Flavoromics approach to identifying the key aroma compounds in traditional Chinese milk fan

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ABSTRACT

To determine the key aroma compounds in Chinese milk fan cheese, samples from 6 of the most popular handmade milk fan workshops in Yunnan province of China were analyzed by gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), sensory evaluation, aroma recombination and omission experiments. Seventy-one aroma compounds were identified by GC-MS, and 31 odor-active compounds were detected by GC-O. The relationships between the 31 odor-active compounds and 10 sensory evaluation properties were explored by partial least squares discriminant analysis. The results identified 23 initial key aroma compounds, which were used to simulate the aroma profile of milk fan in aroma recombination experiments. Aroma omission experiments identified propanoic acid, butanoic acid, octanoic acid, octanal, nonanal, 2-nonanone, and ethyl hexanoate as the key aroma compounds. Propanoic acid and butanoic acid contributed to cheese and rancid aromas, octanal and nonanal to the fat aroma, octanoic acid and 2-nonanone to the cheese aroma, and ethyl hexanoate to the fruity aroma of milk fan.

Key words: milk fan, key aroma compounds, gas chromatography-mass spectrometry, gas chromatography-olfactometry, aroma recombination and omission

INTRODUCTION

Milk fan is a traditional Chinese cheese that has been produced for more than one thousand years, and it is a famous delicacy of the Bai ethnic minority in Yunnan Province (Liu et al., 2009). Named after its characteristic paper fan shape, milk fan is about 30 to 40 cm long, 8 to 10 cm wide, and <2 mm thick (Liu et al., 2011). It is made from the milk of the Dengchuan yak, a breed of dairy cattle in southern China (Ma et al., 2012). At

an altitude of about 1,900 m, the Dengchuan region has a temperate climate throughout the year with plentiful rain, which increases the types and quantities of microorganisms. The processing technology of milk fan differs from that of curd-coagulated cheese. Milk fan is a traditional acid-curd cheese made by adding papaya acid juice to raw milk (which alters the pH value), curdling, blanching, stretching, forming, and drying (Zhang et al., 2014). The unique raw materials and processing technology give milk fan its unique flavor.

The flavor compounds in cheese have been extensively investigated, with studies having identified more than 3,000 volatile and nonvolatile components (Fox and Wallace, 1997). The aroma characteristics of cheese are mainly provided by the volatile components, including fatty acids, alcohols, aldehydes, esters, ketones, lactones, pyrazines, sulfur compounds, amines, and other flavor-active compounds (Masotti et al., 2017; Pelin et al., 2018). However, not all flavor compounds can be perceived by human olfactory receptors and thus contribute to the aroma characteristics (Bertuzzi et al., 2018).

The key aroma compounds can produce the perception of aromas in the human brain through interaction with olfactory organs (Dunkel et al., 2014). In recent years, the flavoromics approach to food flavor analysis has emerged to identify key aroma compounds in food (Gracka et al., 2016). The approach uses high-resolution instruments to collect sample data, which are then analyzed in combination with stoichiometric methods to fit the artificial sensory evaluation data (Karametsi et al., 2014). The gas chromatography-olfactory (GC-O) method combines instrument data with sensory data to provide a valuable tool for analyzing odor-active compounds and studying the activity of key aroma compounds (Song and Liu, 2018). The GC-O method has been used to investigate the odor-active compounds of milk (Jo et al., 2018b), beverages (Zhao et al., 2018), and other foods (Zhu and Xiao, 2018). Majcher et al. (2014) and Jo et al. (2018a) identified the odor-active compounds in cheese using GC-O. Stoichiometric methods have often been used to analyze large amounts of data to reveal hidden information (Medvedev et al.,

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2017). For example, the relationships between the relative abundance of odor-active compounds in cheese and their sensory properties were investigated by partial least squares regression (PLSR; Zabaleta et al., 2016). Zhang et al. (2018) analyzed the relationship between sensory indicators and GC-O data using PLSR and found that some substances with low content, such as methyl 2-methyl-3-furanyl disulfide, had significant effects on the flavor characteristics of semi-hard cheeses (Zhang et al., 2018).

Aroma recombination and omission experiments can be used to verify results of key detected aroma compounds and the contribution of such compounds to the overall aroma. Majcher et al. (2018) verified that methyl ketones were not key aroma compounds in Lazur cheese by aroma recombination and addition experiments. Gao et al. (2014) determined that β -damascenone and ethyl acetate were the key aroma compounds in the typical pleasant, fruity, and floral aromas of Chinese liquor by means of GC-O, odor activity values (OAV), and omission experiments. However, there have been few investigations of the key aroma compounds in milk fan.

The main aim of this study was to identify the key aroma compounds in Chinese traditional milk fan using a flavoromics approach. A flowchart of the experimental process is presented in Figure 1. Sensory evaluation was used to describe the sensory characteristics of milk fan. The aroma components were analyzed by GC-MS, and the odor-active components were identified by GC-O. Then, partial least squares discriminant analysis (PLS-DA) was used to assess correlations between the odor-active compounds and sensory properties to obtain the initial key aroma compounds. Finally, aroma recombination and omission experiments were used to further confirm the key aroma compounds and their contributions to the sensory characteristics. The results can be used as a reference for the quality evaluation, further promotion, and development of Chinese traditional milk fan.

MATERIALS AND METHODS

Samples

Six milk fan samples (Y1, Y2, Y3, Y4, Y5, and Y6) made from the same materials and using the same processing techniques were purchased from 6 of the most popular handmade workshops in Yunnan Province, China. Although the processing was almost identical, specific parameters, such as pH of the acid juice and curd temperature and time, were different. The samples were transported to the laboratory in ice boxes the day

after production (March 14, 2018) and frozen at -20°C until they were analyzed.

Chemicals

Propionic acid, butanoic acid, 3-methyl-butyric acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid, 3-methylbutyraldehyde, octanal, nonanal, (E)-2-octenal aldehyde, 1-pentanol, 1-hexanol, 1,6-octadiene-3-alcohol, nonanol, *para*-cresol, 2-heptanone, 3-octanone, 2-nonanone, acetophenone, ethyl butyrate, ethyl caproate, ethyl octanate, and D-limonene were purchased from Sigma-Aldrich (St. Louis, MO). 2-Octanol (internal standard, **IS**) and *n*-alkane standards (C_6 – C_{30}) were purchased from Dr. Ehrenstorfer GmbH Co. Ltd., Germany. All of the chemicals were of chromatographic grade.

