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The Global Volatile Signature of Veal via Solid-phase Microextraction and Gas Chromatography-mass Spectrometry

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Abstract

The volatile composition of veal has yet to be reported and is one of the important factors determining meat character and quality. To identify the most important aroma compounds in veal from Holstein bull calves fed one of three diets, samples were subjected to solid-phase microextraction (SPME) combined with gas chromatography-quadrupole mass spectrometry (GC-MS). Most of the important odorants were aldehydes and alcohols. For group A (veal calves fed entirely on milk for 90 d before slaughter), the most abundant compound class was the aldehydes (52.231%), while that was alcohols (26.260%) in group C (veal calves fed starter diet for at least 60 d before slaughter). In both classes the absolute percentages of the volatile compounds in veal were different indicating that the veal diet significantly ($p<0.05$) affected headspace volatile composition in veal as determined by principal component analysis (PCA). Twenty three volatile compounds showed significance by using a partial least-squared discriminate analysis (PLS-DA) (VIP>1). The establishment of the global volatile signature of veal may be a useful tool to define the beef diet that improves the organoleptic characteristics of the meat and consequently impacts both its taste and economic value.

Keywords: veal, volatile, SPME-GC-MS, PCA, PLS-DA

Introduction

Veal is more tender and juicy than fully aged beef, yet a detailed characterization of its volatile components is currently missing in the literature (Mao *et al.*, 2011). The volatile composition of meat is one of the most important factors that determine its taste, character, and quality (Gorraiz *et al.*, 2002; Tan *et al.*, 2004). Consumers continue to demand high-quality and consistent meats at a reasonable price (Stetzer *et al.*, 2008). The sensory traits that mostly affect consumer acceptability of beef are tenderness and flavor (Robbins *et al.*, 2003a, b). To date, more than 1000 volatile compounds contributing to the odor of cooked meat have been identified (Lee *et al.*, 2012; Rivas-Cañedo *et al.,* 2009; Wang *et al.*, 2006; Watkins *et al.*, 2012). As with many food products the aroma of beef is influenced by hundreds of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) belonging to different chemical families includ-

ing ketones, esters, aldehydes, alcohols, acids, carbonyl compounds, sulphur compounds, and many others (Moon *et al.*, 2006; Tan *et al.*, 2004).

The Maillard reaction, thiamin degradation, and lipid oxidation are the main reactions that generate flavor when meat is cooked (Calkins *et al.*, 2007; Koutsidis *et al.*, 2008a, b; Varavinit *et al.*, 2000). It showed that benzene and furan compounds were stewed beef flavor components (Shi *et al.*, 2011) and 4-hydroxy-2, 5-dimethyl-3 (2H)-furanone and 4-hydroxyl-5-methyl-3 (2H)-furanone were very important for high impact beef flavor (Guth *et al.*, 1994). Then, many of the VOCs and SVOCs are generally present in trace amounts (from several mg/L to a few ng/L, or even less), which means that an effective extraction technique to enrich or concentrate the analytes as well as a sensitive detection scheme are required for proper measurement. Previously, several methodologies involving static headspace analysis (Guth *et al.*, 1994), purge and trap (Insausti *et al.*, 2005), distillation/extraction techniques (Gasser *et al.*, 1988), and concentration on solid-phase supports (solid-phase extraction-SPE) followed by analysis using gas chromatography-mass spectrometry (GC-MS) (Ruiz *et al.*, 1998) have been widely proposed and used to establish the aroma profile of beef. An artifi-

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cial nose (an electronic nose) was used to give an objective basis for odor and flavor identification that correlates with human sensory experience (Wilkes *et al.*, 2000).

Nevertheless, these methodologies have important limitations including their relatively low reproducibility, low selectivity, and loss of analytes during concentration. Additionally, they require the use of toxic and expensive solvents, and are time consuming and labor-intensive. More recently, solvent-free methodologies that overcome these drawbacks have been widely used, such as solid-phase microextraction (SPME) (Watkins *et al.*, 2012). The SPME methodology is very suitable for organic analytical chemistry because it is a fast, simple, and sensitive procedure that eliminates the use of organic solvents and combines sampling, extraction, and concentration into a single step, substantially shortening analysis time. For these reasons, there are many applications of SPME in food analysis. While this approach has been used to analyze the volatile composition of beef, to the best of our knowledge, there were not the documents which determined the volatile composition in veal, yet. This study reports the development of a reliable and solvent-free SPME-GC-MS methodology to establish the global volatile signature of veal. "Unsupervised" principal component analysis (PCA) and "Supervised" partial least-squared discriminate analysis (PLS-DA) were used to process the GC-MS data to determine which volatile compounds show significance in veal.

