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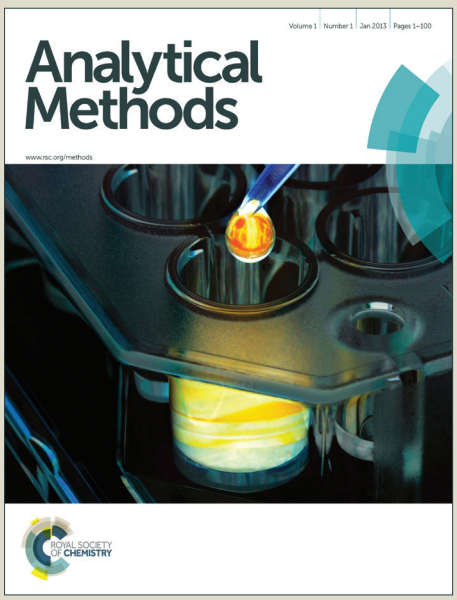

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Triple-channel comparative analysis of volatile flavour composition in raw whole and skim milk via Electronic Nose, GC-MS and GC-O

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Abstract

A novel triple-channel comparative analysis of volatile composition in raw whole and skim milk (RWM, RSM) were developed via electronic nose (E-Nose), headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS), and gas chromatography-olfactometry (GC-O). Volatile flavour compounds from RWM and RSM caused the differences in mouthfeel characteristics by the human sensors, and also affected consumer preference. Solid state sensor technology, E-Nose, was applied to distinguish the differences in volatiles between RWM and RSM. Headspace volatiles adsorbed by HS-SPME fiber (CAR/PDMS) was detected by GC-MS. Aroma compounds were identified by GC-O. Nine and three compounds were found to be aroma-active compounds from RWM and RSM, respectively. Octanoic acid, butanoic acid, and 3-hydroxy-2-butanone were found to be the most important aroma-active compounds in RWM, while the most important aroma-active compound of RSM was octanoic acid.

Keywords: Raw whole milk; Raw skim milk; Flavours; Electronic nose; Gas chromatography-mass spectrometry; Gas chromatography-olfactometry

1. Introduction

The growing demand for milk with improved nutritional qualities has prompted the food processing industry to cut down on ingredients such as fat. Milk with lower fat content such as skim milk (< 0.5% fat) is often preferred by consumers compared to normal fat content of whole milk (3-5% fat). Skim milk while lacking the desirable flavour derived from milk fat has consumer perceived healthful properties such as reducing weight gain, decreasing the risk of cardiovascular disease and related disorders [1,2]. Some studies suggest that the intake of low-fat dairy products is considered to be beneficial [3]. However, fat composition is one of the most important factors for the flavour of milk products. Many consumers prefer the flavour of whole milk to skim milk or low-fat dairy products [4], due to abundant milk fat in whole milk. Therefore, flavour is an important attribute of consumer acceptance and preference for milk products. It is also important to develop a rapid method to determine flavour quality differences for the dairy industry.

It is a challenge for the flavour analysis due to the low concentration of aroma compounds which are the main compositions of milk fat. The flavour properties of milk and dairy products have been previously reported [5], which can be subsequently modified by heat treatment, packaging material, and storage time [6-8]. Volatile flavour compounds from dairy products have been extracted by head space solid-phase micro-extraction (HS-SPME) [9], simultaneous distillation extraction (SDE) [10], solvent assisted flavour evaporation (SAFE) [11], and supercritical CO_2 fluid extraction [12].

HS-SPME is a solvent free, simple, economical and efficient technique compared with conventional extraction [13]. The adsorption of headspace volatiles depends on its chemistry and affinity for the volatile by the polymer coating the HS-SPME. Recently, many researchers have used HS-SPME coupled with gas chromatography-mass spectrometry (GC-MS) for detection. Reasons for the greater popularity of HS-SPME-GC-MS include low detection limits, high efficiency, accuracy and good reproducibility. However, expensive instrumentation, slow turnaround times for multitudinous screening and the need for skilled operators are often cited as problems.

Gas chromatography-olfactometry (GC-O) is essential to identify compounds with odor because they are usually a minor set of eluting compounds, which depends on the human nose and shows high sensitivity and reproducibility. GC-O has been used to characterize the aroma profile of dairy products [14] and identify the milk flavour from cows fed different diets [15]. In recent years, statistical analysis of responses of solid state sensors sensitive to specific functional groups have been applied to flavour analysis. It has the advantages of rapid results, low cost, and simplicity compared with traditional chromatographic methods. Electronic nose (E-Nose) has been used for the analysis and classification of various food products such as beer [16], meat [17], and tea [18]. The new sensor technology of E-Nose may be able to replace human sensory evaluation of some food products.

In this study, we proposed a novel triple-channel comparative analysis of volatile flavour composition in RWM and RSM via GC-MS, GC-O and E-Nose. Here, GC-O and E-Nose have been applied to flavour analysis with rapid and simple processing compared with

GC-MS. GC-MS has high efficiency and accuracy but a number of shortcomings still restrict the practicability. We analyzed the difference of volatile constituents from RWM and RSM by the three technologies for the first time. On the one hand, we analyzed the difference of volatile constituents from RWM and RSM by SPME fiber (carboxen/polydimethylsiloxane (CAR/PDMS) combined with GC-MS and GC-O. On the other hand, E-Nose with solid state sensors was used to assist in quality assessment of RWM and RSM. Each approach has many advantages, which are described and discussed in detail below.

