix of the meat (Echegaray et al., 2021).

Multivariate analysis can help interpret the data for classification. Principal component analysis (PCA) and cluster analysis (CA) are often used to simplify large amounts of data for a better understanding. However, as these tools are unsupervised statistical methods, it is inappropriate to correlate the content of bioactive compounds with *in vitro* functional properties (Granato et al., 2018; Nunes et al., 2015). PCA has been widely applied as an adaptive descriptive data analysis tool to investigate the authenticity of food and to determine some intrinsic and extrinsic effects on food quality based on their chemical traits, including the aroma volatile compounds (Kebede et al., 2018; Procida et al., 2005; Suslick et al., 2010; Wang et al., 2014). In addition, hierarchical clustering, a part of CA, helps to identify the origin of the food, the diversity of microorganisms in the food, and ensures the authenticity and quality of the food (Danezis et al., 2016; Granato et al., 2018). Therefore, the objective of this study was to investigate, using sensor data from an electronic nose and a chemometrics approach, whether the differences in the fat to lean muscle ratio (carcass QG) of highly marbled beef contribute to the distinct aroma profile.

Materials and Methods

Sample preparation

The *M. longissimus lumborum* (striploin) of grade 1++, 1+, 1, and 2 Hanwoo steers (n=6), finished under identical feeding systems on a similar farm, were removed from the left side of the carcasses after 24 h of chilling. The striploin was chosen because this cut is usually used for roasts and grills. Samples were vacuum-packed and distributed to the laboratory in an icebox. Proximate composition, pH, color, and fatty acid analyses were performed on day 4 after postmortem. The remaining sample was lyophilized using a benchtop freeze dryer (Eyela FDU-1200, Tokyo Rikakikai, Tokyo, Japan) and stored at −24°C for analysis of volatile compounds and aroma patterns. The dry sample was used to avoid the effect of different moisture contents among QGs.

Proximate composition analysis

Samples were ground using a food blender at minimum speed for 10 s (HMF-1600PB, Hanil Electric, Wonju, Korea). The proximate composition was determined using the AOAC official methods (AOAC, 2002). Moisture content was determined by dry-heating the samples at 105°C for 24 h and calculating the proportion of weight loss during heating per fresh weight. Crude fat content was determined by ether extraction using a Soxhlet system. Nitrogen content was determined using the

Kjeltec system (2200 Kjeltec Auto Distillation Unit, Foss, Huddinge, Sweden), and crude protein was calculated by multiplying the nitrogen content by 6.25. The ash content was determined by burning the samples in a muffle furnace at 550°C for 16 h.

Fatty acid composition analysis

Meat fat was extracted from the samples using a chloroform-methanol (2:1 v/v) solution and prepared in triplicate (Folch et al., 1957). Fatty acid methyl esters (FAMEs) were prepared in hexane by mixing saponified fat (added with 1 N KOH) with boron trifluoride at 80°C. The fatty acid composition of beef fat was determined using an Agilent gas chromatography system (6890N, Agilent Technologies, Santa Clara, CA, USA). The sample (1 μL) was injected into the GC port using an autosampler (7683, Agilent Technologies). A split ratio of 100:1 was programmed for the inlet and the temperature was set to 250°C. FAMEs were separated using a WCOT-fused silica capillary column (100 m×0.25 mm i.d., 0.20 μm film thickness; Varian Medical Systems, Palo Alto, CA, USA) with a 1.0 mL/min helium flow. The oven temperature and holding-time were programmed as follows: 150°C/1 min, 150°C–200°C at 7°C/min, 200°C/5 min, 200°C–250°C at 5°C/min, and 250°C/10 min. The temperature of the detector was set to 280°C. The peaks were identified as fatty acids using the retention time of the fatty acid standards (47015-U, Sigma-Aldrich, Saint Louis, MO, USA). The peak area of each identified fatty acid was used to calculate the proportion (%) of the total identified peak area.