Materials and Methods

Materials

The divinylbenzene/Carboxen®/polydimethylsilicone (50/30 μm DVB/CAR/PDMS) SPME fiber (Cat. no. 57329-U) glass vials were purchased from Supelco, Inc. (Australia). The SPME fiber was pre-conditioned at 250°C for 1 h according to the manufacturer's recommendation.

Experimental animals and samples

Fifteen 2-3 d old Holstein bull calves with the birth weight from 35.01 to 37.77 kg (mean live weight [LW] = 36.14 kg) were selected, pre-fed entirely on milk for 5-8 d and randomly divided into three groups: (A) whole milk feeding group; (B) quantitative feeding group of whole milk and calf starter diet; (C) *ad libitum* feeding group of whole milk and calf starter diet. Each group contained 5 animals. The quantities of milk and starter were offered at 8% LW of calves (the exact experimental quantity for milk and starter diet was shown in Table 1). Whole milk was purchased from the dairy farm of Gansu Agricultural University for daily feeding of the calves. Calf no. 1 pellet diet was purchased from the Boya Feed Co., Ltd, China. Calf no. 1 pellet diet contained (as fed basis): corn grain (460 g/kg), wheat bran (150 g/kg), whey powder (50 g/kg), soybean meal (250 g/kg), molasses (55 g/kg), CaHPO4(10 g/kg) mineral/vitamin premix (20 g/kg), and limestone (5 g/kg).

All animals were slaughtered after 90 d. A hundred grams of fresh veal longissimus dorsi muscle where the branched-chain fatty acids are present at subthreshold odour levels (Brennand *et al.*, 1992) were collected, homogenized, mixed together in each feeding group. Samples were stored at -18°C for later use.

This study was performed in the dairy division of the animal science training center at Gansu Agricultural University, Lanzhou, China. Experimental procedures and animal holding facilities respected the relevant animal protection legislation of China (Regulations on the management of laboratory animal 1988, State Scientific and Technological Commission of the People's Republic of China; Measures for the management of animal quarantine 2010, Ministry of Agriculture of the People's Republic of China). Animals were slaughtered at the experimental abattoir of Gansu Agricultural University (Lanzhou, China) by exsanguination after stunning with a cartridge-fired captive bolt stunner.

SPME procedure

The samples were removed from the freezer and allowed to reach room temperature prior to analysis. A 10 g sample was placed in a vial to which 10 g of sodium chloride (NaCl) was added. NaCl had salting-out effect, in order to increase the organic volatile (Shi *et al.*, 2011; Wei *et al.*, 2011). The vials and their contents were pre-heated at 90

Table 1. Grouping of Holstein bull calves and daily average feeding per animal

Group	No. of calves	1st month		2nd month		3rd month	
		Milk/kg	Starter/kg	Milk/kg	Starter/kg	Milk/kg	Starter/kg
A	5	4.35	-	6.35	-	9.68	-
B	5	4.25	-	5.14	0.32	6.11	1.85
C	5	4.30	-	5.36	0.48	*ad libitum*	*ad libitum*

±1°C for 60 min in a thermostatted bath. Then the temperature was adjusted to 60±1°C prior to the insertion of the DVB/CAR/PDMS SPME fiber into the headspace where it was held for 40 min (Vasta *et al.*, 2007). The fiber was then withdrawn and manually inserted into the GC injector to allow the adsorbed compounds to be transferred to the analytical column. The fiber was held in the injector for 15 min.

GC-MS analysis

The volatile compounds were separated using an OV-1701 MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness, Chromatographic Technology R&D, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences) in an Agilent GC-MS system (Agilent Technologies, USA) utilizing a model 6890N gas chromatograph and a model 5973N mass selective detector. The GC oven temperature was initially held at 50°C for 1 min, increased at a rate of 3.5°C/min to 220°C where it was held for a further 20 min. For SPME, the injector, heated at 260°C, was held in the splitless mode for the first 2 min of the analysis and then in the split mode (20:1) for the remainder of the analysis. The SPME fiber remained in the injector for 15 min to clean the fiber with a solvent delay time of 3.5 min. Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The MS was operated in electron ionisation mode (70 eV) and data was acquired in full scan mode for range of 30 to 550 m/z. The temperature of the source and the detector were 150 and 230°C, respectively, while the MS transfer line was 280°C.