2. Experimental

2.1 Materials and sample collection

Authentic standard chemicals, 3-hydroxy-2-butanone, nonanal, acetic acid, decanal, 1-octanol, butanoic acid, hexanoic acid, octanoic acid, and decanoic acid and the internal standard, 1,2-dichlorobenzene, were all HPLC grade and purchased from Beijing Chemical Reagents Company (Beijing, China). Authentic standard chemicals were dissolved in HPLC grade methanol with the final concentration of 2μg L^{-1}, respectively. Authentic standard chemicals were used to carry out qualitative analysis and identify peaks by retention times.

The mixed RWM sample was from Holstein cows of thirty farm factories of Sanyuan Milk Products Co., LTD of Beijing, which was transferred into caramel glass bottles and then placed in a portable refrigerator (T2-DC-40Y, TunTo Green Power Technology Co., LTD of Guangzhou). RWM sample was transported from the factory to our laboratory within two hours. After that, RWM sample was kept at 4 ± 1 ℃ for 30 min until further preprocessing.

RWM sample was centrifuged at 8, 000×g for 10 min at 4 ℃, and then the upper layer

of fat was skimmed. The obtained sample was RSM. The compositions of RWM and RSM were 3.02% (w/w) and 2.99% (w/w) protein, 3.78% (w/w) and 3.78% (w/w) lactose, and 3.53% (w/w) and 0.16% (w/w) fat. The compositions of samples were analyzed by MilkoScan^{TM} Minor (Fossomatic, Foss electric, Hillerød, Denmark).

2.2 Preference evaluation of RWM and RSM

Preference evaluation of RWM and RSM was performed under normal light in clear glasses (approximaley 50 mL) in the sensory laboratory at Beijing Technology and Business University. A panel consisting of twenty panellists was used for the evaluation. RWM and RSM were tasted by each panelists and the preference evaluation was evaluated and recorded by accepting RWM or RSM.

2.3 E-Nose analysis

An electronic nose device PEN 2 E-Nose, provided by (Win Muster Airsense Analytic Inc.) Schwerin, Germany, was used. The sensor array system was composed of 10 metal oxide semiconductors (MOS) of different chemical compositions and thicknesses to provide selectivity towards volatile compound classes as indicated by the instrument supplier: W1C (aromatic compounds), W5S (broad-range compounds), W3C (ammonia, aromatic compounds), W6S (hydrogen), W5C (aromatic-aliphatic), W1S (methane, broad-range compounds), W1W (sulphur compounds), W2S (broad-alcohol compounds), W2W (sulphur-chlorine), and W3S (methane-aliphatic). The instrumentation also included software for data storage and multivariate statistical processing (pattern recognition system).

Three different samples were collected for RWM and RSM. Each analysis was

performed in triplicate for each sampling. Ten milliliter of RWM and RSM were sampled in an airtight vials with a volume of 20 mL (concentration chamber), the sample was stirred magnetically for 5 min at 40±2℃, and then one luer-lock needle (20 g) connected to a Teflon-tubing (3 mm) was used to perforate the seal (plastic) of the vial and to absorb the air accumulated inside it. Clean air to replace sampled air was furnished through a second needle connected to a charcoal filter. Adsorption and desorption of volatiles on the sensors are not instantaneous. Adsorption was measured for 60 s and a standby for 300 s was used for desorption. Software controlled electronic valves were used to move the sample or clean air to the different sensors. Irrespective of the phase, airflow in the measurement chamber was kept constant. During the measurement phase, the sampling unit "inhaled" the volatile gases present in the headspace at a constant rate (6.67 mL s^{-1}) causing changes in the sensor's conductance. This phase lasted 60 s, which was long enough for the sensor signals to reach a stable value. When a measurement was completed, a standby phase of 300 s was initiated. During the standby phase the circuit and the measurement chamber were cleaned and the sensor signals returned to baseline. During this phase, clean air entered the circuit, crossing the measurement chamber first and pushing the remaining volatiles out of the circuit itself.

2.4 Isolation of volatile components from RWM and RSM by HS-SPME

SPME fiber: CAR/PDMS (75 μm thickness, black color) was purchased from Supelco (Supelco, Inc., Bellefonte, PA, USA). The SPME fiber was preconditioned in the injection port of 280 ºC of an Agilent 6890N gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium

was used as the carrier gas with a constant flow of 1 mL min^{-1}. The oven temperature was programmed from 40 ºC (0 min hold), ramped at 10 ºC min^{-1} to 280 ºC, held for 60 min. Samples (10 mL), 13 μg mL^{-1} 1, 2-dichlorobenzene methanol solution (20 μL) as the internal standard, and 2 g of NaCl were added to a 15 mL vial. The vial was tightly capped with a polytetrafluoroethylene (PTFE) septum. The sample was stirred magnetically for 30 min at 40 ± 2 ºC. The needle of the SPME was then inserted through the septum and the SPME fiber exposed to the headspace 5 mm above the sample surface. After 30 min incubation the fiber was retracted into the needle and ejected from the sample vial. The needle was inserted into the GC-MS injector port with for desorption for 3 min.

2.5 GC-MS analysis

Volatiles were identified on an Agilent Technologies 5973i mass spectrometer coupled to an Agilent 6890N gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA, USA). Helium was used as the carrier gas with a constant flow of 1 mL min^{-1}. Diameter of the inlet liner was 0.75 mm. For the SPME analysis, desorption was at 250 ºC for 5 min in splitless mode. Samples went through a DB-Wax (30 m × 0.25 mm × 0.25 μm) capillary column (Agilent Technologies, Inc., Palo Alto, CA, USA). The oven temperature was programmed from 40 ºC (0 min hold), ramped at 5 ºC min^{-1} to 230 ºC, held for 10 min, solvent delay for 3 min. The transfer line temperature was 250 ºC. The mass detector was operated at 150 ºC in electron impact mode at 70 eV, and the ion source temperature was 230 ºC. The chromatograms were recorded by monitoring the total ion current (TIC) in the mass range of 30-300 m z^{-1}, with 5 scan s^{-1}.