Volatile compound identification and aroma profiling

The volatile compounds from heated samples were separated and identified by gas chromatography-mass spectrometry using a modified version of the method described by Ba et al. (2010). Approximately 1 g of dry sample (prepared in duplicate) was immediately placed in a 50 mL headspace vial and heated at 105°C in a drying oven for 10 min to release the volatile compounds. Prior to extraction, the sample was calibrated to 60°C in a drying oven for 10 min. The carboxen®/polydimethylsiloxane fiber (Supelco, Sigma-Aldrich) with a diameter of 75 μm was injected into the vial for extraction for 30 min. Following extraction, the fiber was injected into the inlet, which was set to 250°C. The split ration of 1:5 was used for desorbing the volatile compounds for 5 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Separation of the individual compound was performed using a DB5 fused silica column (30 m×0.25 mm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) in a gas chromatograph (7890A, Agilent Technologies). The GC oven was set to operate at an initial temperature of 40°C for 2 min, increased to 160°C (at rate of 5°C/min), then to 180°C (at rate of 6°C/min, holding time of 5 min), and finally to 200°C (at rate of 10°C/min, holding time of 5 min). The interface and quadruple temperatures were set at 280°C and 150°C, respectively. Volatile compounds were detected using a mass spectrometer (5975C, Agilent Technologies). The ion source temperature of the MS was set to 280°C with an electron impact of 70 eV. A scanning mass range of 50–450 m/z with a scan rate of 1 scan/s was used. Identification was performed by comparing the experimental spectra with the National Institute of Standards and Technology (NIST) mass spectral library. Data are presented as area units (AU)×10^6/g.

An electronic nose (FOX3000, Alpha MOS, Toulouse, France) was used for analyzing the aroma pattern. Dry and heated samples (0.5 g) were placed in a 10 mL headspace vial and prepared in duplicate. The vial was sealed with a rubber septa cap (Supelco 29176-U, Sigma-Aldrich). The samples were heated at 60°C for 600 s at an agitation speed of 500 rpm. The 2.5 mL of headspace gas was extracted with an automatic sampler syringe (HS 100, Alpha MOS) at 65°C, flow-injected into the port of the electronic nose with synthetic air as the carrier gas (pressure was set to 0.5 bar with 150 mL/min flow rate) and

detected by a metal oxide sensor array system with an acquisition time of 150 s. The following sensors were chosen (T30/1, P10/1, P10/2, P40/1, T70/2, PA2) as the sensitivity against fat-derived volatile compounds are high. The sensor resistance ratio $(r-r_0)/r_0$ was calculated (r is the real-time resistance and r_0 is the sensor's resistance baseline). The time taken to return to baseline after acquisition was 1,080 s. The maximum resistance ratio was considered as the data value of a single measurement.

Statistical analysis

The statistically significant difference between the mean values from different QGs was determined using a one-way analysis of variance (ANOVA). The mean values were then separated by Duncan's multiple range test at a 5% significance level. Correlation coefficients between the resistance ratio of the six metal sensors of the electronic nose and the peak area of the volatile groups were determined using Pearson's method. Multiple regression analysis was also performed to determine the multiple correlations between the resistance ratio of the six metal sensors of the electronic nose and the peak area of the volatile groups. Two-dimensional PCA and cluster dendrograms were used to discriminate the aroma profile according to the sensor resistance ratio. Analyses were performed using R-version 3.3.3 (R Core Team, 2018) with the "agricolae" package for Duncan's multiple range test (De Mandiburu, 2017) and with the "dendextend", "ggfortify", and "ggplot2" packages for plotting the PCA and cluster dendrogram (Galili, 2015; Tang et al., 2016; Wickham, 2016).

Results and Discussion

The proximate composition of beef striploins of different QGs is presented in Table 1. Among the QGs, moisture and protein content decreased as the QG increased. In contrast, the crude fat content increased as the QG increased. Different carcass QGs showed different fat-to-lean muscle ratios, and the ratio increased linearly as the QG increased. No differences were found in ash content among the QGs. These findings are in accordance with those of previous reports by Piao et al. (2017) and Koh et al. (2019).

The fatty acid composition of Hanwoo beef, categorized by different QGs, is shown in Table 2. No differences were found in the proportions of saturated fatty acids. However, QG 1++ had the lowest proportion of palmitic acid (C16:0; p=0.04). The highest proportion of monounsaturated fatty acids (MUFAs) was found in beef with the highest QG (1++). A higher oleic acid (C18:1n9) proportion was observed in grade 1++ striploin than in lower QGs, contributing to the increased proportion of MUFAs. In contrast with MUFA, the polyunsaturated fatty acid (PUFA) proportion was found to be lower in higher QGs.

Table 1. Proximate composition of beef striploin as affected by carcass quality grade

Variable	Quality grade				SEM	p-value
	1++	1+	1	2		
Moisture (%)	61.4[c]	64.2[b]	66.8[a]	68.0[a]	1.11	<0.001
Crude fat (%)	24.5[a]	19.7[b]	14.4[c]	12.2[c]	1.53	<0.001
Crude protein (%)	12.9[c]	15.0[bc]	17.6[ab]	18.7[a]	0.75	0.01
Ash (%)	1.17	1.08	1.12	1.14	0.02	0.39

Sample size; each quality grade (n=6).
Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).
[a-c] Different superscripts in the same row indicate differences among quality grades (p<0.05).

