AROMA PATTERN AND VOLATILE COMPOUNDS OF BEEF FROM GRAIN-FED HANWOO AND GRASS-FED HOLSTEIN STEERS

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Abstract – This study was aim to identify aroma volatile compounds and distinguish the aroma pattern and intensity of *longissimus lumborum* from grain-fed Hanwoo (grade 1, n = 5) and grass-fed Holstein (undergrade, n = 5) steers. Samples were collected from commercial meat plant 48-h postmortem. Aroma analyses of freeze-dried samples were done using electronic nose and gas chromatography-mass spectrometry (GC-MS). Samples were preheated at 105 °C for 30 min prior to analysis. Electronic nose successfully discriminated the aroma pattern of Hanwoo and Holstein beef. Although the beef of grain-fed Hanwoo had higher fat content than grass-fed Holstein ($p<0.001$), stronger aroma intensity was detected in grass-fed Holstein beef; it was then observed as the effect of the abundance of polyunsaturated fatty acids in grass-fed beef. Among identified volatile compounds, 2-methylpyrazine, 2,5-dimethylpyrazine and 2-butyl-3-methylpyrazine were three most abundant volatile compounds detected from Hanwoo beef, while 2,5-dimethyl-3-ethylpyrazine and 3-dimethyl-aminopyridine were more abundant in grass-fed Holstein beef. Different proportion of meat fatty acids, particularly monounsatureated and polyunsaturated fatty acids, may influence the relative composition of aroma volatile compounds. However, fat content did not have any contribution to aroma intensity.

Key Words – Aroma, beef, Hanwoo, Holstein.

I. INTRODUCTION

Appearance, flavor and tenderness of the meat are the main factors affecting consumer's decision to purchase meat. Flavor is a very complex attribute influencing the palatability of food. It comprises of taste and aroma. Some review articles mentioned that breed, sex, age, diet, processing and storage condition influence meat flavor [1, 2]. As feeding high-energy diet in order to produce high-quality beef from dairy industry is quite expensive, utilizing grass haylage from existing pasture around the industry to dairy steers is considered more efficient and tended to give advantage in producing lean and healthy beef with recommended omega-6 to omega-3 ratio. However, the flavor of grass-fed beef may be different to that of grain-fed beef. Hanwoo beef is renowned as the most favorable and exclusive among Korean society with premium price compared with beef from other breeds. This study was aim to identify the differences of aroma pattern and volatile compounds of beef from grain-fed Hanwoo and grass-fed dairy steers.

II. MATERIALS AND METHODS

Sample preparation and proximate analyses

Animals were slaughtered in a commercial meat plant using standard procedure. The *longissimus lumborum* muscle of grain-fed Hanwoo (grade 1, n = 3) and grass-fed Holstein (undergrade, n = 5) steers was removed from carcasses 48-h postmortem. Samples were vacuum-packed, distributed to laboratory within ice box and immediately arranged for proximate and fatty acid composition. Proximate composition was determined by AOAC official methods [3]. The remaining samples were ground and freeze-dried. The freeze-dried samples were used for aroma analyses through electronic nose and gas chromatography coupled with mass spectrometry (GC-MS).

Fatty acid composition

Fatty acid composition was determined using a gas chromatograph (YL6500, YL Instrument, Korea). Meat fat was extracted according to Folch et al. [4] using chloroform-methanol (2:1 v/v). Sample was prepared in duplicate. Fatty acids were converted into methyl esters as described by AOAC method [3]. Fatty acid methyl esters were dissolved in 2 mL of hexane. One μL of sample was injected into the column with split mode (1:5). Fatty acid methyl esters were separated using a WCOT fused silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness; Varian Inc., Lake Forest, CA, USA) with a 1.0 mL/min of helium flow. The oven temperature was increased from 150 to 250 °C at increasing rate of 10 °C/min. Temperatures of the injector and detector were 250 °C and 275 °C, respectively. The fatty acid peaks were identified and quantified by comparison with the retention time and peak area of fatty acid standards (47015-U, Supelco, Bellefonte, PA, USA).

Aroma pattern

A total of 0.5 g of freeze-dried sample was weighed into 10 ml-headspace vial and prepared in triplicate. Samples were heated in oven for 30 min at 105 °C. The vials were then adjusted to 60 °C within electronic nose-coupled oven for 10 min. The 2.5 mL-gas in the headspace of the samples was extracted by the automatic sampler syringe (HS 100, Alpha MOS, France) and detected using metal oxide sensors (MOS) array system (Alpha MOS, FOX 3000, France). Acquisition time and flow rate were 150 s and 150 mL/min, respectively. Synthetic air was used as carrier gas. Radar fingerprint and principal component analysis (PCA) were used for data processing using Alpha Soft package version 8.01.