Identification of volatile compounds was based on the comparison of their mass spectra with spectra from the National Institute of Standards and Technology (NIST) 2011 database. A series of n-alkanes (C6-C30) was run under the same chromatographic conditions in order to calculate the retention index (RI) of detected compounds for comparison with the RI in the NIST 2011 database using the same capillary column. Authentic standard chemicals were used to confirm the identifications of volatile compounds.

2.6 GC-O analysis

GC-O analysis was performed on an Agilent 6890N gas chromatograph (Agilent Technologies, Inc. Santa Clara, U. S. A.) with a capillary column DB-Wax (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Inc. , Palo Alto, CA, USA) equipped with a sniff port (Sniffer 9000, Brechbühler Scientific Analytical Solutions Inc., Switzerland). Nitrogen was used as the carrier gas with a constant flow of 1 mL min^{-1}. Chromatographic conditions utilized were identical to those of GC/MS analysis. The column effluent was divided (ratio 1:1) between the FID detector and the sniff port by a ‘‘Y’’ shaped glass splitter. The effluent to the odor port was enclosed with a stream of humidified air flowing at 16 mL min^{-1} and transferred to a glass detection cone by a length of capillary column kept at 200 ºC.

Samples (10 mL) and 2 g of NaCl were added to a 15 mL vial. The vial was tightly capped with a polytetrafluoroethylene (PTFE) septum. The sample was stirred magnetically for 30 min at 40 ± 2 ºC. The needle of the SPME was then inserted through the septum and the SPME fiber exposed to the headspace 5 mm above the sample surface. After 30 min incubation the fiber was retracted into the needle and ejected from the sample vial. The needle

was inserted into the GC-O injector port with for desorption for 3 min.

By smelling and recording the odour descriptions, eight well trained panellists performed the GC-O analysis. The perceived aroma intensity was evaluated and recorded by using the degree of "light" (L), "middle" (M), and "strong" (S). Each sample was sniffed three times by each panelist [11]. The RI of the aroma-active compounds was calculated against the retention time of a series of n-alkanes (C6-C30) obtained under the same GC-O conditions and was compared with the RI by GC/MS. The aroma-active compounds were confirmed if the relative error between the sniffed RI and the GC/MS RI was less than 0.1%.

2.7 Statistical analysis

The experimental results of categories for the major volatile flavour compounds were performed using t-tests and all data were analyzed using the SPSS software package (version 17, SPSS Inc., Chicago, IL, USA). Statistical significance was determined at $P < 0.05$. Data were standardize before pre-treated prior to principle component analysis (PCA) using the SPSS software package. In our case, all initial data were expressed as the means and standard deviation of triplicate determinations.

3. Results and discussion

3.1 E-Nose responds to milk volatiles

Typical response signals of ten sensors to RWM and RSM are shown in Figure 1A and B. For RWM and RSM, the conductivity of ten sensors changed quickly at the first 10 s, then changed gradually, and finally reached a stable equilibrium. In Figure 1A and B, we also observed that the ratio of conductance (G/G_0) of each sensor was close to 1.0 in the initial

period, and changed continuously. Finally, the trend was tending towards stabilization after approximately 50 s. The response signals of each sensor at 50-53 s were used in the subsequent analyses. For RWM, the G/G_0 value of the broad range and aromatic sensors W1S, W2S, W5S, W1C, W3C, and W3S reached maximum while the G/G_0 value of hydrogen sensor W6S reached a minimum during the first 10 s. However, the G/G_0 value of sulfur and aromatic sensors W1W, W2W, and W5C changed very little (0.057-0.186). For RSM, the G/G_0 value of sensors W1C, W2S, W3C, W1S, W5S, W5C, W1W, and W2W reached a maximum while the G/G_0 value of sensors W6S merely changed and the G/G_0 value of sensors W3S reached minimum during the first 10 s (Figure 1B). The broad range and aromatic sensors W1S, W2S, W3C, W5S, and W1C had more significant change in their G/G_0 values, indicating that these four sensors were more sensitive to the volatile flavour compounds of RWM and RSM.

Principle component analysis (PCA) was performed with the SPSS software and the cross validation was used to compare multiple independent groups. The PCA results reduced the dimensionality of the original data set by explaining the correlation among a large number of variables in terms of a smaller set of underlying factors without losing much information [19]. Factor score plots were used to indicate similar, dissimilar, or typical data.

The PCA analysis by E-Nose is shown in Figure 2. There was a distinct separation between RWM and RSM. The separation between the two groups occurred in the first principal component where more than 72% of the total data variance was plotted. Moreover, the result of PCA also showed that RWM was located on the negative of PC2 and kept

separate from the sample cluster of RSM. Through visual observation of the PCA results, the flavour profiles of RWM and RSM could be distinguished. In order to evaluate the ability of E-Nose to discriminate among different milk samples, the sensor sensitivities were elaborated (Table 1). Meanwhile, the PCA results of the sensitivity of the ten sensors to the volatile flavour gas of RWM and RSM are listed in Figure 3. Sensors W1S and W6S were more sensitive to RWM on the PC1 component. In contrast, W1C and W3C sensors were more sensitive towards RSM on the PC2 component. It would be seen that the different sensors in the array were sensitive to the different volatile compounds released from RWM and RSM. From the PCA analysis of E-Nose, RWM and RSM could be distinguished by a relatively few volatile components.