Table 2. Fatty acid composition (%) of beef striploin as affected by carcass quality grade

Fatty acid	Quality grade				SEM	p-value
	1++	1+	1	2		
C14:0	3.34	3.11	2.88	2.81	0.07	0.35
C16:0	27.9[b]	29.3[a]	29.6[a]	29.2[a]	0.21	0.04
C16:1n7	4.80	4.32	4.73	4.15	0.09	0.74
C18:0	11.08	12.24	12.2	13.6	0.30	0.42
C18:1n9	50.4[a]	49.4[ab]	48.9[ab]	47.8[b]	0.39	0.01
C18:2n6	1.16[b]	1.21[b]	1.22[b]	1.78[a]	0.08	0.02
C18:3n6	0.07	0.08	0.09	0.09	0.00	0.33
C18:3n3	0.10[c]	0.11[c]	0.15[b]	0.25[a]	0.02	<0.001
C20:4n6	0.09[b]	0.09[b]	0.10[b]	0.13[a]	0.01	0.03
C22:4n6	0.04	0.04	0.05	0.05	0.00	0.14
SFA	42.4	44.7	44.7	45.6	0.40	0.21
MUFA	56.2[a]	53.8[ab]	53.6[ab]	52.0[b]	0.49	0.02
PUFA	1.45[b]	1.54[b]	1.62[b]	2.30[a]	0.11	0.02
n6	1.36[b]	1.43[b]	1.46[b]	2.05[a]	0.09	0.03
n3	0.10[c]	0.11[c]	0.15[b]	0.25[a]	0.02	<0.001
n6/n3	14.3[a]	13.3[a]	9.60[ab]	8.25[b]	0.83	0.01

Sample size; each quality grade (n=6).
Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).
[a–c] Different superscripts in the same row indicate differences among quality grades (p<0.05).
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

This is mainly attributed to the higher proportion of linoleic acid (C18:2n6) and arachidonic acid (C20:4n6) in lower-grade striploin. The ratio of omega-6 to omega-3 was found to be higher in beef with higher QG as the α-linolenic acid (C18:3n3) content decreased. Wood et al. (2008) mentioned that neutral lipids are predominantly deposited into intramuscular adipose tissue to build marbling, whereas PUFAs are mostly deposited into the membrane of muscle cells as cell membranes are built by phospholipids. Cho et al. (2020) reported that coarsely marbled Hanwoo beef loins contain higher proportions of PUFAs than the finer ones, which corresponds to linoleic acid (C18:2n6) and eicosapentaenoic acid (C20:5n3). In other words, the proportion of PUFA increases as the meat cut has more muscle area or tends to be coarse in appearance. PUFAs have a lower melting point and are stable in liquid form at ambient temperature, thus establishing the elasticity of muscle cells to contract and relax (Abbott et al., 2012). Previous studies have reported that oleic acid is the major fatty acid in highly marbled Hanwoo beef, and this fatty acid may contribute to a more acceptable flavor (Jo et al., 2013). Furthermore, this study confirms that the proportion of oleic acid in Hanwoo striploin increases with an increase in IMF content or carcass QG, as previously reported (Joo et al., 2017; Lim et al., 2014; Piao et al., 2017).

Three major groups of volatile compounds were identified from different QGs of Hanwoo beef striploin (Table 3). Pyrazine and aldehyde were the two predominant volatile compounds, as the peak areas of these volatile groups were higher than those of hydrocarbons. Lyophilized samples (dry, with low water activity) were used in this study, and the occurrence of the Maillard reaction, which produces pyrazines, was high. Water activity is one of the many factors affecting the rate of the Maillard reaction. The maximum reaction can occur under low water activity conditions (Labuza and Saltmarch, 1981). The

Table 3. Aroma volatile compounds (area unit x 10^6) released from beef striploin as affected by carcass quality grade