Multivariate data analysis and data processing

Compounds were identified by comparing the mass spectra to those found in the NIST 05 mass spectral library (NIST, 2005 software, Mass Spectral Search Program V.2.0 d; NIST 2005, USA) by the match and the relative match (R. match) 80% (Qi *et al.*, 2010). The GC peak areas for each compound were calculated using the total ion chromatogram, assuming a relative response factor of one for each compound. The results of the volatile analysis were reported as percentages, representing the proportion of each identified peak to the total area of identified peaks in each chromatogram.

The resulting datasets were then imported into SIMCA-P 11.5 (Umetrics, Sweden) for multivariate statistical analyses. All imported data were Pareto-scaled for the multivariate analysis. First, principal components analysis (PCA) was conducted on the spectra of veal samples to discern the presence of inherent similarities in spectral profiles. Next, a PLS-DA was used to maximize covariance between the measured data (peak intensities in GC-MS spectra, X matrix) and the response variable (predictive classifications, Y matrix) by searching for X variables that correlated with class membership and thus indicated that the response variable Y can be predicted from X.

PLS-DA was also applied to the relative concentration data of assigned volatile compounds. The variable importance in the projection (VIP) plot was then used to identify which volatile compound contributed most to clustering or trends observed in the data.

Statistical analyses

The statistical analysis was conducted using SPSS 19 for Windows (SPSS Inc., IBM Corporation, USA). One-way Analysis of Variance (ANOVA) was applied to test differences between groups (A, B and C) for all variables studied by VIP>1. Differences with a *p*-value of 0.05 were considered significant. Assays were performed in quintuplicate, and the results were expressed as mean±SD.

Results and Discussion

Comparison of samples

More than 125 compounds were detected from longissimus dorsi muscle of veal fed 3 different feeding treatment using SPME with GC-MS. Through comparison to the NIST spectral library, a total of 9 ketones, 16 aldehydes, 11 alcohols, 9 hydrocarbons and 9 other compounds were identified. Fourty seven of the 125 peaks gave a definitive match in group A. These peaks accounted for 92.71% of all the compounds. Similarly, 50 and 44 compounds were identified in group B and C; their content accounted for 96.05% and 76.91%, respectively (Table 2).

Aldehydes, ketones, alcohols, and hydrocarbons were the four main classes of compounds identified in the veal samples. For group A, the most abundant compound class was the aldehydes (52.231%), followed by the alcohols (20.890%), hydrocarbons (5.296%), and the ketones (3.749%). A similar trend was observed with group B and C, but the percentages of four classes were different. In group B, the aldehydes, alcohols, hydrocarbons and the ketones were 49.631%, 22.558%, 6.308% and 3.053%, respectively. They were 31.247%, 22.260%, 8.105% and 2.497% in group C, respectively. That is, the total GC peak area percentage content (%) of the aldehydes was the highest in group A and lowest in group C. The ketones showed the same trend as the aldehydes. However, the alco-

hols and hydrocarbons showed the opposite trend. The percentage content (%) of alcohols was in the highest in group C and lowest in group A, and hydrocarbons' was as the same. It suggested that marked differences existed between the proportions of the extracted compounds by SPME that were common to three groups.

PCA and PLS-DA

Here, "Unsupervised" PCA and "Supervised" PLS-DA were used to process the GC-MS data. PCA and PLS-DA were used because they cope well with highly multivariate, noisy, collinear, and possibly incomplete data. PCA is an unsupervised pattern recognition method initially used

Table 2. Volatile compounds of muscle *longissimus dorsi* from veal fed different diets[a]