Solid-Phase Microextraction of Aroma Compounds in Milk Fan

The milk fan samples (4 g) were thawed for 24 h at 4°C , kept at room temperature for 1 h, and then cut evenly into cubes. The cube samples and 100 μL of IS solution (13 mg/L) were placed in a 15-mL vial and covered with Teflon covers. The aroma compounds were extracted by using a solid-phase microextraction (SPME) fiber (Supelco Inc., Bellefonte, PA). The main parameters for SPME analysis were optimized based on the method of Jo et al. (2018b), including the type of SPME fiber [50-/30- μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS),

Figure 1. Process of identifying the key aroma compounds of milk fan. GC-O = gas chromatography-olfactometry; PLS-DA = partial least squares discriminant analysis.

75- μ m CAR/PDMS, and 65- μ m PDMS/DVB], extraction temperature (50, 60, 70, and 80°C), and extraction time (20, 30, 40, and 50 min). The sample vials were kept in a water bath at 60°C for 40 min for the GC-MS and GC-O analyses.

GC-MS Analysis

Volatile compounds were detected by an Agilent 7890-5973 GC-MS (Agilent Technologies, Santa Clara, CA) system and an HP-Innowax analytical fused-silica capillary column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m; Agilent Technologies). The injection was in splitless mode. The oven temperature was held at 40°C for 4 min, increased to 100°C at a rate of 3°C/min and held for 2 min, increased to 150°C at 4°C/min, and then increased to 230°C at 10°C/min and maintained for 5 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The electron ionization energy of the MS detector was 70 eV, the ion source temperature was 230°C, with the quadrupole in scan mode (total ion currents from 30 to 450 m/z at 1 scan/s).

The volatile compounds were identified by retention indices, which were determined by straight-chain alkanes (C₆–C₃₀) and by comparing them with MS images in the NIST11 mass spectral database library. Volatile compounds were quantified using internal standards. Before quantification, an IS curve was prepared by adding 100 μ L of different concentrations of propionic acid and 100 μ L of IS solution (13 mg/L) to milk fan samples to confirm whether there was a linear relationship between the ratio of the concentration of the tested compounds (C_i) to IS (C_{IS}) ($x = C_i/C_{IS}$) and the ratio of their peak areas (A) ($y = A_i/A_{IS}$) under certain chromatographic conditions. The standard curve was $y = 0.7269x + 0.0004$ and the linear correlation factor R^2 (0.993) of the standard curve met the experimental requirements. The formula for the relative content of volatile compounds is as follows: $C_i = f_i \times C_{IS} \times A_i/A_{IS}$, where $f_i = (A_i/A_{IS})/(C_i/C_{IS})$, representing the slope of the internal standard curve.

GC-O Analysis

Aroma compounds were identified using an Agilent 7890 GC system coupled with an olfactory detection port (Gerstel ODP-2, Mulheim an der Ruhr, Germany). The GC effluent was set to be separated equally between the flame-ionization detector and the sniffer. Milk fan samples were separated on an HP-Innowax (60 m $\times$ 0.25 mm $\times$ 0.25 μ m; Agilent Technologies) analytical fused-silica capillary column.

The air carried the capillary column effluent into a glass funnel where odor-specific magnitude estimation

(OSME) analysis was conducted, which is a time-intensity method for GC-O. Fifteen panelists were trained before sniffing and were familiarized with the odor descriptions using solutions of the reference odorants. Panelists were required to note the onset and end time, odor characteristic, and intensity while sniffing the effluent from the sniffing mask. The aroma intensity was evaluated using a 5-point intensity scale from 0 to 5, where 0 was none, 3 was medium, and 5 was extremely strong. Each experiment was performed in triplicate by each panelist. The aroma intensity value was the average of the 15 panelists.

Odor Activity Values

Odor activity values were used to evaluate the contribution of each compound to overall milk fan aroma, which was calculated as the ratio of the concentration of a single aroma compound to its detection threshold in air. The threshold values were taken from the literature.

Sensory Evaluation

The sensory evaluations, GC-O analysis, and aroma recombination and omission experiments were performed by 15 panelists (8 men and 7 women, average age 23 yr). All had previously received professional training in sensory evaluation. The odor descriptors (fruity, cheesy, milky, sour, rancid, musty, nutty, umami, sulfurous, and fatty) were chosen in preliminary tests by the panelists (Majcher et al., 2018). The 10 descriptors were defined as follows, and relevant material objects or chemical standards were used as references: 20 mg/kg ethyl hexanoate for “fruity”; fresh butter for “cheesy”; fresh milk for “milky”; 0.08% citric acid solution in water for “sour”; butanoic acid for “rancid”; potting soil for “musty”; unsalted raw nuts for “nutty”; 1% monosodium glutamate solution in water for “umami”; mashed boiled egg for “sulfurous”; and salad oil for “fatty” (Aday and Yuceer, 2014). The 8-g milk fan samples or recombinants were kept in an odor-proof container (100 mL) until each panelist's evaluation. The odor properties were rated from 0 (none) to 10 (very strong). The tests were performed in triplicate.

Aroma Recombination Experiments

Aroma recombination was undertaken to further confirm the key aroma compounds. The 23 initial key aroma compounds with OAV ≥ 1 were dissolved in glycerol triacetate matrix in their natural concentrations (in Y6) and then equilibrated at an ambient temperature for 10 min, and then a complete recombination

model Y was obtained. A descriptive profile test was performed by the 15 panelists. The method was the same as described in the Sensory Evaluation section.

Aroma Omission Experiments

The contribution of aroma compounds to the overall aroma of milk fan was investigated by the triangle test, in which one or a group of compounds was omitted from the recombinant aroma model Y to obtain an omission model. Every omission model together with 2 recombinants (5 g each) was given to the panelists for sensory evaluation at random.

Statistical Analysis

Descriptive data and the differences between samples were analyzed by ANOVA and Duncan's test using SPSS (version 13.0; SPSS Inc., Chicago, IL). Then, PLS-DA was used to identify the contribution of the odor-active compounds to the sensory evaluation of milk fan by using R software with the mixOmics package (<http://mixomics.org>). Partial least squares discriminant analysis applies a quantitative partial least squares method to discriminant analysis and is suitable for multidimensional data analysis.

RESULTS AND DISCUSSION

Optimization of Headspace-SPME Conditions

Three headspace-SPME variables (type of SPME fiber, extraction temperature, and extraction time) were investigated. The efficiency of the extraction process is critically dependent on the fiber coating (Risticvic et al., 2010). The highest number of aroma compounds and highest total peak areas were obtained with the 75- μ m CAR/PDMS fiber. The polarity of this coating material is more consistent with the volatile compounds in milk fan and has a larger partition coefficient, so the extraction and flavor enrichment ability of the milk fan is stronger (Lucey, 2011). In general, the number of adsorbed compounds increases sharply at the beginning of the extraction process, and the number of components tends to stabilize after reaching a certain equilibrium point. Extraction time strongly influences the extraction effect. The results showed that the number of aroma compounds and the total peak areas obtained after 40 min of extraction were greater than those obtained after 50 min, indicating that extraction reached equilibrium after 40 min. If the extraction time is too long, the accuracy of the relative content of the analyte will be reduced because the compounds compete for position on the fiber. If the fiber stays in the headspace

too long, this competition can cause inaccuracies in the relative amounts of analytes (Howard et al., 2005). Extraction temperature also has a strong effect on the type and quantity of volatile compounds in the samples (Rocha et al., 2001). The results showed that the number of aroma compounds and the total peak areas were maximized when the extraction temperature reached 50 to 60°C, and then remained steady as the temperature increased to 80°C. The aroma extraction of milk fan was therefore carried out with the 75- μ m CAR/PDMS SPME fiber at 60°C with an extraction time of 40 min.