3.2 Difference on the amount of volatile profiles between RWM and RSM

The results from ANOVA showed significant ($P < 0.05$) differences among RWM and RSM about the preference (Figure 4). To determine the differences of volatile compounds in RWM and RSM, HS-SPME adsorption of the headspace was combined with GC-MS. A significant ($P < 0.05$) difference in the headspace concentration of volatile profiles between RWM and RSM was observed (Table 1). Fifteen (13) compounds were extracted from RWM, while 11 compounds were extracted from RSM by CAR/PDMS, a polar coating fiber. CAR/PDMS has the property of extracting more small analytes and can adsorb the trace level of volatile components. CAR/PDMS-coated fiber has been used previously by Karatapanis *et al*. [7] and Marsili [20] to analyze milk volatile flavours.

3.3 Identification of volatile flavour compounds from RWM and RSM by GC-MS

The detailed volatile components found in RWM and RSM samples were summarised by chemical groups: fatty acids (FAs, from C2:0 to C16:0), alcohols, aldehydes, and ketones (Figure 5). The results indicated that FAs were the major volatile compounds in RWM and RSM (Figure 6), which agreed with a previous report [21]. The FA content of RWM was significantly ($P < 0.05$) higher (4.66%) than that of RSM (Figure 7). The FA composition of RWM included C2:0 to C16:0 while the range of FA composition of RSM was C2:0 to C14:0. Meanwhile, the FAs contents of C2:0 to C14:0 in RWM were significantly ($P < 0.05$) higher (2.22-6.24%) than those in RSM (Figure 7). As for the flavour compounds of alcohols, ketones, and aldehydes, the concentrations of these compounds were significantly ($P < 0.05$) lower in RSM than that in RWM, which might contribute to the reduced consumer acceptability of RSM.

3.4 Identification of aroma-active compounds from RWM and RSM by GC-O

Aroma-active compounds of RWM and RSM were analyzed by the SPME extracts coupled with GC-O. A total of 9 aroma-active compounds were detected by GC-O in RWM and 3 in RSM, including 7 acids, 1 aldehyde, and 1 ketone (Table 1). Accounting for 69% of all the volatile compounds identified by GC-MS were aroma-active compounds, which have fatty, vinegar, sweet, fruity, green, sour, rancid, and tallow odor. Odor descriptions of all flavour compounds identified in the volatile compounds of RWM and RSM were basically similar to a previous report [15].

As can be seen from Table 1, octanoic acid, butanoic acid, and 3-hydroxy-2-butanone showed “strong” aroma-active flavour intensity, which were the most important aroma-active

compounds among the 9 compounds in RWM. However, among the volatile compounds of RSM, only octanoic acid showed “strong” aroma-active flavour intensity.

4. Conclusions

In conclusion, we developed a novel triple-channel comparative analysis of volatile composition in RWM and RSM via GC-MS, GC-O and E-Nose. This is the first time on the application of non-destructive analytical method to compare the analysis of volatile flavour composition in RWM and RSM via E-Nose, GC-MS, and GC-O. The E-Nose analysis can represent a valid supplement with the chance of becoming an alternative to the traditional analytical methods used in the fight against counterfeiting of foods. Further studies will be conducted to improve the flavour of RSM to understand the contribution of individual components to increase consumer acceptance of RSM.

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Figure legends

Figure 1. Response curves of ten sensors (W1C: aromatic compounds, W5S: broad-range compounds, W3C: aromatic, W6S: hydrogen, W5C: aromatic-aliphatic, W1S: broad-methane, W1W: sulphur-organic, W2S: broad-alcohol, W2W: sulphur-chlorine, and W3S: methane-aliphatic) with to raw milk volatiles by electronic nose (E-Nose): (A) raw whole milk (RWM) and raw skim milk (RSM). The x-axis represents time and the y-axis represents sensor's ratio of conductance (G/G_0, where G and G_0 are the conductivities of the sensors when exposed to the sample gas and the zero gas, respectively). Each curve represented the variation of conductivity of each sensor with time when the milk volatile flavour compounds reached the measurement chamber.

Figure 2. Principle component analysis (PCA) results of raw whole milk (RWM) and raw skim milk (RSM) by electronic nose (E-Nose).

Figure 3. Principle component analysis (PCA) results of the sensitivity of the ten sensors (W1C: aromatic compounds, W5S: broad-range compounds, W3C: aromatic, W6S: hydrogen, W5C: aromatic-aliphatic, W1S: broad-methane, W1W: sulphur-organic, W2S: broad-alcohol, W2W: sulphur-chlorine, and W3S: methane-aliphatic) to the volatile flavour gas of raw whole milk (RWM) and raw skim milk (RSM).

Figure 4. Percentage preference evaluation of raw whole milk (RWM) and raw skim milk (RSM). Statistical significance of Percentage preference between RWM and RSM was calculated using t-tests, * $P < 0.05$, and ** $P < 0.01$.

Figure 5. Categories and contents of the major volatile compounds in raw whole milk (RWM) and raw skim milk (RSM). Statistical significance of contents of the major volatile compounds between RWM and RSM was calculated using t-tests, * $P < 0.05$, and ** $P < 0.01$.

Figure 6. Total ion chromatogram of milk volatile flavors. A: RWM, B: RSM

Figure 7. Contents of fatty acids **(**FAs) from raw whole milk (RWM) and raw skim milk (RSM). Statistical significance of the FAs contents between RWM and RSM was calculated using t-tests, * $P < 0.05$, and ** $P < 0.01$.