Aroma volatile compounds

A total of 1 g of freeze-dried sample was weighed into 50 ml-headspace vial, prepared in duplicate, closed with silicone septa magnetic cap (29176-U, Supelco, Bellefonte, PA, USA) and heated in oven for 30 min at 105 °C. The vials were then adjusted to 60 °C within another oven while 75 µm Carboxen/PDMS fiber was injected into the vial. After 40 min of extraction, fiber was injected to GC port set at 250 °C for 5 min desorption at 1:5 split ratio and 4 mL/min flow rate. Separation was done using a DB-5 column (30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Folcom, CA, USA) within a gas chromatograph (6890N Agilent Technologies, Santa Clara, CA, USA) and compounds were detected by mass spectrometer (5973, Agilent Technologies, Santa Clara, CA, USA). Helium at 1 mL/min was used as carrier gas. GC oven was programmed to 45 °C (2 min), 150 °C (5 °C/min), 180 °C (6 °C/min, holding time 11 min), 200 °C (10 °C/min, holding time 5 min). The electron ionization temperature of MS was set at 200 °C with electron impact of 70 eV. Interface and quadruple temperature were 280 °C and 150 °C, respectively. Scanning mass range 50 to 450 m/z with scan rate of 1 scan/s. Identification was done using Mass Spectra Library (Wiley Registry of Mass Spectral Data 7th ed. Agilent part No. G1035B). Present data are the relative composition (%) based on peak area of total identified compounds.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the effects of different breeds. All analyses were performed using R-version 3.2.0 with "Agricolae" library (The R-foundation for Statistical Computing, Austria).

III. RESULTS AND DISCUSSION

The differences in proximate composition as affected by different breeds are shown in Table 1. Beef from grain-fed Hanwoo had more fat but less moisture and ash than beef from grass-fed Holstein. These was caused by different energy taken from diet. Concentrate feeding, which provides higher metabolisable energy than grass feeding, is commonly used in feed-lot industry in Korea for producing high-marbled Hanwoo beef. No significant differences were found on crude protein and ash content.

Table 1 Meat proximate composition (%)

	Hanwoo	Holstein	SEM	SL
Moisture	67.78	78.95	1.24	***
Crude fat	10.72	1.07	1.06	***
Crude protein	20.84	19.41	0.31	Ns
Ash	0.96	0.94	0.003	Ns

SEM, standard error of the means; SL, significant levels; *** ($p<0.001$); Ns (not significant, $p>0.05$).

The fatty acid composition of *longissimus lumborum* fat for Hanwoo and Holstein are presented in Table 2. Significant differences were found on all identified fatty acids, except for gamma linolenic acid (C18:3n6). Hanwoo beef contained higher myristate (C14:0), palmitate (C16:0), palmitoleate (C16:1n7) and oleate

(C18:1n9), while Holstein beef contained higher stearate (C18:0), alpha linolenate (C18:3n3), and other long-chain polyunsaturated fatty acids. Furthermore, grass-fed Holstein beef was observed containing remarkably higher omega-3 fatty acids and lower omega-6 fatty acids, resulting lower omega-6 to omega-3 ratio. These indicate that grass-fed Holstein beef had recommended ratio of omega-6 and omega-3 fatty acids (less than 4:1) for daily intake. However, oleic acid in grain-fed Hanwoo beef was higher than grass-fed Holstein. Gilmore et al. [5] reported that consuming high-oleic acid ground beef increased serum high-density lipoprotein cholesterol. Higher monounsaturated fatty acids, particularly oleic acid in beef also associate with good palatability [6].

Table 2 Meat fatty acid composition (%)

	Hanwoo	Holstein	SEM	SL
C14:0	3.72	2.48	0.25	***
C16:0	30.48	25.65	0.90	***
C16:1n7	6.11	2.67	0.58	***
C18:0	10.20	17.43	1.74	***
C18:1n9	47.80	44.53	1.08	*
C18:2n6	1.31	3.37	0.34	***
C18:3n6	0.01	0.08	0.01	Ns
C18:3n3	0.15	1.06	0.15	***
C20:4n6	0.19	1.49	0.25	***
C20:5n3	0.02	0.89	0.16	***
C22:4n6	0.01	0.20	0.03	***
C22:6n3	0.01	0.15	0.03	***
SFA	44.40	45.56	1.03	Ns
MUFA	53.91	47.20	1.47	*
PUFA	1.68	7.24	0.95	***
n6	0.18	2.10	0.33	***
n3	1.51	5.14	0.62	***
n6/n3	8.67	2.44	1.23	***

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SEM, standard error of the means; SL, significant levels; *** (p<0.001); * (p<0.05); Ns (not significant, p>0.05).

The major groups of volatile compounds found in recent study are shown in Table 3. Aldehydes, pyrazines, hydrocarbons and furans were more abundant in grain-fed Hanwoo beef than grass-fed Holstein. No significant differences were found on ketones. Higher pyridines and sulfur-containing compounds were observed in grass-fed Holstein beef. Xie et al. [7] mentioned that pleasant roasted meat-like aroma are from oleic acid-derived aldehydes and pyrazines, which are produced from Maillard reactions.