Quantitative Analysis of Aroma Compounds of Milk Fan

Seventy-one aroma compounds were detected, including 10 acids, 8 aldehydes, 9 alcohols, 9 ketones, 19 esters, 11 aromatic and heterocyclic compounds, and 5 other compounds. Acids (30.8–71.6%; percentages indicate the ratio of the content of this substance to the total aroma content), esters (3.94–67.6%), and ketones (2.73–17.9%) were the most abundant groups. The main aroma compounds analyzed by GC-MS were consistent with other types of cheeses analyzed by this method, including Cheddar, Camembert, Grana Padano, and Gruyère (Curioni and Bosset, 2002).

Acids were the compounds present at the highest levels. The fatty acid content in milk fan (total acids ~9,400 to 21,100 μ g/kg) was significantly higher than that in rennet-curd cheese (total acids 3,028 μ g/kg; Hayaloglu et al., 2013). The high fatty acid content may be due to the unique acidulation processing technology of milk fan. Unlike a curd-coagulated cheese, milk fan is a traditional acid-curd cheese made by adding papaya acid juice to the raw milk (Zhang et al., 2014). The acid juice is also known as whey. It contains many probiotic strains and normally contains *Lactobacillus acidophilus* to promote acid development (Gallardo-Escamilla et al., 2005). The butanoic acid content of the milk fan (3,190–10,700 μ g/kg) was higher than that of Gokceada cheese (677 μ g/kg), as was the hexanoic acid content (milk fan: 2,900–6,500 μ g/kg; Gokceada: 820 μ g/kg) and octanoic acid content (milk fan: 1,400–2,800 μ g/kg; Gokceada: 305 μ g/kg). From a biochemical point of view, electrophoretic patterns have demonstrated that β -LG is the main protein present in ricotta forte (a type of acid-curd cheese), indicating that intermediate levels of proteolysis took place in acid-curd cheese. The main biochemical event was lipolysis and many short-chain fatty acids were produced (Faccia et al., 2018). However, some of the acids (propanoic and butanoic) in milk fan in the current study were present at levels lower than in Cheddar (Drake et al., 2010). Fatty acids play an important role in the aroma of milk

fan. Butanoic acid (3,190–10,700 $\mu\text{g/kg}$), hexanoic acid (2,900–6,500 $\mu\text{g/kg}$), octanoic acid (1,400–2,800 $\mu\text{g/kg}$), and decanoic acid (340–600 $\mu\text{g/kg}$) were the most abundant acids in the 6 milk fan samples. Butanoic acid has a rancid cheese-like odor that plays an important role in the flavor in many cheeses, such as Camembert, Cheddar, Grana Padano, and Gruyère. Hexanoic acid is also a characteristic flavor component of Grana Padano and Roncal. Octanoic acid and decanoic acids are the main odorants of the latter cheese (Curioni and Bosset, 2002).

Other major volatile groups were esters and ketones. A total of 19 esters were detected in the milk fan, the most abundant of which were ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl caprylate, and ethyl caprate. It is well known that esters are important contributors to the flavor of cheese. Based on a low threshold, they were capable of masking some bad odors caused by large amounts of short-chain free amino acids, methyl ketones, and amines (Holland et al., 2005). Ethyl acetate, ethyl butyrate, and ethyl hexanoate play important roles in the formation of the fruity properties of Parmigiano-Reggiano, Cheddar, and other cheeses (Fenster et al., 2003). The ethyl acetate content of the milk fan (210–1,220 $\mu\text{g/kg}$) was higher than that of Parmigiano-Reggiano (50–250 $\mu\text{g/kg}$). Ethyl butyrate and ethyl hexanoate (300–3,500 $\mu\text{g/kg}$) are considered key aroma compounds of cheese. The ethyl butyrate content of the milk fan (500–7,000 $\mu\text{g/kg}$) was higher than that of Mozzarella (700 $\mu\text{g/kg}$) and Emmental (260–500 $\mu\text{g/kg}$) (Liu et al., 2004). Other ester compounds may help to produce a very mild sweet background aroma (Majcher et al., 2014). Ketones are a rich constituent of many dairy products and have characteristic odors and low perception thresholds (Frank et al., 2004). Nine ketones (mostly methyl ketones) were detected in the milk fan, with 2-pentanone, 2-heptanone, 2-octanone, and 2-nonanone being the most abundant. The main sources of flavor in raw and semi-ripe cheeses are the corresponding volatile constituents of methyl ketones, which are produced by β -oxidation of acyl lipids (Matera et al., 2018). Fatty acids are the substrate for ester and methyl ketone formation (Law and Wigmore, 2007). Because of the high content of free fatty acids in milk fan, the contents of ketones and esters were also relatively high (Table 1).

Fruity, floral, and musty notes are associated with various methyl ketones, such as 2-octanone, 2-heptanone, 2-nonanone, and 2-undecanone. The herbal odor of 2-heptanone may play an important role in the formation of the fruit properties of milk fan, consistent with Emmental and Gorgonzola cheeses. These aroma compounds are believed to have a positive effect on the aroma of cheese (Lee et al., 2003).

Odor-Active Compounds Identified by GC-O

Gas-chromatography olfactometry analysis is an effective means of identifying odor-active compounds in milk fan. The olfactometry results are provided in Table 2. Thirty-one aroma compounds were detected in total. The aroma intensities of the compounds ranged from 1.00 to 4.89. Compounds with a medium or strong aroma intensity of ≥ 3 , which are likely to contribute to the aroma characteristics of milk fan, included propionic acid, butyric acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, hexanal, pentanol, hexanol, 1-octen-3-ol, 2-pentanone, ethyl butyrate, ethyl hexanoate, ethyl caprylate, and ethyl caprate. Acids were the most abundant compounds in milk fan. Eight acids were identified (Table 2): propanoic acid (aroma intensity: 1.18–4.51), butanoic acid (4.10–4.82), pentanoic acid (1.31–3.86), hexanoic acid (1.76–4.62), heptanoic acid (1.25–3.50), octanoic acid (1.50–4.73), nonanoic acid (1.55), and *n*-decanoic acid (1.39–2.70). The panelists' sensory descriptions suggested that these acids generally contributed "cheese," "rancid," "fat," and "soy" notes. These acids are widely found by GC-O in many other cheeses (Liu et al., 2004). Short- and medium-chain fatty acids (C_4 – C_{12}) have low perception thresholds and provide typical aroma notes to cheeses such as Cheddar, Gruyère, Roncal, Emmental, Camembert, and Grana Padano (Curioni and Bosset, 2002). These compounds were the main acids in milk fan, which has strong and rich flavors of milk and acid decay.