Peak n	RT[b] (min)	Compounds	Molecular formula	GC peak area (%)[c]±RSD[d] A	B	C
Ketones						
1	6.97	2-Butanone	C_4H_8O	-	-	0.279±0.012
6	12.15	3-Hydroxy-2-Butanone	$C_4H_8O_2$	0.295±0.007	0.583±0.027	0.262±0.0280
10	17.84	2-Heptanone	$C_7H_{14}O$	0.878±0.069	0.577±0.012	0.539±0.031
18	22.41	2-Octanone	$C_8H_{16}O$	0.566±0.109	0.439±0.040	0.409±0.010
24	26.88	2-Nonanone	$C_9H_{18}O$	0.641±0.070	0.282±0.015	0.498±0.010
31	31.17	2-Decanone	$C_{10}H_{20}O$	0.351±0.089	0.192±0.005	-
44	42.01	3-(3-Hydroxy-1-Propenyl)- Cyclopentanone	$C_8H_{12}O_2$	0.411±0.051	0.544±0.021	-
46	42.48	Dihydro-5-Propyl-2(3H)-Furanone	$C_7H_{12}O_2$	0.336±0.025	0.434±0.046	0.510±0.012
52	52.81	Butylidene-1(3H)-Isobenzofuranone	$C_{12}H_{12}O_2$	0.271±0.031	-	-
		Subtotal (GC peak area)		3.749±0.035	3.053±0.017	2.497±0.011
		Subtotal compounds number		8	7	6
Aldehydes						
3	9.58	Pentanal	$C_5H_{10}O$	0.940±0.049	0.623±0.062	0.326±0.022
8	13.56	Hexanal	$C_6H_{12}O$	17.665±0.393	21.424±0.261	10.158±0.145
11	18.07	Heptanal	$C_7H_{14}O$	4.632±0.252	3.601±0.154	2.647±0.088
12	18.28	(Z)-4-Heptenal	$C_7H_{12}O$	0.150±0.029	-	-
15	21.83	(E)-2-Heptenal	$C_7H_{12}O$	-	0.237±0.031	0.083±0.005
19	22.71	Octanal	$C_8H_{16}O$	9.314±0.211	6.789±0.308	5.525±0.241
25	27.2	Nonanal	$C_9H_{18}O$	16.562±0.484	12.268±0.240	9.359±0.261
30	30.79	(E)-2-Nonenal	$C_9H_{16}O$	0.136±0.011	0.936±0.092	0.361±0.015
32	31.32	cis-4-Decenal	$C_{10}H_{18}O$	0.203±0.020	0.242±0.033	-
33	31.68	3-Ethyl-Benzaldehyde	$C_9H_{10}O$	0.144±0.010	0.279±0.017	0.164±0.015
37	34.92	(E)-2-Decenal	$C_{10}H_{18}O$	0.239±0.040	0.480±0.035	0.178±0.013
39	37.43	4-(1-Methylethyl)-1-Cyclohexene-1-Carboxaldehyde	$C_{10}H_{16}O$	0.319±0.028	0.319±0.027	0.479±0.029
41	38.82	2-Dodecenal	$C_{12}H_{22}O$	-	0.275±0.014	0.166±0.016
49	46.3	Undecanal	$C_{11}H_{22}O$	0.291±0.015	0.439±0.039	0.284±0.009
51	49.53	Pentadecanal	$C_{15}H_{30}O$	0.271±0.012	0.456±0.044	0.356±0.054
53	52.95	Tetradecanal	$C_{14}H_{28}O$	1.364±0.041	1.266±0.047	1.163±0.068
		Subtotal (GC peak area)		52.231±0.152	49.631±0.098	31.247±0.084
		Subtotal compounds number		14	15	14
Alcohols						
7	13.05	1-Pentanol	$C_5H_{12}O$	0.998±0.074	0.992±0.064	0.585±0.027
9	17.42	1-Hexanol	$C_6H_{14}O$	0.560±0.031	0.509±0.075	0.445±0.050
17	22.06	1-Octen-3-ol	$C_8H_{16}O$	8.447±0.318	8.448±0.400	6.360±0.309
21	25.2	2,4-Dimethyl-Cyclohexanol	$C_8H_{16}O$	-	0.143±0.008	0.108±0.016
22	26.34	1-Octanol	$C_8H_{18}O$	1.464±0.072	2.373±0.182	1.191±0.119
23	26.67	(E)-2-Octen-1-ol	$C_8H_{16}O$	0.986±0.063	1.637±0.086	0.941±0.092
29	29.07	6-Undecanol	$C_{11}H_{24}O$	0.205±0.028	0.263±0.034	0.134±0.017
36	33.75	6,10,13-Trimethyltetradecanol	$C_{17}H_{36}O$	0.154±0.012	0.191±0.022	0.229±0.039
40	38.46	1-[2-Methyl-3-(methylthio)allyl]- Cyclohex-2-enol	$C_{11}H_{18}OS$	1.190±0.101	1.210±0.087	2.478±0.163
42	39.02	2-Hexyl-1-Decanol	$C_{16}H_{34}O$	0.467±0.040	0.500±0.033	0.440±0.026
43	39.16	1-(2-Nitropropyl)-Cyclohexanol	$C_9H_{17}NO_3$	6.418±0.144	6.292±0.175	13.349±0.239
		Subtotal (GC peak area)		20.890±0.090	22.558±0.113	26.260±0.099
		Subtotal compounds number		10	11	11