Compound name	Quality grade				SEM	p-value
	1++	1+	1	2		
Aldehydes						
2-Methyl butanal	11.0[ab]	15.5[a]	8.06[b]	16.9[a]	1.26	0.03
3-Methyl butanal	14.4	19.3	14.5	21.9	1.54	0.23
Hexanal	18.7	25.6	27.0	28.5	1.68	0.18
Heptanal	3.55	3.19	3.19	4.26	0.19	0.17
Benzaldehyde	3.69[c]	5.95[b]	6.63[b]	14.1[a]	1.17	<0.01
Nonanal	3.32	3.34	3.30	3.44	0.16	0.99
Hydrocarbons						
Toluene	4.84[c]	7.90[bc]	9.50[b]	13.9[a]	0.91	<0.001
Styrene	5.02[b]	5.75[b]	8.40[ab]	11.3[a]	0.70	0.02
Dodecane	1.38	1.48	1.05	1.26	0.10	0.21
Pentadecane	0.87[a]	0.82[a]	0.43[b]	0.50[b]	0.05	0.03
Pyrazines						
Pyrazine	1.29[bc]	1.88[a]	1.01[c]	1.80[ab]	0.11	0.01
2-Methyl pyrazine	23.0[b]	30.9[b]	32.9[b]	56.0[a]	4.07	0.01
2,5-Dimethyl pyrazine	18.3[b]	26.0[b]	72.6[a]	62.9[a]	6.72	<0.01
2-Ethyl-6-methyl pyrazine	0.77[b]	0.68[b]	1.56[a]	1.59[a]	0.13	<0.01
2,3,5-Trimethyl pyrazine	2.14[b]	2.89[b]	2.93[b]	6.80[a]	0.54	0.03
3-Ethyl-2,5-dimethyl pyrazine	0.62[c]	0.87[c]	2.26[b]	6.10[a]	0.63	<0.01
Total	112.9	152.1	195.3	251.3	16.1	<0.01

Sample size; each quality grade (n=6).
Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).
[a–c] Different superscripts in the same row indicate differences among quality grades (p<0.05).

proportions of pyrazine and aldehyde ranged from 41%–58% and 32%–48%, respectively. The hydrocarbon content was the third most abundant, ranging from 10%–11%.

Lower grade (QG1 and QG2) striploin released more fatty and meaty flavor aldehydes and hydrocarbons (in AUs), such as 2- and 3-methyl butanal, hexanal, heptanal, nonanal, dodecane, and pentadecane, although the proportion of aldehyde groups was higher in higher quality grades (QG1++ and QG1+). The proportion of aldehydes increased as the QG (IMF content) increased. The fat content in emulsion systems and meat products slows down the release of polar volatile compounds, such as aldehydes, ketones, and alcohols (Jo and Ahn, 1999; Jo et al., 1999). Thus, the present results confirm previous findings (Jo and Ahn, 1999; Jo et al., 1999). Aldehyde is also one of the products of the Maillard reaction at high temperatures and is derived from the thermal degradation of unsaturated fatty acids, such as linoleic and linolenic acids (Elmore et al., 2004). Some aldehydes possess pleasant flavors, such as fatty, roasted meat, and an almond-like aroma based on olfactory analysis (Xie et al., 2008). Ba et al. (2012) found that the *longissimus* tissue of Hanwoo released high amounts of aldehydes. Furthermore, Frank et al. (2016) reported that the proportion of most aliphatic aldehydes increases as the polar lipid content increases. These results indicate that the major aldehydes from leaner striploins were mainly derived from lipid oxidation of

muscle cell membrane phospholipids.

Among pyrazines, 2,5-dimethylpyrazine was the most abundant volatile in leaner striploin, comprising more than 30% of the total volatile compounds, followed by styrene, a hydrocarbon, which was remarkably higher than that of higher QGs. Pyrazines are generally the products of the Maillard reaction between free amino acids and reducing sugars (Yu et al., 2021). The flavor characteristics of pyrazines are roasted and nutty, and are mostly found in roasted beef, coffee beans and nuts (Mortzfeld et al., 2020). This suggests that the roasted aroma from aldehydes in lower-QG Hanwoo striploin was obtained from pyrazines. Mottram and Edwards (1983) reported that the amount of pyrazines is negatively associated with the presence of the lipid fraction in beef. Therefore, the present results are in line with those of previous reports. Hydrocarbons, which are the main products of the oxidation of polyunsaturated fatty acids through thermal degradation, were higher in leaner striploins. This can also be associated with the higher proportion of PUFAs in lower QG striploin than in higher QGs. Legako et al. (2015) and Hunt et al. (2016) reported that higher QG beef is associated with more neutral lipids (MUFA) than polar lipids (PUFA).

From electronic nose sensor data, the findings from gas chromatography can be associated with the highest intensity of beef volatile compounds released from the lowest QG group, wherein a significant proportion of pyrazines was observed. The sensor resistance ratios of the volatile compounds in the headspace derived from the heated samples are shown in Fig. 1. The resistance ratios of T30/1, P10/1, P10/2, P40/1, T70/2, and PA2 were significantly higher in the lower QG, indicating significant differences in the intensity of volatile compounds. The clustering is clear, indicating statistical discrimination (Utama et al., 2017). Among the volatile groups, aldehydes were positively correlated with the resistance ratio of the T30/1, P10/2, T70/2, and PA2 sensors, while hydrocarbons and pyrazines were positively correlated with the resistance ratio of all sensors (Table 4). Multiple regression models revealed that the combination of all volatile groups showed significant regression with the resistance ratio of each sensor (Table 5). However, each volatile group independently affected the resistance ratio of all sensors. Although the regression model is significant, the linearity or accuracy ($0.57 < r^2 < 0.62$) shows that the model is not good enough to predict the response of the sensor using the abundance of volatile groups.