Aldehydes, esters, and ketones were also found to be important odor-active compounds of milk fan. Isovaleraldehyde, hexanal, octanal, (E)-2-heptenal, nonanal, (E)-2-octenal, 2-pentanone, 2-heptanone, 3-octanone, 2-nonanone, ethyl acetate, ethyl butyrate, ethyl valerate, and ethyl hexanoate were the odor-active compounds of milk fan. In addition to (E)-2-heptenal, (E)-2-octenal, and ethyl valerate, these compounds were also odor-active compounds in Cheddar, Mozzarella, Grana Padano, Ragusano, Emmental, and Gorgonzola cheeses (Curioni and Bosset, 2002).

Correlation of Odor-Active Compounds with Sensory Characteristics of Milk Fan

The relationships between the 31 odor-active compounds and the 10 sensory evaluation properties were explored using a PLS-DA algorithm to investigate the effect and contribution of compounds on the sensory characteristics of milk fan. The statistical results of the sensory profile analysis of the 10 odor properties are shown in Figure 2. It is well known that factors such as geography, climate, and cattle diet affect the chemical

Table 1. Average concentrations (μg/kg), relative standard deviations (RSD), and odor activity values of the volatile compounds detected in the 6 milk fan samples (Y1 to Y6)

No.	Compound ¹	RI ²		Concentration (μg/kg; RSD in % in parentheses)						Threshold (μg/kg)	Odor activity value						
		Calc	Ref	ID ³	Y1	Y2	Y3	Y4	Y5		Y6	Y1	Y2	Y3	Y4	Y5	Y6
1	Propanoic acid	1,544	1,535	MS, RI	176 (21.5)	71.5 (4.71)	373 (5.62)	— ⁴	100 (5.19)	347 (2.30)	3.00	59	24	124	—	34	116
2	Isobutyric acid	1,573	1,564	MS, RI	34.9 (2.97)	17.1 (8.18)	57.2 (11.2)	—	—	35.0 (7.47)	10.0	3	2	6	—	—	4
3	Butanoic acid	1,631	1,624	MS, RI	10,700 ^{de} (2.01)	4,510 ^b (6.11)	3,190 ^a (6.77)	6,060 ^{bc} (3.98)	6,500 (3.10)	7,030 ^d (0.980)	13.0	825	347	246	466	500	55
4	Isovaleric acid	1,677	1,682	MS, RI	129 (3.98)	31.1 (2.21)	75.5 (6.46)	—	—	65.1 (4.94)	1.00	129	32	76	—	—	65
5	Pentanoic acid	1,745	1,743	MS, RI	334 ^d (6.54)	136 ^b (3.05)	94.0 ^a (11.6)	217 ^c (5.71)	167 (4.78)	2 ^c (10.3)	0.150	2,229	92	627	1,452	1,114	1,655
6	Hexanoic acid	1,851	1,855	MS, RI	654 ^d (3.96)	3,750 ^b (0.680)	2,940 ^a (10.2)	7,090 ^d (7.85)	6,550 (3.69)	5,220 ^c (1.49)	10.0	655	375	294	80	655	523
7	Heptanoic acid	1,958	1,953	MS, RI	197 ^c (5.31)	99.8 ^a (1.23)	110 ^a (4.31)	291 ^d (4.99)	136 (11.4)	173 ^{bc} (9.94)	22.0	9	5	6	13	6	8
8	Octanoic acid	2,063	2,070	MS, RI	2,340 ^d (3.37)	1,400 ^a (4.03)	2,060 ^c (1.42)	2,750 ^c (8.35)	2,820 (2.29)	1,720 ^b (1.75)	5.10	459	275	43	538	552	337
9	Nonanoic acid	2,169	2,169	MS, RI	56.1 (8.58)	32.8 (7.82)	32.2 (9.77)	—	—	13.1 (6.67)	1.60	35	3	2	—	—	8
10	n-Decanoic acid	2,275	2,272	MS, RI	569 ^b (11.6)	360 ^a (5.93)	529 ^b (3.18)	602 ^b (1.97)	520 (2.63)	345 ^a (7.50)	5.00	114	72	16	12	15	70
11	2-Ethylpropanal	919	912	MS, RI	10.4 (5.81)	—	25.3 (5.22)	—	—	24.1 (2.66)	100	<1	—	<1	—	—	<1
12	Isovaleraldehyde	923	932	MS, RI	—	—	—	—	—	31.3 (18.0)	2.00	—	—	—	—	—	16
13	Hexanal	1,085	1,084	MS, RI	—	—	—	315 (0.580)	—	—	11.0	—	—	—	29	—	—
14	(E)-2-Hexenal	1,223	1,219	MS, RI	59.5 (1.75)	—	—	—	—	13.5 (1.43)	3.10	19	—	—	—	—	4
15	Octanal	1,292	1,299	MS, RI	83.3 (8.90)	18.2 (4.21)	27.6 (12.1)	—	12.3 (5.22)	29.3 (5.08)	0.880	95	3	31	—	14	33
16	(E)-2-Heptenal	1,328	1,332	MS, RI	—	37.0 (7.59)	—	116 (2.35)	8.44 (7.35)	44.8 (5.72)	13.0	—	3	—	9	1	3
17	Nonanal	1,397	1,396	MS, RI	169 ^c (4.91)	99.4 ^b (3.39)	184 ^{cd} (5.50)	220 ^d (9.56)	29.7 (5.63)	199 ^{cd} (11.9)	1.10	154	9	168	2	27	19
18	(E)-2-Octenal	1,436	1,434	MS, RI	45.5 (9.85)	—	14.0 (6.94)	41.1 (3.71)	—	29.9 (5.85)	3.00	15	—	5	14	—	10