Table 2. Volatile compounds of muscle *longissimus dorsi* from veal fed different diets[a] (Continued)

Peak n	RT[b] (min)	Compounds	Molecular formula	GC peak area (%)[c]±RSD[d]		
				A	B	C
Hydrocarbons						
4	10.22	Octane	C_8H_{18}	0.631±0.037	0.151±0.011	-
5	11.06	Toluene	C_7H_8	0.284±0.023	0.154±0.012	0.182±0.010
13	18.57	Decane	$C_{10}H_{22}$	0.162±0.029	0.112±0.011	-
20	23.32	1,2,4-Trimethyl-Cyclohexane	C_9H_{18}	-	0.133±0.004	-
26	27.35	Dodecane	$C_{12}H_{26}$	1.769±0.026	1.902±0.109	2.461±0.206
27	27.58	1,2-Dipentylcyclopropene	$C_{13}H_{24}$	0.886±0.067	0.777±0.033	0.518±0.010
34	32.31	Hexyl-Benzene	$C_{12}H_{18}$	0.348±0.036	0.445±0.048	0.790±0.064
38	35.34	Tetradecane	$C_{14}H_{30}$	1.216±0.018	1.514±0.030	2.169±0.163
50	46.95	1,11-Tridecadiene	$C_{13}H_{24}$	-	-	0.062±0.005
		Subtotal (GC peak area)		5.296±0.019	5.188±0.037	6.182±0.075
		Subtotal compounds number		7	8	6
Others						
2	8.21	o-Methylisourea hydrogen sulfate	$C_2H_6N_2O$	2.808±0.063	2.773±0.045	1.889±0.124
14	20.21	2-Pentyl-Furan	$C_9H_{14}O$	2.851±0.093	5.090±0.129	2.168±0.162
16	21.99	n-Caproic acid vinyl ester	$C_8H_{14}O_2$	0.179±0.014	0.195±0.027	-
28	28.98	Hexanoic acid, anhydride	$C_{12}H_{22}O_3$	0.167±0.009	0.198±0.033	0.230±0.041
35	32.77	4-(1,1-Dimethylethyl)-2-Methyl-Phenol	$C_{11}H_{16}O$	0.158±0.009	0.323±0.031	0.113±0.025
45	42.38	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	3.268±0.109	3.663±0.217	3.794±0.197
47	42.87	Butylated Hydroxytoluene	$C_{15}H_{24}O$	0.825±0.033	1.120±0.088	1.923±0.042
48	45.32	2,5-bis(1,1-Dimethylethyl)-Phenol	$C_{14}H_{22}O$	0.290±0.012	0.342±0.035	0.687±0.037
54	64.92	N-butyl-Benzenesulfonamide	$C_{10}H_{15}NO_2S$	-	1.916±0.090	-
		Subtotal (GC peak area)		10.545±0.043	15.62±0.067	10.805±0.077
		Subtotal compounds number		8	9	7

[a]Whole milk feeding group (A); quantitative feeding group of whole milk and calf starter diet (B); *ad libitum* feeding group of whole milk and calf starter diet (C).
[b]Retention times (min).
[c]Mean of five replicates (%).
[d]Relative standard deviation.

to discern the presence of inherent similarities in spectral profiles (Rivas-Cañedo *et al.*, 2009). The results are displayed as score plots indicating the scatter of the samples-similar volatile compositions are clustered together and different compositions are dispersed. In the PCA scores, each point represents an individual sample, and further composition differences are indicated by the PCA loading plots. Therefore, PCA offers a clear visual representation of the differences among the samples. It shows clear separation among the groups (R^2X=0.909, Q^2=0.871; Fig. 1). R^2X and Q^2 expressed matrix of X explained and predictive ability of matrix model established, respectively. Their values were closer to 1 that showed the model was more reliable. Here samples A1-A5 clustered together similar to B1-B5 and C1-C5. There were some compounds that did not definitely belong in groups A, B, or C while others did. The scatter 3D plot shows a clear discrimination between group A, group B, and group C (Fig. 2).