The PCA plot (Fig. 2) and cluster dendrogram (Fig. 3) revealed that the aroma profile differed according to QG. The
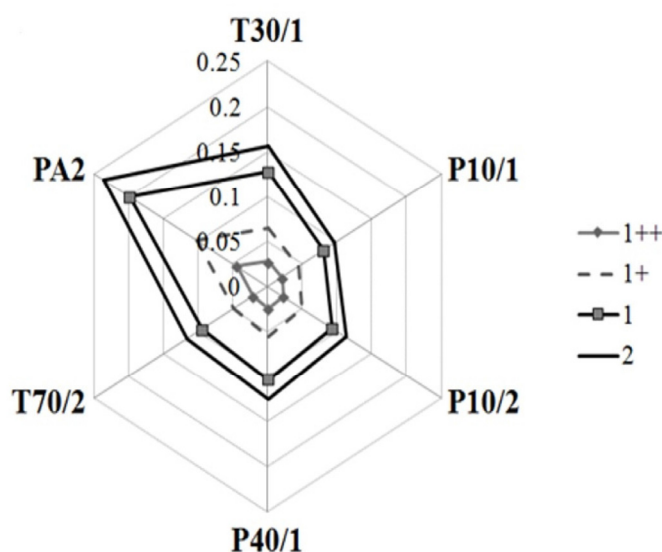

Fig. 1. Differences in aroma intensity among quality grades. Data are shown as mean of each sensor's resistance ratio. Metal oxide sensors; T30/1, P10/1, P10/2, P40/1, T70/2, PA2. Sample size for each quality grade (n=6). Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).

Table 4. Correlation coefficients between the volatile groups and resistance ratio of six metal sensors of electronic nose

Major volatile group	Sensor					
	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Aldehydes	0.34[*]	0.32	0.33[*]	0.33	0.34[*]	0.36[*]
Hydrocarbons	0.56[***]	0.55[***]	0.56[***]	0.56[***]	0.56[***]	0.58[***]
Pyrazines	0.61[***]	0.58[***]	0.59[***]	0.59[***]	0.61[***]	0.63[***]

Sample size for each quality grade (n=6).
[*] $p<0.05$, [***] $p<0.001$.

Table 5. Multiple regression models for resistance ratio of six metal sensors of electronic nose using the measured peak area of volatile groups as covariate

Sensor	Intercept	Covariate			r^2	p-value
		Aldehydes	Hydrocarbons	Pyrazines		
T30/1	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.60	<0.001
P10/1	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.57	<0.001
P10/2	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.59	<0.001
P40/1	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.58	<0.001
T70/2	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.60	<0.001
PA2	<0.001	<0.001[*]	<0.001[***]	<0.001[***]	0.62	<0.001