19	2-Methyl-1-propanol	1,401	1,092	MS, RI	—	—	67.4 (7.39)	—	—	32.8 (7.56)	100	—	—	1	—	—	<1
20	Butanol	1,148	1,150	MS, RI	33.3 (1.88)	—	—	—	—	—	78.0	<1	—	—	—	—	—
21	3-Methyl-1-butanol	1,209	1,205	MS, RI	243 (5.02)	—	982 (4.75)	—	273 (4.90)	883 (2.64)	6.10	40	—	17	—	45	145
22	Pentanol	1,252	1,255	MS, RI	106 (3.01)	54.5 (6.90)	162 (8.54)	80.5 (4.88)	—	74.8 (1.84)	20.0	5	3	8	4	—	4
23	Hexanol	1,355	1,359	MS, RI	230 ^{ab} (14.5)	223 ^{ab} (5.26)	265 ^{bc} (9.14)	203 ^a (3.58)	180 (6.35)	314 ^c (4.75)	34.0	7	7	8	6	5	9
24	1-Octen-3-ol	1,452	1,456	MS, RI	—	41.8 (8.69)	—	27.2 (2.04)	—	—	1.00	—	42	—	27	—	—
25	2-Ethylhexanol	1,491	1,486	MS, RI	25.7 ^c (6.91)	16.6 ^{ab} (10.21)	23.8 ^{bc} (7.74)	15.7 ^c (0.830)	19.5 (2.67)	12.8 ^a (2.39)	740	<1	<1	<1	<1	<1	<1
26	Linalool	1,551	1,552	MS, RI	—	—	—	—	—	14.0 (3.12)	4.40	—	—	—	—	—	3
27	Nonanol	1,665	1,666	MS, RI	—	—	27.3 (5.92)	177 (6.06)	46.5 (5.46)	29.7 (9.56)	5.30	—	5	3	9	6	6
28	p-Cresol	2,099	2,091	MS, RI	—	—	19.7 (7.35)	53.8 (7.83)	—	17.3 (9.53)	0.240	—	—	82	224	—	72
29	2-Pentanone	984	980	MS, RI	—	1,210 (3.01)	777 (7.88)	1,940 (2.61)	—	2,050 (1.63)	98.0	—	12	8	20	—	3
30	2-Heptanone	1,186	1,180	MS, RI	4,540 ^c (9.44)	1,450 ^b (7.04)	1,250 ^b (11.4)	3,790 ^d (7.22)	20.1 (8.98)	2,090 ^c (1.68)	3.50	1,300	427	357	183	6	598
31	3-Octanone	1,253	1,259	MS, RI	—	10.1 (2.48)	—	9.65 (12.2)	8.17 (1.49)	15.7 (1.55)	1.30	—	8	—	7	6	12
32	2-Octanone	1,288	1,283	MS, RI	170 ^d (2.14)	95.8 ^b (3.41)	76.9 ^a (8.73)	138 ^c (6.64)	67.1 (4.49)	78.1 ^a (10.2)	60.0	3	2	1	2	1	1
33	6-Methyl-5-hepten-2-one	1,338	1,336	MS, RI	—	13.5 (8.26)	13.4 (6.62)	12.5 (3.74)	—	32.0 (7.81)	68.0	—	<1	<1	<1	—	—
34	γ-Nonanone	1,393	1,393	MS, RI	552 ^c (5.06)	219 ^c (7.05)	122 ^b (5.61)	470 ^d (2.17)	35.9 (3.49)	244 ^c (4.81)	32.0	17	7	4	15	1	8
35	3-Octen-2-one	1,414	1,416	MS, RI	—	—	—	—	—	9.45 (0.670)	0.0300	—	—	—	—	—	315
36	2-Decanone	1,498	1,493	MS, RI	—	—	12.3 (5.05)	28.1 (2.06)	—	64.3 (9.50)	60.0	—	—	<1	<1	—	2
37	2-Undecanone	1,604	1,598	MS, RI	18.6 ^c (8.47)	6.83 ^c (1.89)	11.3 ^b (5.71)	12.8 ^b (6.31)	12.5 (3.94)	11.4 ^b (6.94)	30.0	1	<1	<1	<1	<1	<1
38	2-Pentylfuran	1,231	1,240	MS, RI	212 ^d (8.11)	87.2 ^{bc} (5.49)	61.5 ^b (3.88)	126 ^c (10.1)	13.6 (2.36)	119 ^c (2.91)	19.0	11	5	3	7	1	6
39	Styrene	1,261	1,255	MS, RI	23.9 (8.50)	—	13.9 (5.77)	76.4 (4.56)	7.81 (6.27)	690 (2.68)	12.0	2	—	1	6	1	58
40	Benzaldehyde	1,535	1,530	MS, RI	13.6 ^c (8.52)	12.4 ^a (6.89)	15.2 ^{bc} (3.95)	12.4 ^a (2.89)	17.5 (4.27)	14.8 ^{ab} (6.66)	85.0	<1	<1	<1	<1	<1	<1
41	Acetophenone	1,668	1,669	MS, RI	—	—	13.2 (3.21)	—	—	8.78 (8.58)	1.20	—	—	2	—	—	7
42	γ-Hexanolactone	1,702	1,703	MS, RI	9.24 (1.85)	—	7.49 (3.84)	8.33 (2.53)	—	6.35 (6.50)	—	—	—	—	—	—	—
43	δ-Hexalactone	1,815	1,818	MS, RI	12.0 (3.37)	8.92 (1.36)	13.3 (14.7)	9.48 (3.25)	—	7.02 (6.99)	—	—	—	—	—	—	—
44	Benzyl alcohol	1,890	1,884	MS, RI	28.3 ^b (8.67)	35.8 ^c (10.8)	43.6 ^d (4.99)	41.0 ^{cd} (1.58)	12.7 (1.24)	39.7 ^{cd} (9.94)	250	<1	<1	<1	<1	1	<1
45	Phenylethyl alcohol	1,928	1,925	MS, RI	45.1 ^{bc} (6.87)	23.4 ^a (1.12)	42.3 ^{bc} (2.04)	57.4 ^c (7.18)	32.7 (9.31)	70.4 ^d (4.50)	21.0	2	1	2	3	2	3
46	5-Octanolide	1,998	1,999	MS, RI	14.8 (9.31)	7.24 (8.53)	12.3 (14.1)	8.41 (1.20)	—	—	420	<1	<1	<1	<1	<1	<1
47	5-Decanolide	2,238	2,229	MS, RI	14.0 (5.09)	13.5 (5.20)	—	17.0 (3.27)	13.6 (9.68)	20.7 (1.44)	66.0	<1	<1	<1	<1	<1	<1

Continued

Table 1 (Continued). Average concentrations ($\mu\text{g/kg}$), relative standard deviations (RSD), and odor activity values of the volatile compounds detected in the 6 milk fan samples (Y1 to Y6)