Next, PLS-DA was performed to reduce the possible contribution of intergroup variability and to further imp-

rove the separation between the samples. The PLS-DA analysis calculates models that differentiate groups or classes and among compounds. In the PLS-DA model, the samples from the different groups were sorted into different classes using score plots, and the compounds that contribute to the classification were identified in loading plots. These showed the importance of each variable to the classification. Each score plot has a loading plot associated with it, which makes it possible to identify the spectral regions (compounds) that are responsible for the observed sample clustering. The biplots (score plot and loading plot; Fig. 3) showed a clear differentiation between the groups (R^2Y=0.995, Q^2=0.992), and the scatter 3D plot showed a clear discrimination between controls (Fig. 4). R^2Y and Q^2 expressed matrix of Y explained and predictive ability of matrix model established, respectively. Their values were closer to 1 that showed the model was more reliable. The VIP parameter was essentially a measure of the degree to which a particular variable explained the Y variance.
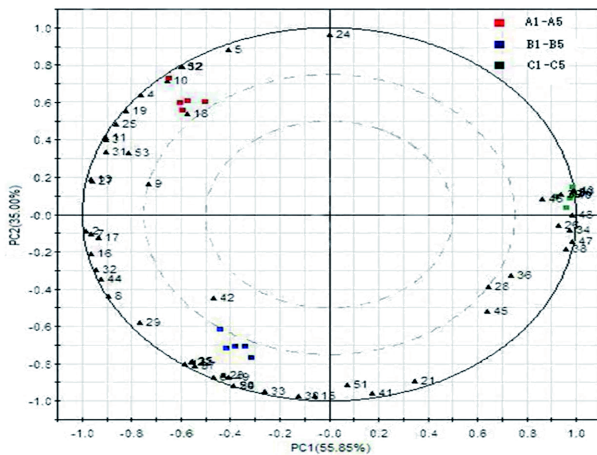
Fig. 1. PCA biplot (score plot and loading plot, PC1×PC2) of the GC-MS peak area (%) of volatiles identified in veal (attribution of the peak number shown in Table 2). A1-A5: whole milk feeding group (A); B1-B5: quantitative feeding group of whole milk and calf starter diet (B); C1-C5: *ad libitum* feeding group of whole milk and calf starter diet (C).
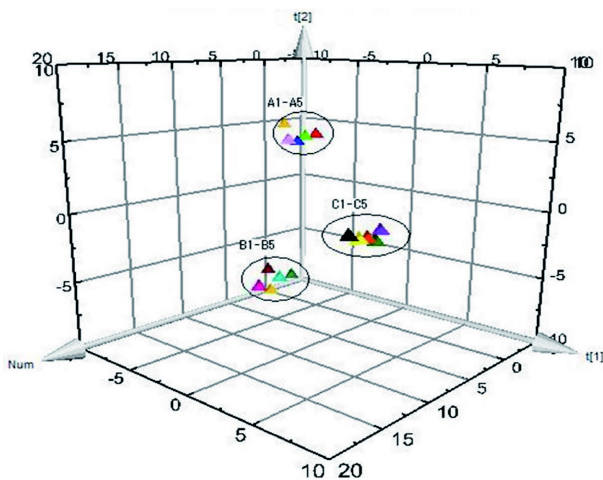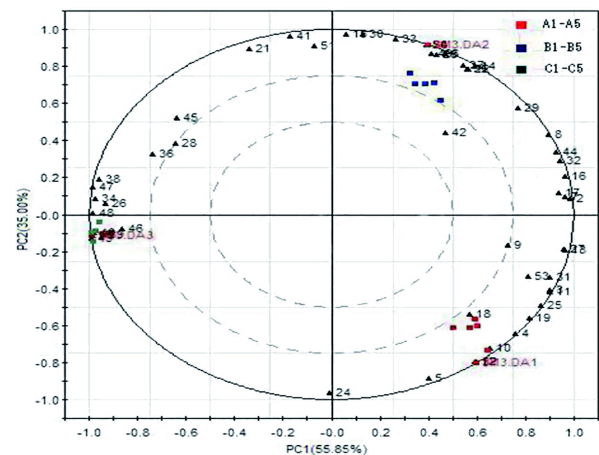

Fig. 3. PLS-DA biplot (score plot and loading plot, PC1×PC2) of GC-MS peak area (%) of volatile identified in veal (attribution of the peak number shown in Table 2). A1-A5: whole milk feeding group (A); B1-B5: quantitative feeding group of whole milk and calf starter diet (B); C1-C5: *ad libitum* feeding group of whole milk and calf starter diet (C).