Table 1 The volatile compounds in RWM and RSM identified by GC-MS, GC-O, and ~~E-Nos~~ E-Nose

NO [c]	RT [d]	RI [e]	Compound	Odor description [f]	CAS	Identified method [g]	HS-SPME [h] (μg L^{-1})		GC-O [i]		E-Nose [j]	
							RWM	RSM	RWM	RSM	RWM	RSM
1	9.88	1272	3-hydroxy-2-butanone	Fatty	000513-86-0	MS, RI, STD	0.24±0.06	0.10±0.04	S	—	***	##
2	12.66	1362	nonanal	Fatty	000124-19-6	MS, RI, STD	0.84±0.11	0.64±0.19	L	L	***	##
3	14.13	1421	acetic acid	Vinegar	000064-19-7	MS, RI, STD	1.23±0.01[a]	0.23±0.01[b]	M	—	***	##
4	15.25	1469	decanal	Sweet	000112-31-2	MS, RI, STD	0.42±0.03	—	—	—	***	##
5	16.82	1533	1-octanol	Fruity	000111-87-5	MS, RI,STD	0.97±0.04[a]	0.12±0.01[b]	—	—	**	**
6	18.38	1595	butanoic acid	Green	000107-92-6	MS, RI, STD	2.46±0.38[a]	0.40±0.02[b]	S	L	***	##
7	21.11	1714	2(5H)-furanone	—	000497-23-4	MS, RI	0.37±0.04	0.12±0.06	—	—	***	##
8	23.21	1811	hexanoic acid	Sour	000142-62-1	MS, RI, STD	3.97±0.05[a]	0.54±0.03[b]	L	—	***	##
9	27.53	2022	octanoic acid	Rancid	000124-07-2	MS, RI, STD	4.90±0.18[a]	1.06±0.20[b]	S	S	***	##
10	31.46	2235	decanoic acid	Tallow	000334-48-5	MS, RI, STD	3.77±0.14[a]	1.18±0.33[b]	L	—	***	##
11	35.07	2445	dodecanoic acid	Fatty	000143-07-7	MS, RI	2.26±0.28[a]	0.39±0.05[b]	M	—	***	##
12	38.39	2656	tetradecanoic acid	Fatty	000544-63-8	MS, RI	6.27±2.00[a]	1.16±0.13[b]	M	—	***	##
13	45.56	2682	hexadecanoic acid	—	000057-10-3	MS, RI	2.37±0.22	—	—	—	***	##
			Aromatic [k]	—	—	—	—	—	—	—	**	###
			Total				30.05±2.70[a]	6.13±0.34[b]				

RWM: raw whole milk, RSM: raw skim milk.

Data in the same row with different superscript letters are significantly different ($P < 0.05$) (statistical analysis was performed using t-tests).

c. Volatile compounds were listed in order of the retention time.

d. The retention time of compounds.

e. The RI of unknown compounds on DB-Wax column calculated against the GC-MS retention time of n-alkanes (C6-C30).

f. The description of the odour from the references: George [22], (http://www.odour.org.uk/odour/index.html) [23], and Angelino [24]. "—"means not found.

g. MS: compared with Nist 2011 Mass Spectral Database or the published mass spectra. RI: agrees with retention index of literatures. STD: agrees with RI and mass spectrum of standard chemical.

h. Content of compounds were calculated by internal standard quantitative identified by HS-SPME-GC-MS, and the data was the ‘‘mean standard deviation’’. “—”: means not detected.

i. The assessment was repeated three times with eight trained and experienced panelists. “L” represents light; “M”, middle;” S: strong. “—” represents not detected.

j. Results of RWM and RSM analyzed by ten sensors of E-Nose (W1C: aromatic compounds, W5S: broad-range compounds, W3C: aromatic, W6S: hydrogen, W5C: aromatic-aliphatic, W1S: broad-methane, W1W: sulphur-organic, W2S: broad-alcohol, W2W: sulphur-chlorine, and W3S: methane-aliphatic). PC1: “Strong” ***, “Moderate” **, PC2: “Strong” ###, “Moderate” ##.

k. Aromatic gas analyzed by E-Nose.