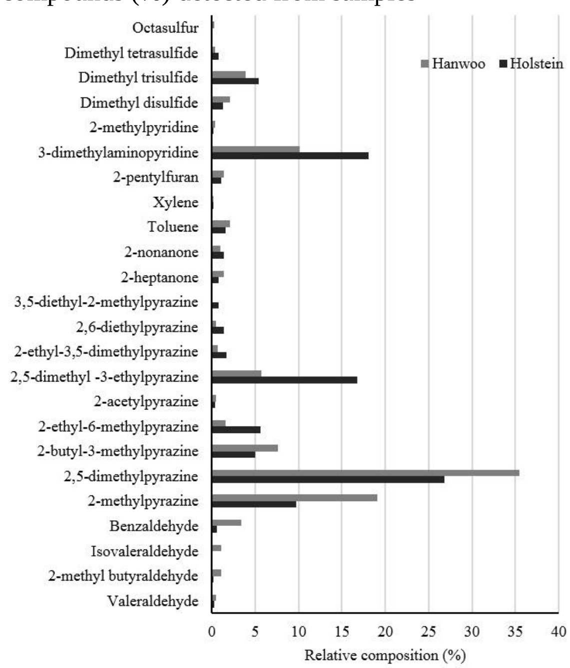
Table 3 Relative composition of major group of volatile compounds (%) identified from samples

Compounds	Hanwoo	Holstein	SEM	SL
Aldehydes	6.04	1.14	0.78	***
Pyrazines	71.07	68.13	0.13	*
Ketones	2.41	2.16	0.13	Ns
Hydrocarbons	2.22	1.74	0.36	*
Furans	1.38	1.05	0.76	*
Pyridines	10.41	18.25	1.42	***
Sulfur-containing compounds	6.60	7.54	5.58	*

SEM, standard error of the means; SL, significant levels; *** (p<0.001); * (p<0.05); Ns (not significant, p>0.05).

Figure 1 shows that among pyrazines, 2-methylpyrazine, 2,5-dimethylpyrazine and 2-butyl-3-methylpyrazine were three most abundant volatile compounds from Hanwoo beef, while 2,5-dimethyl-3-ethylpyrazine was more abundant in grass-fed Holstein beef. Pyridines, particularly 3-dimethylaminopyridine was more dominant in grass-fed Holstein beef than Hanwoo beef. Higher proportion of dimethyl trisulfide was found in grass-fed Holstein beef. These compounds were also previously observed in cooked beef aroma study by Ba et al. [8].

Figure 1. Relative composition of aroma volatile compounds (%) detected from samples



Aroma intensity of grain-fed Hanwoo beef and grass-fed Holstein beef, which was figured out by radar fingerprint chart of 12 sensors, is shown in Figure 2 and the differences on aroma pattern as discriminated by electronic nose is presented in Figure 3. Grass-fed Holstein beef had higher relative values of PA2, T70/2. P40/1, P10/2, P10/1 and T30/1 than grain-fed Hanwoo beef. No significant differences were found on relative values of other sensors. These indicate that beef from grass-fed Holstein possess stronger aroma intensity than Hanwoo beef. The total contribution rate of PCA (C1, 99.84% and C2, 0.12%) is higher than 85%, which is feasible to determine the differences on aroma pattern of samples [9]. These results suggest that different proportion of meat fatty acids may influence aroma, particularly MFA and PUFA. However, higher fat content did not have any effects on aroma intensity.

Figure 2. Aroma intensity detected by 12 metal oxide sensors of electronic nose

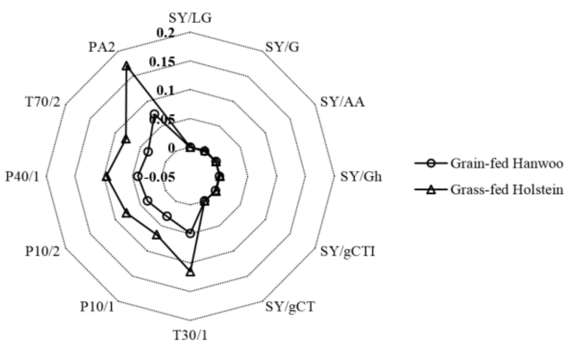
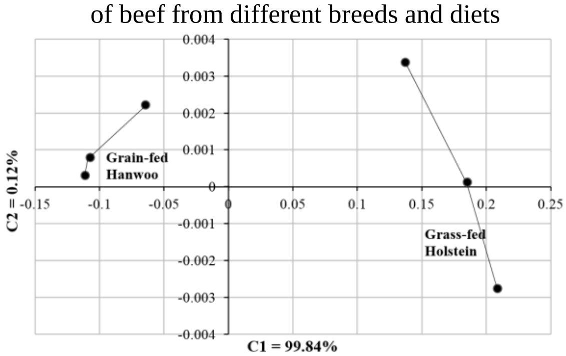


Figure 3. Principle component analysis of aroma pattern of beef from different breeds and diets



IV. CONCLUSION

Aroma pattern and volatile compounds of grain-fed Hanwoo and grass-fed Holstein beef were different. These can be characterized by using the combination analysis through GC-MS and electronic nose. Higher fat content did not affect aroma intensity. However, fatty acid composition may influence the relative composition of aroma volatile compounds and their pattern.

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