Characterization of the key odorants in kurut with aroma recombination and omission studies

B. Wang,^{1,2*} J. Wang,¹ L. Y. Xu,¹ J. H. Zhang,¹ N. S. Ai,^{1,2} and Y. P. Cao^{1,2}

¹School of Food and Health, Beijing Technology and Business University, Beijing, 100048, China

²Beijing Key Laboratory of Flavor Chemistry, Beijing Technology and Business University, Beijing, 100048, China

ABSTRACT

Kurut is a traditional acid-coagulated cheese from the northwest region of China. Using gas chromatography olfactometry and aroma extract dilution analysis, we identified 21 potent odorants from kurut within the flavor dilution factor range of 8 to 256. We developed a kurut matrix to determine the odor thresholds of key aroma compounds in kurut. The odor activity values of these 21 potent volatile compounds revealed that 18 were present at concentrations above their odor threshold values and therefore contributed to the overall aroma of kurut. The result of aroma recombinant experiments prepared by mixing the 18 most important odorants at the concentrations in which they occurred in kurut showed that the overall aroma profile of the recombinant sample was very similar to that of kurut. Omission experiments indicated that acids, furans, esters, and ketones were the most important volatile compounds in kurut. Some aroma compounds—such as butanoic acid, homofuraneol, ethyl hexanoate, and ethyl butanoate—play the most important roles in the overall flavor profile of kurut. Some odorants with a high flavor dilution factor, such as sulfur compounds, may have little effect. The study of key aroma compounds in kurut could provide important information for researching and developing traditional Chinese cheese products.

Key words: kurut, aroma extract dilution analysis, odor activity value, aroma recombination and omission

INTRODUCTION

Kurut is a traditional dairy product from the northwest region of China, and it can also be found in Turkey and some Middle Eastern countries (Kamber, 2008). In some pasture regions of China, it is difficult to ship fresh milk to a milk processing factory in time, so herd-

ers process the fresh milk into kurut to enable storage. Although kurut is sometimes described as dried yogurt (Seçkin et al., 2009), it is considered a hard cheese by most researchers, because its manufacture has a whey draining step, and it has a high dry matter content.

Kurut is a white or light yellow dried, fermented dairy food with unique organoleptic sensations of acidity. In China, kurut is usually made with fresh bovine milk; the processing steps for kurut are shown in Figure 1. Kurut is usually prepared in an open environment at 10 to 20°C for a relatively long fermentation period (2 to 5 d). The initial manufacturing process is similar to that of yogurt, but at the end of production, fermentation is maintained until the kurut naturally coagulates, followed by whey draining. Then, the curd is given a round shape in lumps of 40 to 60 g and 3 to 5 cm in diameter. Salt is added to the kurut curd at the 1.5 to 3% (wt/wt) to preserve it (Liu et al., 2012). Finally, kurut pieces are dried in the sun over 5 to 10 d. The final moisture content of kurut is approximately 10%.

Flavor is one of the key characteristics that determine cheese quality. Numerous research papers have described the volatile profiles of cheeses, such as Cheddar (Arora et al., 1995), blue cheese (Qian et al., 2002), and ewe milk cheese (Majcher and Jeleń, 2011). However, few flavor related-studies on kurut are available; most kurut-related papers have focused on microbiological diversity (Chen et al., 2015; Ispirli and Dertli, 2017) or the manufacturing process (Karabulut et al., 2007). The study of the volatile compounds in kurut could provide important information about traditional Chinese cheese products. The aim of this study was to determine the volatile compounds involved in the flavor of kurut. First, we identified the most potent odorants of kurut using GC olfactometry and aroma extract dilution analysis (AEDA). Then, we determined the odor activity values (OAV) of key aroma compounds identified by AEDA using quantitative measurements. Finally, to assess the effect of the potent odorants identified in kurut, we prepared reconstituted and omitted models and evaluated them in sensory experiments. We developed a novel kurut matrix to determine the

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*Corresponding author: wangbei@th.btbu.edu.cn

Figure 1. The process of making kurut in the Altay region of Xinjiang, China.

odor thresholds in OAV calculations and carry out the reconstitution and omission experiments.

MATERIALS AND METHODS

Materials

We obtained 3 kurut samples (approximately 300 g each) made from bovine raw milk from the Altay region of Xinjiang, all handmade by 3 local herding families. The cheesemaking process was as shown in Figure 1. All samples were collected and kept in ice boxes during transportation. Once in the laboratory, the samples were immediately stored at -80°C .