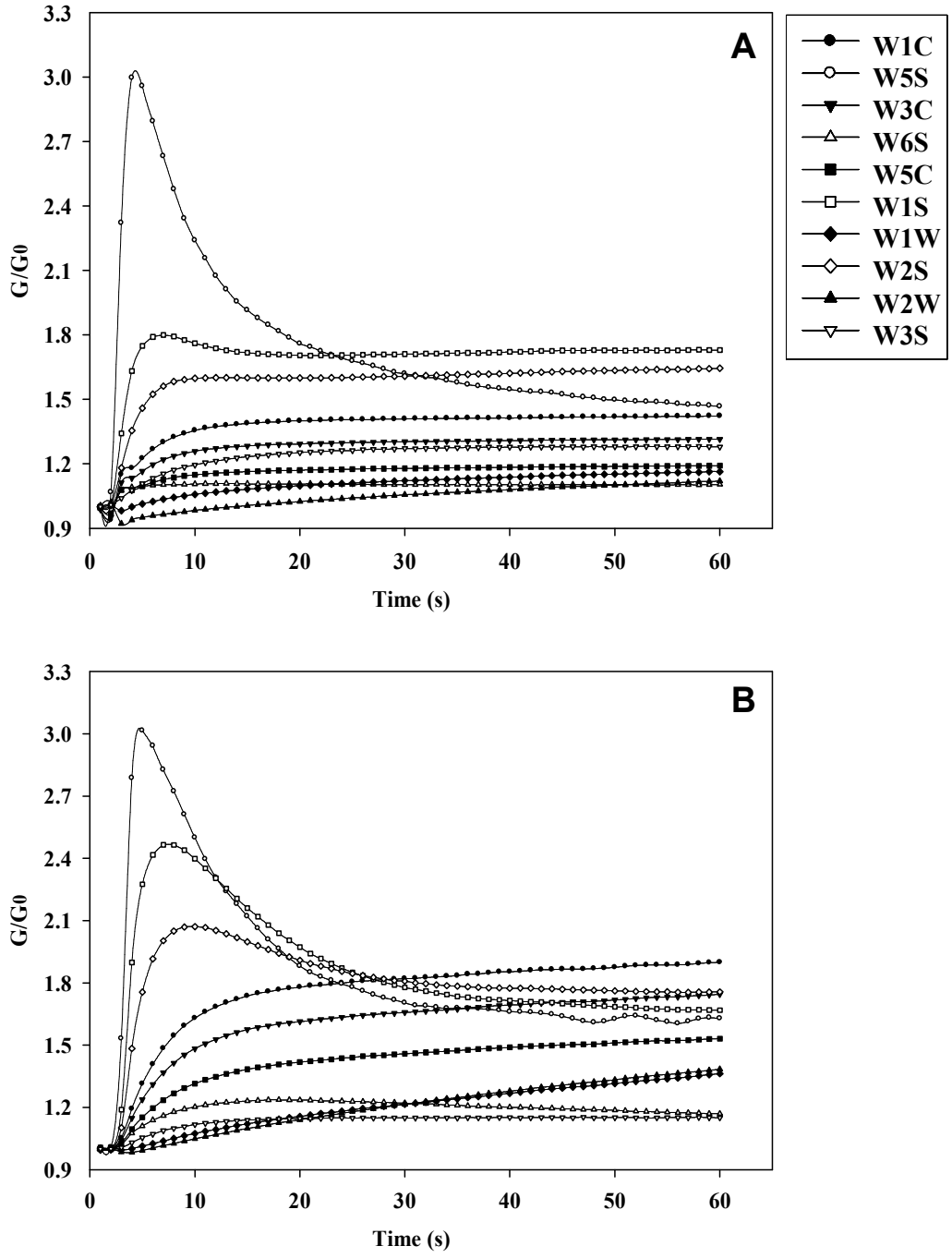


Fig. 1

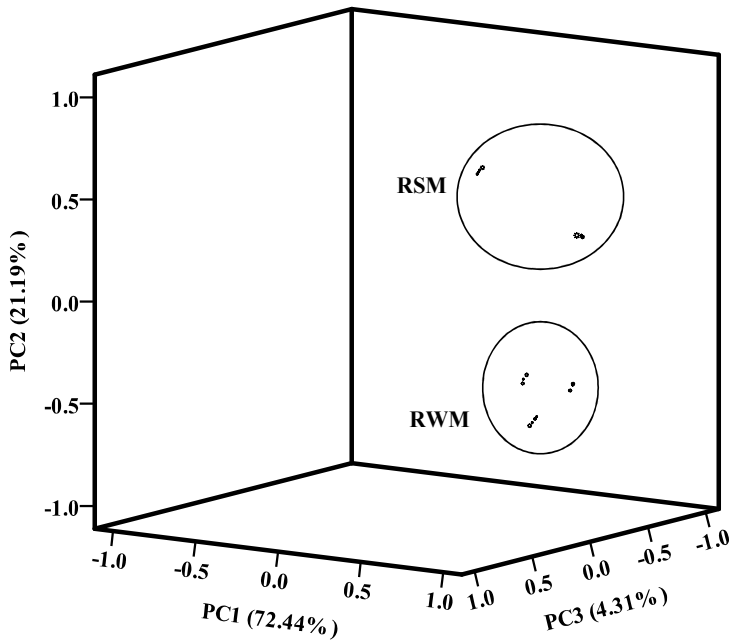


Fig. 2

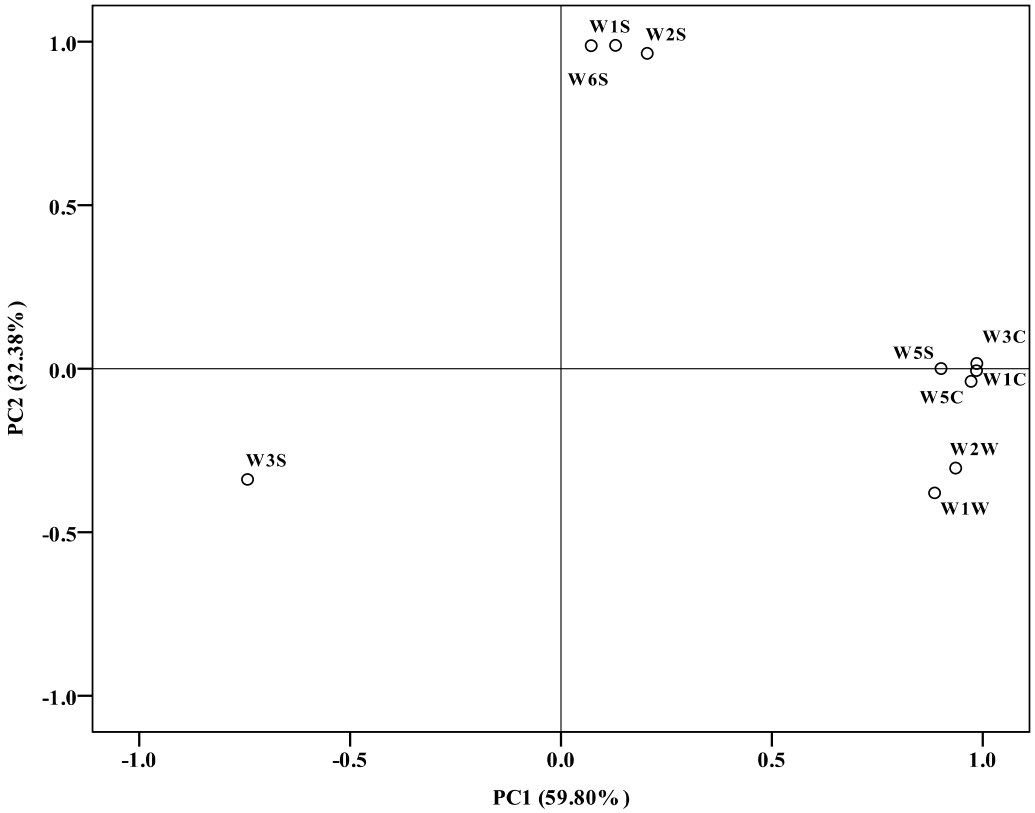