No.	Compound ¹	RI ²		Concentration (μg/kg; RSD in % in parentheses)						Threshold (μg/kg)		Odor activity value					
		Calc	Ref	ID ³	Y1	Y2	Y3	Y4	Y5	Y6	Y1	Y2	Y3	Y4	Y5	Y6	
48	Ethyl acetate	898	891	MS, RI	216 ^a (10.6)	717 ^c (9.52)	1,230 ^d (10.3)	454 ^b (4.05)	734 (5.21)	640 ^c (6.23)	340	1	2	4	1	2	2
49	Ethyl propionate	962	961	MS, RI	—	—	—	—	84.2 (7.58)	—	0.0270	—	—	—	—	3,118	—
50	Propyl acetate	980	977	MS, RI	—	—	49.7 (3.27)	—	42.3 (7.04)	—	200	—	—	<1	—	<1	—
51	Ethyl butyrate	1,040	1,041	MS, RI	529 ^a (7.56)	4,120 ^d (5.43)	1,340 ^b (12.8)	3,750 ^d (3.57)	7,060 (7.43)	2,180 ^c (0.800)	0.0530	9,988	77,727	26,398	7,833	133,199	4,196
52	Pentyl butyrate	1,111	1,094	MS, RI	—	12.1 (4.08)	—	—	—	—	—	—	—	—	—	—	—
53	Pentyl valerate	1,132	1,133	MS, RI	—	77.2 (7.62)	65.5 (10.3)	143 (3.00)	267 (10.7)	—	0.580	—	133	113	246	47	—
54	Isobutyl butyrate	1,162	1,160	MS, RI	—	—	—	—	16.4 (6.93)	11.4 (9.97)	9.4	—	—	—	—	2	1
55	Methyl hexanoate	1,190	1,187	MS, RI	—	—	—	—	10.7 (6.01)	—	700	—	—	—	—	<1	—
56	Butyl butyrate	1,222	1,221	MS, RI	—	—	—	—	23.1 (9.36)	—	28.0	—	—	—	—	1	—
57	Ethyl hexanoate	1,235	1,232	MS, RI	312 ^a (6.32)	2,410 ^c (4.86)	1,130 ^b (3.43)	3,520 ^d (6.55)	18,300 (1.10)	1,000 ^b (2.74)	3.00	14	82	376	1,174	6,114	334
58	Propyl hexanoate	1,317	1,316	MS, RI	—	—	—	8.96 (2.32)	103 (6.25)	—	—	—	—	—	—	—	—
59	Ethyl heptanoate	1,323	1,317	MS, RI	—	48.6 (6.84)	41.5 (5.90)	51.6 (5.66)	353 (7.54)	—	39.0	—	1	2	1	10	—
60	Butyl hexanoate	1,418	1,418	MS, RI	—	—	—	—	15.6 (9.77)	—	700	—	—	—	—	<1	—
61	Ethyl caprylate	1,438	1,440	MS, RI	72.2 ^a (10.1)	488 ^c (1.96)	1,570 ^f (2.25)	923 ^d (4.24)	8,370 (1.38)	205 ^b (7.31)	40.0	2	12	39	24	29	5
62	Propyl octanoate	1,524	1,523	MS, RI	—	—	14.7 (2.07)	—	25.1 (2.65)	—	—	—	—	—	—	—	—
63	Ethyl nonanoate	1,542	1,541	MS, RI	—	—	—	—	77.2 (5.76)	—	10.0	—	—	—	—	8	—
64	Ethyl caprate	1,646	1,648	MS, RI	30.7 ^a (3.21)	85.2 ^b (6.45)	446 ^d (2.80)	337 ^c (5.48)	1,250 (2.42)	79.1 ^b (4.87)	53.0	1	2	8	6	24	1
65	Pentyl octanoate	1,687	1,696	MS, RI	—	—	—	—	7.03 (4.67)	—	—	—	<1	<1	1	<1	<1
66	Ethyl myristate	2,055	2,054	MS, RI	—	8.90 (07.20)	20.5 (5.26)	10.0 (1.01)	57.7 (5.95)	8.73 (3.78)	180	—	<1	—	28	—	—
67	1-Octene	845	842	MS, RI	—	—	—	125 (1.46)	—	—	4.60	—	—	—	—	—	—
68	2-Octene	860	858	MS, RI	—	—	63.1 (6.72)	180 (10.6)	—	—	500	—	—	<1	<1	—	—
69	D-Limonene	1,263	1,264	MS, RI	395 ^d (3.1)	76.1 ^a (1.67)	85.0 ^{ab} (10.5)	105 ^b (0.800)	61.9 (6.64)	215 ^c (12.1)	200	2	<1	<1	1	<1	2
70	Dimethyl disulfide	1,076	1,071	MS, RI	123 (11.1)	46.0 (10.9)	135 (6.29)	25.4 (1.22)	—	83.9 (4.98)	—	—	—	—	—	—	—
71	Dimethyl sulfone	1,925	1,912	MS, RI	31.2 ^{bc} (3.9)	37.3 ^c (4.78)	47.2 ^d (6.69)	24.8 ^b (7.01)	11.7 (9.38)	33.4 ^c (6.35)	—	—	—	—	—	—	—

^{a-c}Values with different letters in a row are significantly different using Duncan's multiple comparison tests ($P < 0.05$).¹Aroma compounds detected in the milk fan samples.²The retention index of volatile compounds on HP-Innowax columns (Agilent Technologies, Santa Clara, CA); Calc = calculated; Ref = reference.³Method of identification: MS = mass spectrum comparison using NIST11.L library; RI = retention index in agreement with literature value.⁴Not detected.

Table 2. Odor-active compounds identified by chromatography-olfactometry analysis in milk fan samples Y1 to Y6 by the aroma intensity method