Reagents and Chemicals

Diethyl ether, sodium bicarbonate, hydrochloric acid, and sodium sulfate were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Serial n-alkanes (C7–C40) were purchased from O2si Smart Solutions (North Charleston, SC). The standards butanoic acid (99%), furaneol (98%), acetic acid (99%), acetoin (98%), methional (99%), 2-heptanone (98%), and homofuraneol (98%) were purchased from Macklin Biochemical Co. Ltd. (Shanghai, China). Hexanoic acid (99%), ethyl caprylate (98%), ethyl caprate (99%), hexanal (98%), furfural (98%), 2-nonanone (99%), (E)-2-nonenal (99%), ethyl hexanoate (98%), phenethyl alcohol (99%), ethyl butanoate (99%), 2,3-butanedione (98%), nonanal (99%), octanoic acid (99%), limonene (95%), methanethiol (2,000 $\mu\text{g/mL}$ in toluene), octanal (99%), 2-undecanone (99%), and dimethyl trisulfide (98%) were obtained from Sigma-Aldrich AccuStandard (Beijing, China).

Sample Preparation

Kurut samples were prepared according to the method described by Majcher and Jeleń (2011) with some modifications. To obtain representative samples, we peeled

approximately 1 cm from the surface of the 3 samples, and then cut the peelings into small pieces and mixed them together. The mixing sample was frozen in liquid nitrogen and ground to obtain a homogenized sample. The main components of the final mixing sample and their analysis methods are as follows: protein content $62.5 \pm 2.87\%$ (Kjeldahl method; Rouch et al., 2008), fat content $11.8 \pm 0.84\%$ (Gerber method; Rahimi et al., 2007), water content $9.6 \pm 0.43\%$ (oven-drying method; Xie and He, 2009).

Next, 100 g of ground kurut was transferred to an Erlenmeyer flask and extracted with diethyl ether (200 mL) at 350 rpm in an incubator shaker at room temperature for 7 h. The residual substances were separated by centrifuging at $3,000 \times g$ for 15 min at 4°C . To further concentrate the volatile compounds, the solvent extract was performed using a solvent-assisted flavor evaporation (SAFE) method.

Solvent-Assisted Flavor Evaporation

Volatile compounds from kurut were distilled using SAFE as described by Engel et al. (1999). The SAFE apparatus was connected to a receiving tube and a waste tube. The glassware was then connected to an Edwards nXDS 6i rotary vane pump (Edwards, West Sussex, UK) as the vacuum source. The SAFE apparatus was thermostatically controlled at 40°C using a circulating water bath. Liquid nitrogen was poured into the cooling trap and the receiver flasks. Distillation was carried out for 2 h under vacuum ($\sim 10^{-4}$ Pa). After distillation, the distillate was dried with anhydrous sodium sulfate overnight, and then concentrated to about 1.0 mL using a Kuderna-Danish concentrator.

Phase Separation

To eliminate the influence of high concentrations of acid compounds on other volatile compounds, a phase separation procedure was applied (Drake et al., 2010). The concentrated distillate was washed 3 times with 1

mL 0.5 M sodium bicarbonate and mixed thoroughly. After each wash, the bottom layer, which was the water phase of the distillate, was removed and collected in a separate test tube. The concentrated solvent was then washed 3 times with 1 mL of saturated sodium chloride solution. Each time, the solution was mixed thoroughly and the water phase removed and collected in the same test tube. The remaining layer was collected as the neutral or basic fraction. Acidic fraction volatiles were recovered by acidifying the water layer collected from the concentrated distillate with hydrochloric acid (18% wt/vol) to a pH of 2.0 to 2.5. The acidified sample was then extracted 3 times with 3 mL of ethyl ether. All extracts were dried over anhydrous sodium sulfate before concentration to 0.5 mL under a nitrogen gas stream.

GC-MS Analysis

We carried out GC-MS analysis using an Agilent 7890B/5977A instrument (Agilent Technologies, Santa Clara, CA). Concentrated fractions of samples (2.0 μ L) were individually analyzed by GC olfactometry and GC-MS. The samples were injected using a PAL 3 RTC autosampler with a 10- μ L direct injection syringe attached. The injector temperature was 250°C. Compounds were separated on DB-5 and DB-Wax (each 30 m long $\times$ 0.32 mm i.d. $\times$ 0.25 μ m film thickness; J&W Scientific, Folsom, CA). The GC oven temperature program used was as follows: DB-5: 35°C for 3 min, increased by 5°C/min to 100°C and held for 2 min, increased by 4°C/min to 150°C, and then increased by 10°C/min to 230°C and held for 3 min to clean the column; DB-WAX: 35°C for 3 min, raised by 4°C/min to 120°C, then 5°C/min to 190°C, and then increased by 10°C/min to 230°C and held for 3 min to clean the column. The GC-MS transfer line temperature was maintained at 250°C. The mass spectrometer was operated in electron impact mode, and the source temperature was set at 220°C. The mass spectrometer operated in full scan mode from 33 to 450 amu. All analyses were performed using 70 eV.

We identified constituents by comparing the mass spectra, retention indices, and characteristic aromas on 2 columns of different polarities with authentic standards. We calculated the retention index using the C7–C40 n-alkane series under the same chromatographic conditions.