Fig. 3

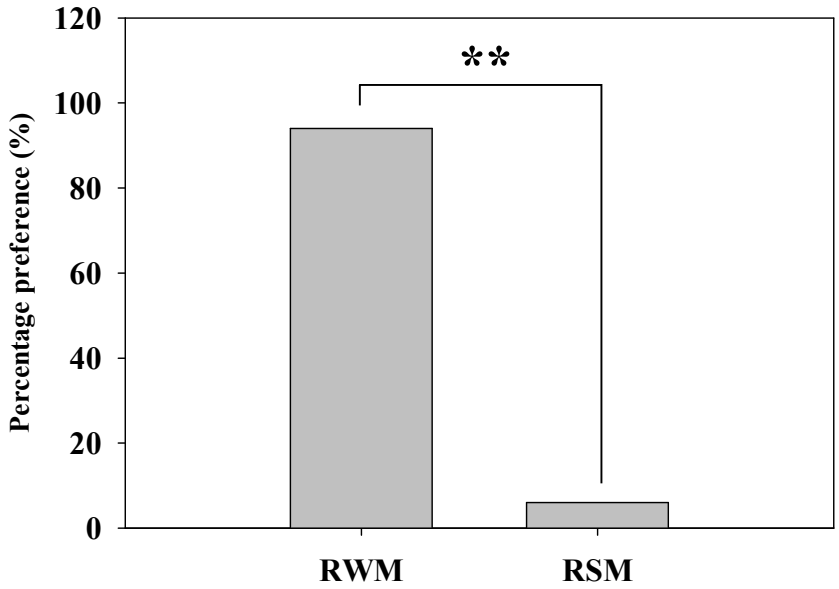


Fig. 4

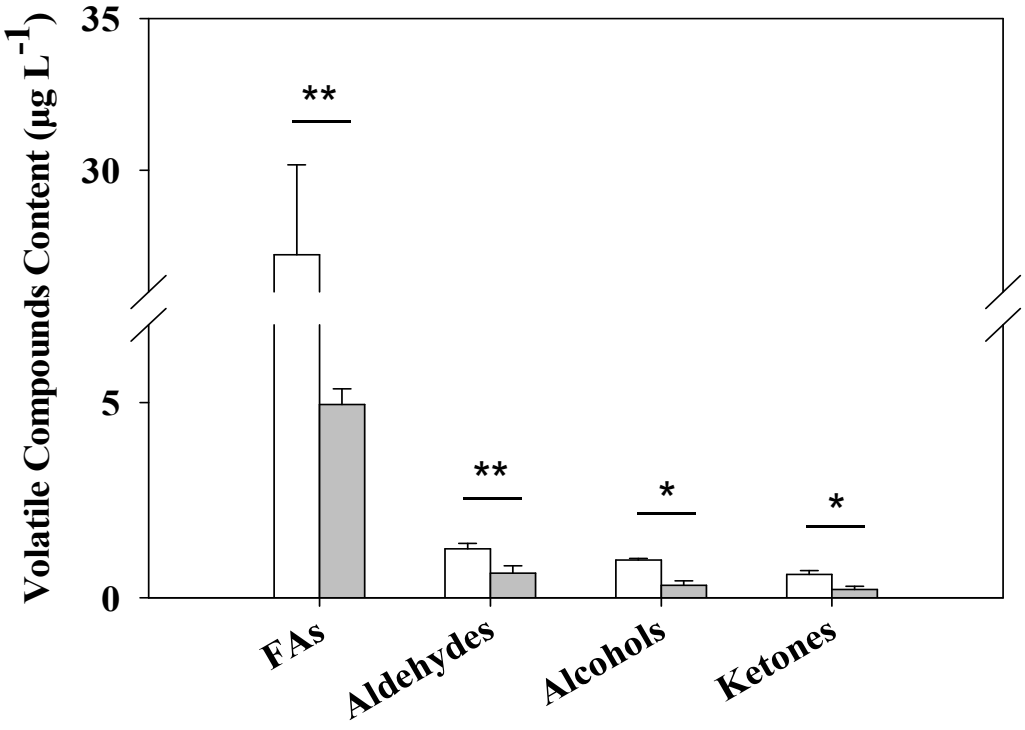


Fig. 5

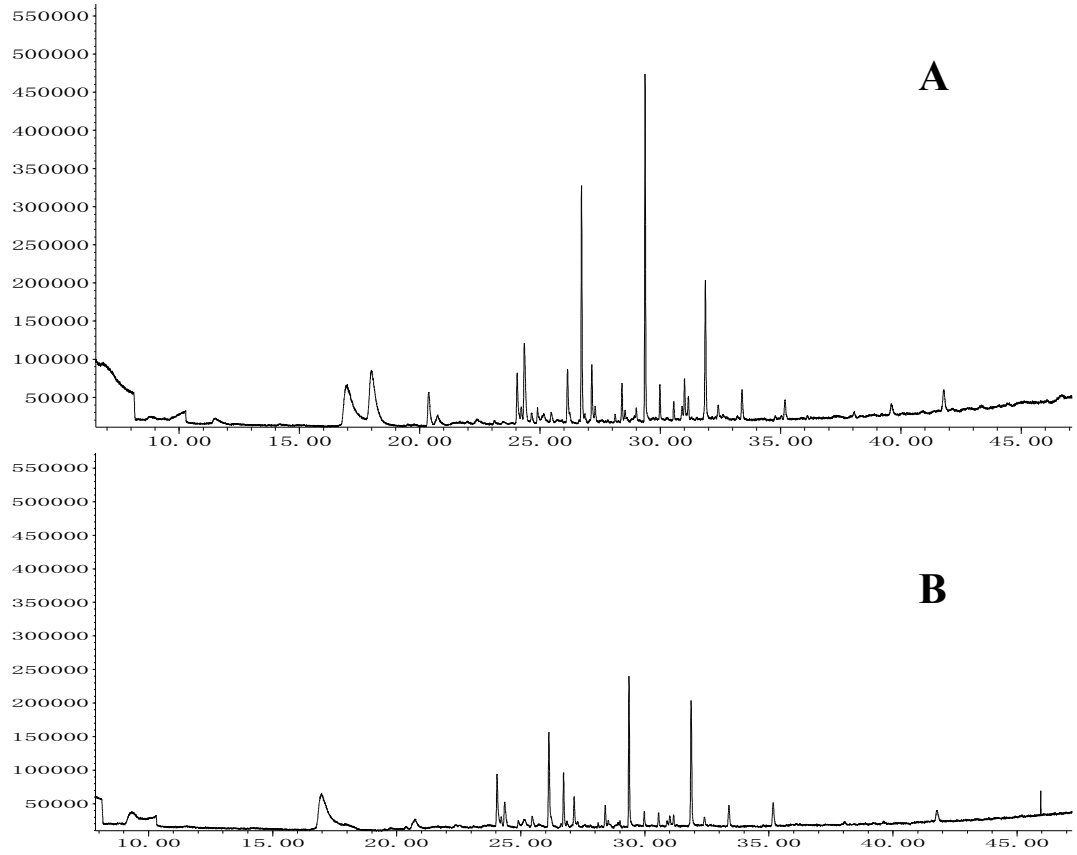