Sample size for each quality grade (n=6).
[*] $p<0.05$, [***] $p<0.001$.
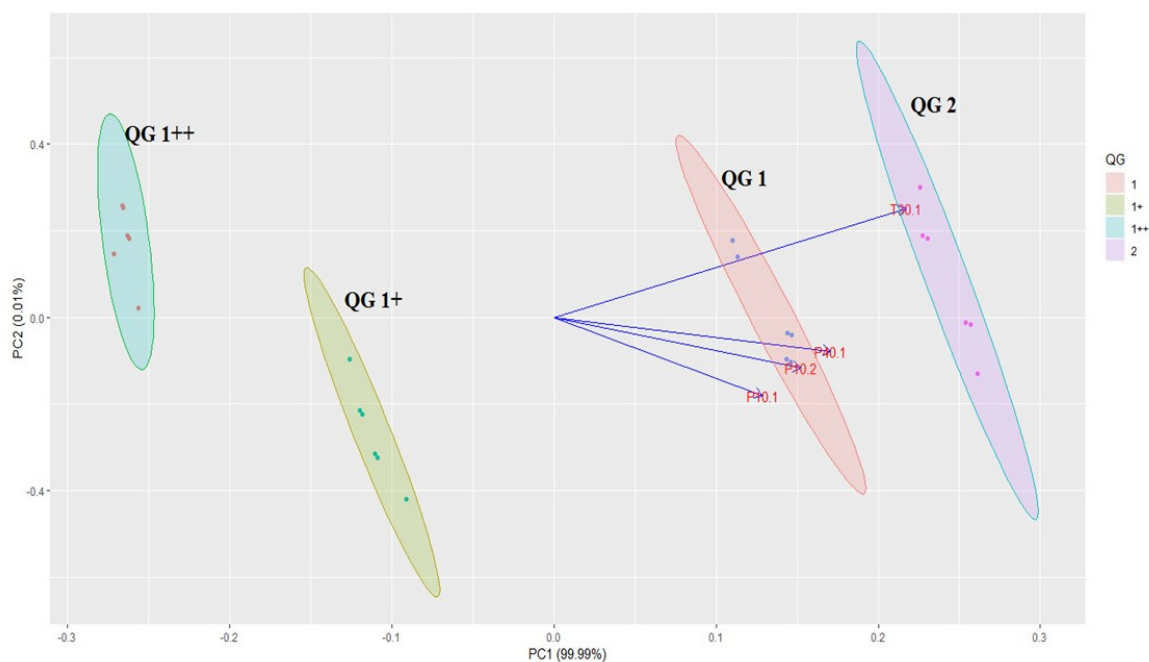
Fig. 2. Principal component analysis plot of the aroma profile of different quality grades (QG). Total contribution of principal component 1 and 2 (PC1 and PC2) is 100%, which means that 100% of data variance is explained. Loading plots; T30/1, P10/1, P10/2, P40/1, T70/2, PA2, are the intensity of the response of the sensor. Sample size for each quality grade (n=6). Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).
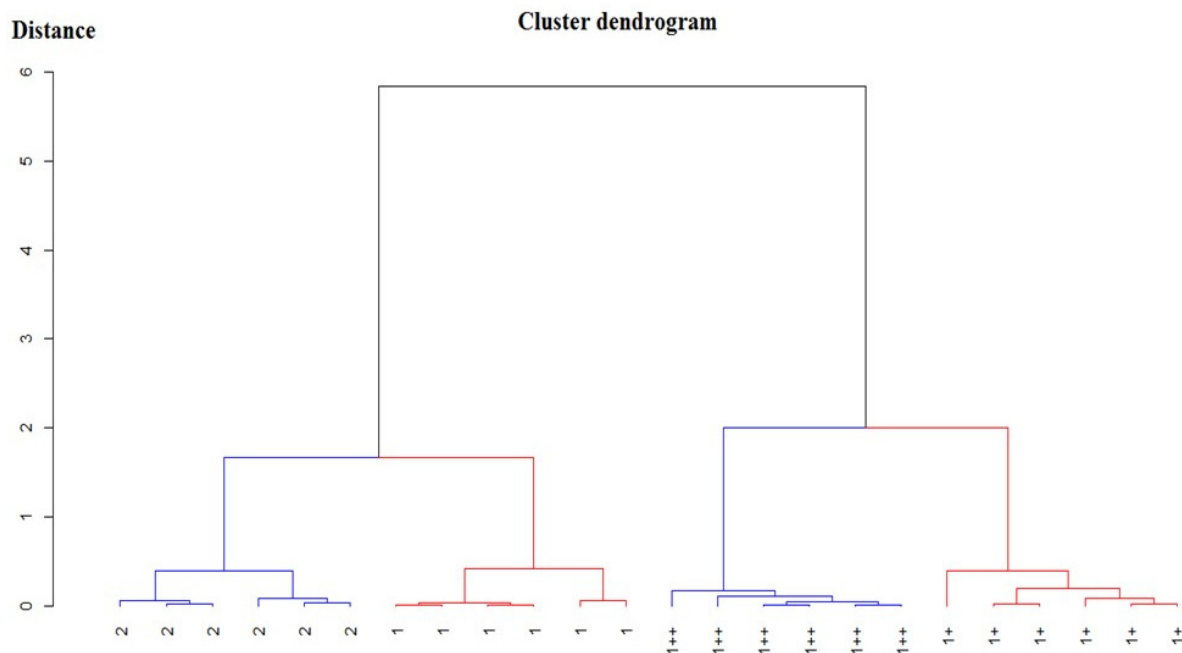
Fig. 3. Cluster dendrogram of the aroma profile of different quality grades. Sample size for each quality grade (n=6). Carcass quality grade (1++, 1+, 1, and 2) was assessed according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2017).

loading plots and the resistance ratio of the sensors led to a group with a high intensity of volatile release. The aroma profile of striploin with different QGs was well-discriminated, indicating that marbling or the fat to lean muscle ratio affects the release of volatile compounds. However, the cluster dendrogram shows that the aroma profile between the higher quality grades (QG1++ and QG1+) and the lower quality grades (QG1 and QG2) is close to each other with a smaller distance than that between the higher QG group and lower QG groups.