No.	Compound ¹	RI ²		ID ³	Aroma description	Aroma intensity ⁴ (relative SD in % in parentheses)					
		Calc	Ref			Y1	Y2	Y3	Y4	Y5	Y6
1	Propanoic acid	1,544	1,535	AD, RI	Spicy, rancid, soy	4.51 (5.40)	4.36 (3.82)	3.31 (4.90)	— ⁵	1.18 (7.41)	3.62 (9.10)
2	Butanoic acid	1,631	1,624	AD, RI	Cheese, rancid	4.21 ^a (5.82)	4.82 ^b (5.15)	4.53 ^{ab} (1.53)	4.75 ^b (3.53)	4.10 ^a (6.45)	4.27 ^a (4.72)
3	Pentanoic acid	1,745	1,743	AD, RI	Sweat	2.10 ^b (7.83)	3.86 ^c (4.38)	1.31 ^a (8.26)	2.96 ^d (2.83)	2.53 ^c (6.58)	3.81 ^c (2.13)
4	Hexanoic acid	1,851	1,855	AD, RI	Lavender, camphor	4.62 ^c (1.84)	4.21 ^c (3.96)	4.51 ^c (1.80)	2.12 ^b (3.94)	1.76 ^a (9.69)	4.58 ^c (1.85)
5	Heptanoic acid	1,958	1,953	AD, RI	Fat	2.66 ^c (6.30)	1.51 ^{ab} (10.9)	1.87 ^b (13.6)	3.50 ^d (7.08)	1.25 ^a (6.88)	2.56 ^c (6.56)
6	Octanoic acid	2,063	2,070	AD, RI	Camphor, nutmeg	1.50 ^a (10.9)	4.24 ^d (7.81)	2.28 ^b (3.71)	4.73 ^d (2.95)	1.60 ^a (10.2)	3.62 ^c (4.54)
7	Nonanoic acid	2,169	2,169	AD, RI	Green, fat	1.55 (10.9)	—	—	—	—	—
8	n-Decanoic acid	2,275	2,272	AD, RI	Rancid, fat	1.39 ^a (6.32)	2.70 ^c (6.03)	1.56 ^{ab} (10.9)	1.52 ^{ab} (0.0100)	1.51 ^{ab} (10.9)	1.89 ^b (9.10)
9	Isovaleraldehyde	923	932	AD, RI	Malt, nut	4.21 (5.85)	—	—	—	—	1.53 (5.45)
10	Hexanal	1,085	1,084	AD, RI	Green, tallow, fat	—	—	—	3.68 (9.17)	—	—
11	Octanal	1,292	1,299	AD, RI	Fat, soap, lemon, green	1.80 (4.57)	—	2.50 (3.31)	—	—	1.82 (4.51)
12	(E)-2-Heptenal	1,328	1,332	AD, RI	Soap, fat, almond	—	1.25 (6.87)	—	—	—	—
13	Nonanal	1,397	1,396	AD, RI	Green, nut, fat	1.55 ^b (5.43)	1.52 ^b (5.40)	1.03 ^a (8.25)	1.20 ^a (6.81)	1.84 ^c (9.12)	1.80 ^c (9.12)
14	(E)-2-Octenal	1,436	1,434	AD, RI	Green, nut, fat	1.63 (10.2)	—	—	1.59 (10.9)	—	1.51 (10.9)
15	Pentanol	1,252	1,255	AD, RI	Balsamic	2.93 (8.41)	4.25 (5.83)	4.52 (1.87)	3.14 (7.93)	—	3.62 (9.11)
16	Hexanol	1,355	1,359	AD, RI	Resin, flower, green	—	—	3.60 (4.51)	—	—	3.43 (2.42)
17	1-Octen-3-ol	1,452	1,456	AD, RI	Mushroom	—	—	—	3.11 (10.5)	—	—
18	Nonanol	1,665	1,666	AD, RI	Fat, green	—	—	1.26 (13.6)	—	1.54 (10.9)	2.36 (10.6)
19	p-Cresol	2,099	2,091	AD, RI	Smoke	—	—	1.18 (7.43)	1.69 (10.2)	—	1.58 (10.9)
20	2-Pentanone	984	980	AD, RI	Fruit	—	4.89 (1.71)	—	4.27 (1.90)	—	—
21	2-Heptanone	1,186	1,180	AD, RI	Soap, butter	1.50 ^a (5.40)	1.35 ^a (12.6)	1.58 ^a (10.9)	1.45 ^a (11.7)	1.26 ^a (6.80)	1.87 ^b (4.50)
22	3-Octanone	1,253	1,259	AD, RI	Herb, butter, resin	—	1.12 (7.48)	1.42 (5.82)	1.82 (9.14)	1.59 (10.9)	1.97 (12.9)
23	2-Nonanone	1,393	1,393	AD, RI	Cheese, butter, strawberry	—	—	1.52 (5.49)	2.83 (11.7)	—	2.89 (5.86)
24	Acetophenone	1,668	1,669	AD, RI	Almond, flower	—	—	1.30 (12.6)	—	—	1.50 (5.48)
25	Ethyl acetate	898	891	AD, RI	Fruit, pineapple, grape	1.22 (13.7)	—	—	1.19 (7.41)	—	—
26	Ethyl butyrate	1,040	1,041	AD, RI	Fruit, pineapple	3.69 ^b (4.52)	4.22 ^c (5.87)	1.64 ^a (10.2)	3.37 ^b (4.90)	4.71 ^d (1.71)	3.54 ^b (2.39)
27	Ethyl valerate	1,132	1,133	AD, RI	Yeast, fruit	—	2.11 (7.89)	—	—	1.44 (5.80)	—
28	Ethyl hexanoate	1,235	1,232	AD, RI	Fruit, apple, pineapple	1.56 ^a (10.9)	4.34 ^c (5.76)	4.64 ^c (3.58)	4.44 ^c (6.04)	3.50 ^b (7.01)	3.85 ^b (2.17)
29	Ethyl caprylate	1,438	1,440	AD, RI	Apricot, banana, pineapple	4.67 ^c (1.81)	4.81 ^c (5.10)	4.18 ^{ab} (4.01)	3.85 ^a (2.19)	4.53 ^{bc} (7.37)	4.41 ^{bc} (1.96)
30	Ethyl caprate	1,646	1,648	AD, RI	Grape	—	—	3.86 (2.15)	3.21 (7.73)	—	—
31	D-Limonene	1,263	1,264	AD, RI	Citrus, mint	1.64 (5.18)	—	—	—	—	1.80 (13.6)

^{a-c}Values with different letters in a row are significantly different using Duncan's multiple comparison tests ($P < 0.05$).

¹Aroma compounds detected in the milk fan samples.

²The retention index of volatile compounds on HP-Innowax columns (Agilent Technologies, Santa Clara, CA); Calc = calculated; Ref = reference.

³Method of identification: AD = aroma descriptor; RI = retention index in agreement with literature value.

⁴Aroma intensity was evaluated by GC-olfactometry.

⁵Not detected.

composition of milk, which in turn affects the sensory quality of milk and cheese (Carpino et al., 2004; Rapisarda et al., 2013). Based on the Dengchuan region's particular geographical climate, rich vegetation, and breed of dairy cattle, the milk from this area has a strong flavor and high fat content, which give milk fan its rich and unique flavor. The prominent aromas of the milk fan are cheesy, milky, sour, fruit, fatty, and rancid. The overall aroma is typically mild. Some pungent odors, such as sulfur and musty, are not obvious. In general, acid and acid- or heat-coagulated cheeses have a mild or acidic aroma. They are usually fresh cheeses. The “sour” and “rancid” descriptors were significantly different between samples ($P < 0.05$), which might be related to the processing technology of milk fan and the amount of papaya acid juice added.