Fig. 6

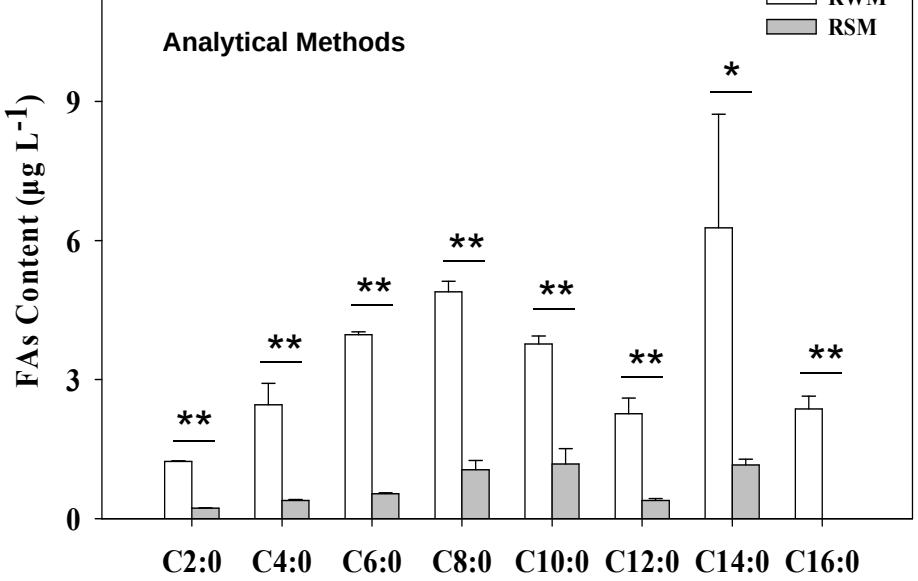
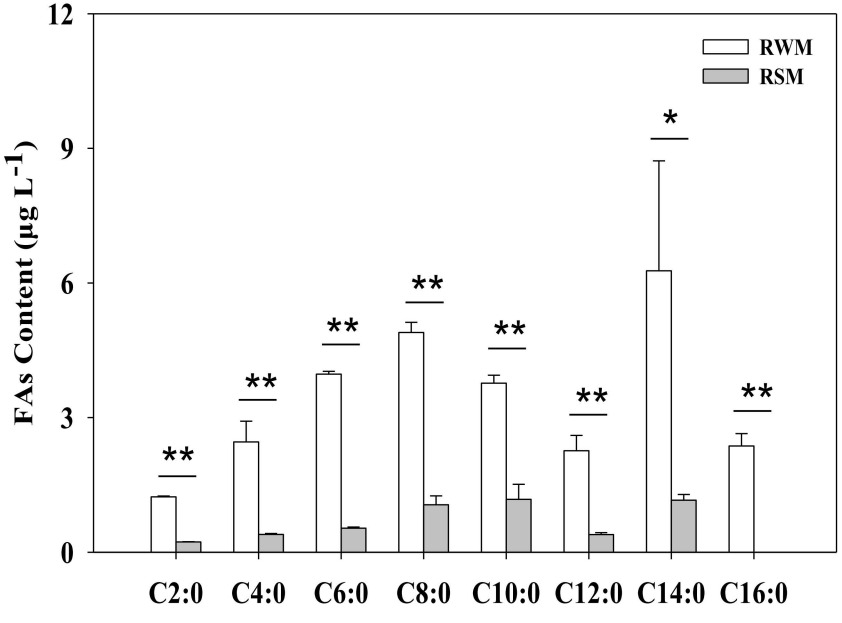


Fig. 7

GC-MS



GC-O

E-Nose