Conclusion

The aroma profile of beef according to carcass QG can be discriminated using chemometrics approach. The higher the QG, the less abundant volatile compounds released from the beef. The chemometrics approach helps to confirm the effect of fat deposition on the differences in the aroma profiles of beef. The correlation between the sensor resistance ratio or the response of the electronic nose and the abundance of volatile compounds is strongly dependent on the intensity of the volatile compounds. Therefore, to predict the abundance of individual volatile compounds using the response of each sensor, pre-treatments, such as temperature adjustment prior to the extraction of volatile compounds, should be considered.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

Author Contributions

Ethics Approval

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

References

Abbott SK, Else PL, Atkins TA, Hulbert AJ. 2012. Fatty acid composition of membrane bilayers: Importance of diet polyunsaturated fat balance. Biochim Biophys Acta Biomembr 1818:1309-1317.

AOAC. 2002. Official methods of analysis of AOAC International. 19th ed. AOAC International. Gaithersburg, MD, USA.

Ba HV, Hwang IH, Jeong D, Touseef A. 2012. Principle of meat aroma flavors and future prospect. In Latest research into quality control. Akyar I (ed). IntechOpen, Rijeka, Croatia. pp 145-176.

Ba HV, Oliveros MC, Ryu KS, Hwang IH. 2010. Development of analysis condition and detection of volatile compounds from cooked Hanwoo beef by SPME-GC/MS analysis. Korean J Food Sci Anim Resour 30:73-86.

Cho S, Lee W, Seol KH, Kim Y, Kang SM, Seo H, Jung Y, Kim J, Van Ba H. 2020. Comparison of storage stability, volatile compounds and sensory properties between coarsely- and finely-marbled 1+ grade Hanwoo beef loins. Food Sci Anim Resour 40:497-511.

Danezis GP, Tsagkaris AS, Camin F, Brusic V, Georgiou CA. 2016. Food authentication: Techniques, trends & emerging approaches. TrAC Trends Analyt Chem 85:123-132.

De Mandiburu F. 2017. Agricolae: Statistical procedures for agricultural research. R package version 1.2-8. Available from https://CRAN.R-project.org/package=agricolae. Accessed at Nov 1, 2018.

Domínguez R, Pateiro M, Agregán R, Lorenzo JM. 2017. Effect of the partial replacement of pork backfat by microencapsulated fish oil or mixed fish and olive oil on the quality of frankfurter type sausage. J Food Sci Technol 54:26-37.

Echegaray N, Domínguez R, Cadavez VAP, Bermúdez R, Pateiro M, Gonzales-Barron U, Lorenzo JM. 2021. Influence of feeding system on *Longissimus thoracis et lumborum* volatile compounds of an Iberian local lamb breed. Small Rumin Res 201:106417.

Elmore JS, Warren HE, Mottram DS, Scollan ND, Enser M, Richardson RI, Wood JD. 2004. A comparison of the aroma volatiles and fatty acid compositions of grilled beef muscle from Aberdeen Angus and Holstein-Friesian steers fed diets based on silage or concentrates. Meat Sci 68:27-33.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497-509.

Frank D, Ball A, Hughes J, Krishnamurthy R, Piyasiri U, Stark J, Watkins P, Warner R. 2016. Sensory and flavor chemistry

characteristics of Australian beef: influence of intramuscular fat, feed, and breed. J Agric Food Chem 64:4299-4311.

Galili T. 2015. Dendextend: An R package for visualizing, adjusting, and comparing trees of hierarchical clustering. Bioinformatics 31:3718-3720.

Granato D, Santos JS, Escher GB, Ferreira BL, Maggio RM. 2018. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. Trends Food Sci Technol 72:83-90.

Hunt MR, Legako JF, Dinh TT, Garmyn AJ, O'quinn TG, Corbin CH, Rathmann RJ, Brooks JC, Miller MF. 2016. Assessment of volatile compounds, neutral and polar lipid fatty acids of four beef muscles from USDA choice and select graded carcasses and their relationships with consumer palatability scores and intramuscular fat content. Meat Sci 116:91-101.

Jo C, Ahn DU. 1999. Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/ gas chromatography. J Food Sci 64:641-643.

Jo C, Ahn DU, Lee JI. 1999. Lipid and cholesterol oxidation, color changes, and volatile production in irradiated raw pork batters with different fat content. J Food Qual 22:641-651.

Jo C, Jayasena DD, Lim DG, Lee KH, Kim JJ, Cha JS, Nam KC. 2013. Effect of intramuscular fat content on the meat quality and antioxidative dipeptides of Hanwoo beef. Korean J Food Nutr 26:117-124.

Joo ST, Joo SH, Hwang YH. 2017. The relationships between muscle fiber characteristics, intramuscular fat content, and fatty acid compositions in *M. longissimus lumborum* of Hanwoo steers. Korean J Food Sci Anim Resour 37:780-786.