Fig. 2. PCA scatter 3D plot. The colors were correlated back to the samples shown in Table 1. A1-A5: whole milk feeding group (A); B1-B5: quantitative feeding group of whole milk and calf starter diet (B); C1-C5: *ad libitum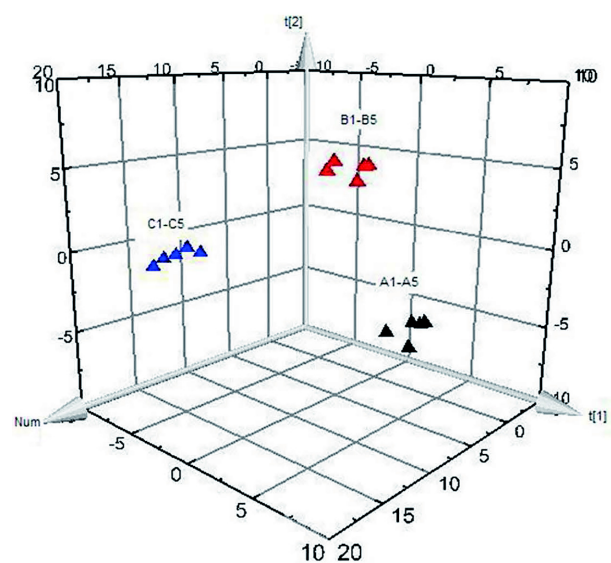* feeding group of whole milk and calf starter diet (C). t[1]: PC1(55.85%), t[2]: PC2(35.00%), Num: quintuplicate for each group.

Fig. 4. PLS-DA scatter 3D plot. A1-A5: whole milk feeding group (A); B1-B5: quantitative feeding group of whole milk and calf starter diet (B); C1-C5: *ad libitum* feeding group of whole milk and calf starter diet (C). t[1]: PC1 (55.85%), t[2]: PC2 (35.00%), Num: quintuplicate for each group.

According to results obtained by PCA and PLS-DA, the group A was mainly characterized by the presence of 2-heptanone, butylidene-1(3H)-isobenzofuranone and octane. Moreover, these carbonyl compounds when present at concentration above their odour threshold could contribute with fruity, sweet and flowery notes. (E)-2-decenal, 1-octanol and 2-pentyl-furan were the important flavor compounds in group B. For group C, 2,5-bis(1,1-dimethylethyl)-phenol, hexyl-benzene and 1-(2-nitropropyl)-cyclohexanol were the main flavor substances. The flavors were dissimilar because of the different flavor substances in three groups.

Twenty three volatile compounds showed significance

through VIP values (VIP>1) (Table 3) including 10 alde-hydes, 4 ketones, 3 alcohols, 3 hydrocarbons and 3 others. Carbonyl compounds were quantitatively the largest group contributed most for flavor in veal. Of these, fifteen were common to the 3 groups. 2-dodecenal, (E)-2-heptenal, 2,4-dimethyl-cyclohexanol did not exist in group A. (E)-2-heptenal, 1,2,4-trimethyl-cyclohexane, n-butyl-benzene-sulfonamide were only in group B, and (Z)- 4-heptenal, 3-butylidene-1(3H)-isobenzofuranone occurred in group A, only. This multivariate statistical analysis showed a clear distinction among the 3 kinds of veal (Table 3).

The volatile compounds detected in the three groups could be produced by two ways-one consisting of volatile components produced by Maillard reactions and the other consisting of volatile components formed through lipid oxidation. The higher peak area values for the products of lipid oxidation could be ascribed to the mild cooking con-ditions (60°C), employed in this study. More severe cook-ing conditions were needed to produce more Maillard reactions products, which are more aromatic than lipid oxidation products (Xu *et al.*, 2008).

Aldehydes have low detection thresholds and small amounts can exert an outsize influence on odor (Liu *et al.*, 2004). Certain volatile aldehydes such as heptanal, (Z)-octanal, and nonanal are formed by oxidation of oleic acid. These volatiles are very important, because they have been found to impart a pleasant sweet and fruity odor to the meat, while heptanal is associated with the odor of potatoes. It reported that oxidation at high temperatures resulted in ketone and alcohol formation (Tan *et al.*, 2004). They are related to sweet odor notes. Aromatic hydrocar-bons played an important role in the overall flavor of meat, even though none is individually responsible for meat odor (Min *et al.*, 1979). In addition, two sulfur com-pounds (N-butyl-benzenesulfonamide, 1-[2-Methyl-3-(me-thylthio) allyl]-cyclohex-2-enol) were identified. Sulfur compounds are important flavor substances with odor th-resholds (Wu *et al.*, 2001). They were very important con-tributors to the flavor profile of cooked meat.