Twenty-three of the pairwise associations between the odor-active compounds and the sensory evaluations were significant (Figure 3). Nonanoic acid was positively correlated with nutty properties; octanoic acid and propanoic acid were positively correlated with cheesy properties; pentanoic acid was negatively correlated with sulfurous properties; butanoic acid, 2-nonanone, and 2-heptanone were positively correlated with rancid properties; nonanol was positively correlated with fatty properties and negatively correlated with fruity properties; octanal was positively correlated with fatty and nutty properties; (E)-2-octenal was positively correlated with rancid properties and negatively correlated with cheesy and sulfurous properties; ethyl caprylate and ethyl hexanoate were positively correlated with fruity properties; ethyl butyrate was positively correlated with cheesy and fruity properties and negatively

correlated with nutty properties; ethyl valerate was positively correlated with fruity properties and negatively correlated with nutty properties; and D-limonene was positively correlated with rancid properties and negatively correlated with cheesy properties. Hence, these compounds (nonanoic acid, octanoic acid, propanoic acid, pentanoic acid, butanoic acid, 2-nonanone, 2-heptanone, nonanol, octanal, (E)-2-octenal, ethyl caprylate, ethyl hexanoate, ethyl butyrate, ethyl valerate, and D-limonene) seem to be the initial key aroma compounds of milk fan and may contribute to the overall aroma of the cheese.

Aroma Recombination and Omission Experiments

An aroma recombination experiment was performed to provide further information about the initial key aroma compounds. Omission experiments were performed to evaluate the contribution of the initial key aroma compounds to the overall aroma of milk fan and to identify the key aroma compounds of milk fan. Figure 4 shows that the aroma profile of the recombinant was similar to that of the original milk fan, sample Y6. The sensory properties (fruity, cheesy, milky, sour, rancid, musty, nutty, umami, sulfurous, and fatty) were generally consistent with those of Y6. Only 2 properties (fruity and rancid) showed a slight but nonsignificant difference ($P > 0.05$), suggesting a higher amount of the corresponding aroma compounds. The results showed that the typical aroma of milk fan was successfully simulated by combining 23 initial odor-active compounds (at their natural concentrations in Y6).

In the omission experiment, 23 aroma omission models were studied. The data (Table 3) suggested that all panelists could detect the missing group of acids in model 1, with a highly significant ($P \leq 0.001$) difference between the omission model and the recombination model. This indicates that these fatty acid odors play a key role in the overall aroma of milk fan. Furthermore, models 1-1, 1-2, and 1-6 (lacking propanoic acid, butanoic acid, and octanoic acid, respectively) were significantly different ($P \leq 0.001$) from the recombination model. Therefore, propanoic acid, butanoic acid, and octanoic acid might be important contributors to the aroma of milk fan. Similar results were found for models 2, 2-2, 2-3, 4, 4-2, 5, and 5-2, indicating that octanal, nonanal, 2-nonanone, and ethyl hexanoate might also play a key role in the aroma. These results provide further evidence that these are indeed the key aroma compounds in milk fan, confirming the GC-O results. Pentanoic acid (OAV of 92–2,230), hexanoic acid (OAV of 80–655), heptanoic acid (OAV of 5–13), and decanoic acid (OAV of 12–114) had high OAV (Table 2). However, in the triangle tests, only 6, 3, 5,

Figure 2. Aroma profiles of milk fan obtained from the Y1, Y2, Y3, Y4, Y5, and Y6 samples. The asterisks indicate that values are significantly different using Duncan's multiple comparison tests ($P < 0.05$).

Figure 3. Correlations between odor-active components and sensory properties of 6 milk fan samples by partial least squares discriminant analysis. Gray lines in the circle represent positive correlations and black lines represent negative correlations between odor-active components and sensory properties. Ten sensory odor properties and 31 odor-active components are shown, as listed in Table 2.

and 6 of the 15 panelists ($P > 0.05$) were correct in detecting the omission models 1–3, 1–4, 1–5, and 1–7, respectively. These 4 compounds were thus not considered to be key aroma contributors. Similar results were

found for models 2–1, 2–4, 3, 4–1, 4–3, 5–1, 5–3, 6, and 7, which were missing isovaleraldehyde, (E)-2-octenal, pentanol, hexanol, nonanol, 2-heptanone, 3-octanone, ethyl butyrate, ethyl caprylate, acetophenone, *p*-cresol, and D-limonene, respectively, indicating that these compounds had no significant effect on the aroma of milk fan.

In summary, the key aroma profile of milk fan consisted of propanoic acid, butanoic acid, octanoic acid, octanal, nonanal, 2-nonanone, and ethyl hexanoate. Propanoic acid and butanoic acid contribute to the cheese and rancid aromas, octanal and nonanal contribute to the fat aroma, octanoic acid and 2-nonanone contribute to the cheese aroma, and ethyl hexanoate contributes to the fruity aroma.

CONCLUSIONS

The aroma characteristics of 6 milk fan cheeses were studied using GC-MS, GC-O, sensory evaluation, PLS-DA, and aroma recombination and omission experiments. Seventy-one aroma compounds were detected by GC-MS, and 31 were identified as odor-active com-

Figure 4. Aroma profile of the Y6 sample of milk fan and the complete aroma recombination.

Table 3. Omission experiments from the complete recombination model

Number	Odorants omitted from the recombination model	N ¹	Significance
1	Propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, and decanoic acid	15	***
1-1	Propanoic acid	9	*
1-2	Butanoic acid	10	**
1-3	Pentanoic acid	6	
1-4	Hexanoic acid	3	
1-5	Heptanoic acid	5	
1-6	Octanoic acid	9	*
1-7	Decanoic acid	6	
2	Isovaleraldehyde, octanal, nonanal, and (E)-2-octenal	9	*
2-1	Isovaleraldehyde	6	
2-2	Octanal	9	*
2-3	Nonanal	9	*
2-4	(E)-2-Octenal	5	
3	Pentanol, hexanol, nonanol	8	
4	2-Heptanone, 2-nonanone, and 3-octanone	10	**
4-1	2-Heptanone	4	
4-2	2-Nonanone	9	*
4-3	3-Octanone	5	
5	Ethyl butyrate, ethyl hexanoate, and ethyl caprylate	9	*
5-1	Ethyl butyrate	6	
5-2	Ethyl hexanoate	9	*
5-3	Ethyl caprylate	3	
6	Acetophenone and <i>p</i> -cresol	6	
7	D-Limonene	6	

¹Number of correct judgments from 15 panelists evaluating the aroma difference by means of a triangle test.

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

pounds by GC-O. The PLS-DA results showed that the initial key aroma compounds had good correlations with sensory properties. Based on the aroma recombination and omission experiments, 7 odor-active compounds were further identified as the key aroma compounds: 3 acids (propanoic, butanoic, and octanoic), 2 aldehydes (octanal and nonanal), and 2-nonanone and ethyl hexanoate. In future work, nonvolatile flavor compounds should be analyzed to better understand the flavor characteristics of milk fan.

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