Kebede B, Lee PY, Leong SY, Kethireddy V, Ma Q, Aganovic K, Eyres GT, Hamid N, Oey I. 2018. A chemometrics approach comparing volatile changes during the shelf life of apple juice processed by pulsed electric fields, high pressure and thermal pasteurization. Foods 7:169.

Koh KC, Chung KY, Kim HS, Kang SJ, Choi CB, Jo C, Choe J. 2019. Determination of point of sale and consumption for Hanwoo beef based on quality grade and aging time. Food Sci Anim Resour 39:139-150.

Korea Institute for Animal Products Quality Evaluation [KAPE]. 2017. The beef carcass grading. Available from http://www.ekapepia.com/. Accessed at Nov 6, 2018.

Labuza TP, Saltmarch M. 1981. The nonenzymatic browning reaction as affected by water in foods. In Water activity: Influences on food quality: A treatise on the influence of bound and free water on the quality and stability of foods and other natural products. Rockland LB, Stewart GF (ed). Academic Press, New York, NY, USA. pp 605-650.

Legako JF, Dinh TTN, Miller MF, Brooks JC. 2015. Effects of USDA beef quality grade and cooking on fatty acid composition of neutral and polar lipid fractions. Meat Sci 100:246-255.

Lim DG, Cha JS, Jo C, Lee KH, Kim JJ, Nam KC. 2014. Comparison of physicochemical and functional traits of Hanwoo steer beef by the quality grade. Korean J Food Sci Anim Resour 34:287-296.

Mortzfeld FB, Hashem C, Vranková K, Winkler M, Rudroff F. 2020. Pyrazines: Synthesis and industrial application of these valuable flavor and fragrance compounds. Biotechnol J 15:2000064.

Mottram DS, Edwards RA. 1983. The role of triglycerides and phospholipids in the aroma of cooked beef. J Sci Food Agric 34:517-522.

Nunes CA, Alvarenga VO, de Souza Sant'Ana A, Santos JS, Granato D. 2015. The use of statistical software in food science and technology: Advantages, limitations and misuses. Food Res Int 75:270-280.

Piao MY, Yong HI, Lee HJ, Fassah DM, Kim HJ, Jo C, Baik M. 2017. Comparison of fatty acid profiles and volatile compounds among quality grades and their association with carcass characteristics in *longissimus dorsi* and

semimembranosus muscles of Korean cattle steer. Livest Sci 198:147-156.

Procida G, Giomo A, Cichelli A, Conte LS. 2005. Study of volatile compounds of defective virgin olive oils and sensory evaluation: A chemometric approach. J Sci Food Agric 85:2175-2183.

R Core Team. 2018. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. Available from: http://www.R-project.org. Accessed at Nov 1, 2018.

Sirtori F, Aquilani C, Dimauro C, Bozzi R, Franci O, Calamai L, Pezzati A, Pugliese C. 2021. Characterization of subcutaneous fat of Toscano dry-cured ham and identification of processing stage by multivariate analysis approach based on volatile profile. Animals 11:13.

Suslick BA, Feng L, Suslick KS. 2010. Discrimination of complex mixtures by a colorimetric sensor array: Coffee aromas. Anal Chem 82:2067-2073.

Tang Y, Horikoshi M, Li W. 2016. Ggfortify: Unified interface to visualize statistical result of popular R packages. R J 8:474-485.

Utama DT, Lee CW, Park YS, Jang A, Lee SK. 2018. Comparison of meat quality, fatty acid composition and aroma volatiles of Chikso and Hanwoo beef. Asian-Australas J Anim Sci 31:1500-1506.

Utama DT, Lee SG, Baek KH, Chung WS, Chung IA, Jeon JT, Lee SK. 2017. High pressure processing for dark-firm-dry beef: Effect on physical properties and oxidative deterioration during refrigerated storage. Asian-Australas J Anim Sci 30:424-431.

Wang Q, Jin G, Jin Y, Ma M, Wang N, Liu C, He L. 2014. Discriminating eggs from different poultry species by fatty acids and volatiles profiling: Comparison of SPME-GC/MS, electronic nose, and principal component analysis method. Eur J Lipid Sci Technol 116:1044-1053.

Wickham H. 2016. Ggplot2: Elegant graphics for data analysis. Springer Publishing, NY, USA. pp 244-247.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci 78:343-358.

Xie J, Sun B, Zheng F, Wang S. 2008. Volatile flavor constituents in roasted pork of mini-pig. Food Chem 109:506-514.

Yu H, Zhang R, Yang F, Xie Y, Guo Y, Yao W, Zhou W. 2021. Control strategies of pyrazines generation from Maillard reaction. Trends Food Sci Technol 112:795-807.