Conclusion

The composition of volatile flavor substances differs between the three veal groups; this may be due to the dif-

Table 3. The volatile compounds contributed most by VIP values (VIP > 1)

peak n	Compounds	GC peak area (%) [a] ± RSD [b]			VIP[c]
		A	B	C	
41	2-Dodecenal	-	0.275±0.014*	0.166±0.016*	1.1833
30	(E)-2-Nonenal	0.136±0.011*	0.936±0.0928*	0.361±0.015*	1.1804
15	(E)-2-Heptenal	-	0.237±0.031*	0.083±0.005*	1.1794
20	1,2,4-Trimethyl-Cyclohexane	-	0.133±0.004*	-	1.1606
54	N-butyl-Benzenesulfonamide	-	1.916±0.090*	-	1.1597
24	2-Nonanone	0.641±0.070*	0.282±0.015*	0.498±0.010*	1.1596
33	3-Ethyl-Benzaldehyde	0.144±0.010*	0.279±0.017*	0.164±0.015*	1.1545
21	2,4-Dimethyl-Cyclohexanol	-	0.143±0.008*	0.108±0.016*	1.1526
6	3-Hydroxy-2-Butanone	0.295±0.007*	0.583±0.027*	0.262±0.0280*	1.1329
23	(E)-2-Octen-1-ol	0.986±0.063	1.637±0.086*	0.941±0.092	1.1221
5	Toluene	0.284±0.023*	0.154±0.012*	0.182±0.010*	1.1200
14	2-pentyl-furan	2.851±0.093*	5.090±0.129*	2.168±0.162*	1.1117
52	butylidene-1(3H)-isobenzofuranone	0.271±0.031*	-	-	1.1M58
49	undecanal	0.291±0.015	0.439±0.039*	0.284±0.009	1.1018
37	(E)-2-decenal	0.239±0.040*	0.480±0.035*	0.178±0.013*	1.0989
35	4-(1,1-dimethylethyl)-2-methyl-Phenol	0.158±0.009*	0.323±0.031*	0.113±0.025*	1.0924
12	(Z)-4-heptenal	0.150±0.029*	-	-	1.0899
22	1-octanol	1.464±0.072*	2.373±0.182*	1.191±0.119*	1.0879
51	pentadecanal	0.271±0.012*	0.456±0.044*	0.356±0.054*	1.0752
4	octane	0.631±0.037*	0.151±0.011*	-	1.0558
10	2-heptanone	0.878±0.069*	0.577±0.012	0.539±0.031	1.0545
19	octanal	9.314±0.211*	6.789±0.308*	5.525±0.241*	1.0228
25	nonanal	16.562±0.484*	12.268±0.240*	9.359±0.261*	1.0098

[a]Mean of five replicates (%).
[b]Relative standard deviation.
[c]The parameters of the variable importance in the projection.
*The mean difference is significant according to Fisher's protected LSD test, at a *p* value of 0.05.

ferent diets. It showed similar result with Resconi *et al.* (2010) and Vasta *et al.* (2011). Our preliminary data suggested that the main flavor components of veal including (E)-2-nonenal, (E)-2-heptenal, 1,2,4-trimethyl-cyclohexane, N-butyl-benzenesulfonamide, 2-nonanone, 3-ethylbenzaldehyde, 2,4-dimethyl-cyclohexanol, 3-hydroxy-2-butanone, (E)-2-octen-1-ol, toluene, 2-pentyl-furan, 3-butylidene-1(3H)-isobenzofuranone, undecanal, (E)-2-decenal, 4-(1,1-dimethylethyl)-2-methyl-Phenol, (Z)-4-heptenal, 1-octanol, pentadecanal, octane, 2-heptanone, octanal, and nonanal.

Our study has improved the understanding of the influence of the finishing diet on the volatile components contained in veal and contributes to the development of diets for veal that meet consumer expectations in different markets. It also provided a scientific reference for the future production of high-quality veal. However, the study of volatile substances of veal is still in its infancy. Further research using gas chromatography-olfactometry (GC-O) is needed for confirmation of our results.

Acknowledgements

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