Aroma Extract Dilution Analysis

Flavor dilution (FD) factors were determined for the neutral or basic fraction and the acidic fraction by GC olfactometry and AEDA analysis on a capillary column

with DB-WAX. Odor active regions were detected by GC olfactometry equipped with an ODP2 sniffing port (Olfactory Detector Port; Gerstel, Beijing, China). The extract was diluted stepwise 2-fold with solvent by volume to obtain dilutions of 1:2, 1:4, 1:8, and so on, with dilutions injected into the GC olfactometer, and each sample of the dilution series was analyzed until no odor was clearly identified at the sniffing port.

Four experienced panelists described the GC olfactometry volatiles. Each panelist evaluated the sample once in 2 time segments of 45 min to avoid fatigue. All analyses were repeated in triplicate by each panelist. Before experiments, these panelists had been trained, including sniffing 57 reference compounds determined by some pre-experiments to contribute to kurut flavor; each reference compound was prepared for panelists in amounts adjusted to 3- to 5-fold above their odor thresholds in pure solvent (water or sunflower oil; Van Gemert, 2011).

Quantitation of Key Aroma Compounds

The isolates obtained by SAFE were quantitated, and 3-methy-pentanoic acid (for the carboxylic acids quantitation) and 2-methyl-3-heptanone (for the other compounds quantitation) were used as internal standards. The standard curve for individual volatile aroma compound was built up by plotting the response ratio of target compounds and corresponding internal standards against the concentration ratio (Gao et al., 2014). The limits of quantification (LOQ) and detection (LOD) were estimated as the concentrations of the analyte of a standard that produced a signal-to-noise ratio of 10 and 3 times, respectively. Selective ion monitoring MS was used to quantitate the aroma compounds.

Determination of Odor Detection Threshold in Cheese Matrix

To calculate the OAV of key aroma compounds identified by AEDA, we determined their odor detection thresholds in kurut matrix based on Czerny et al. (2008). A sensory panel of 10 panelists was asked to determine the thresholds of key aroma compounds in kurut matrix. The sensory panel (6 women and 4 men, average age 28 yr) was experienced in orthonasal odor perception.

The kurut matrix used in threshold determination was prepared as follows (Wang et al., 2018): milk protein concentrate powder (MPC 80 Saishang Dairy Company, Ningxia, China) and extra pure water were mixed and pasteurized as the substrate. The substrate was incubated with rennet (CHY-MAX Power NB,

Chr. Hansen, Horsholm, Denmark) at 37°C until it coagulated. After draining the whey, we prepared a fresh rennet curd as the primary kurut matrix.

To establish an ideal matrix system, we built the kurut matrix to simulate its consumption process. Odorless sunflower oil (fat phase), ultra-pure water (water phase), and rennet curd (protein phase) were mixed at the same concentration as in kurut (kurut:water = 1:1 wt/wt). The pH value of the kurut matrix system was adjusted to pH 4.0 by adding a trace amount of a 1% aqueous solution of formic acid (Toelstede and Hofmann, 2008). After homogenization, the final kurut matrix was prepared.

Descriptive Profile Tests

The same sensory panel applied in threshold tests was asked to sniff the flavor profile of different samples. The 7 odor descriptors discussed by the sensory panel were chosen as the most relevant odors to describe the overall aroma of kurut, as shown in Table 1. The assessors were asked to evaluate the odor intensity of these attributes by orthonasally scoring 8 odor qualities on a 5-cm linear scale anchored on either side for the intensity of attributes as “none” and “very strong” (Majcher and Jeleń, 2011). The obtained results were averaged for each odor note and plotted in a spider web diagram. The values, judged by a single assessor, differed by no more than 20%. All sensory tests were conducted in a room with a temperature of approximately 25°C.

Aroma Reconstitution

The matrix of the reconstituted kurut model (**RKM**) was prepared in the same way as the kurut matrix for threshold determination. As a reference, the kurut model (**KM**) was blended and diluted twice with extra-pure water. Then, 30 mL RKM and 30 mL KM were placed in glass vessels (50 mL) and presented to the sensory panel. In the first session, the aroma profile of the RKM was evaluated; in the second session, the

aroma profile of the KM was evaluated. All evaluations were performed in triplicate.

Omission Experiments

Triangle tests were performed to determine the significance of certain compounds according to Buettner and Schieberle (2001). To do this, 16 omission models were prepared in the same manner as the RKM, omitting one component or a group of components each time. Three samples, including 1 omission model and 2 completely reconstituted models, were presented simultaneously to sensory assessors. The same sensory panel as for the reconstitution experiment, was used, but this time they were asked whether differences were detectable. Sensory data were analyzed using 1-way ANOVA in SPSS 17.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Odor Active Compounds in Kurut Identified by AEDA

As a geographically specific dairy food, kurut is a very complicated physical and biochemical matrix, and its flavor develops in this system through a series of chemical and biochemical reactions during the cheese-making process. We evaluated the volatile concentrates of this cheese product by combining solvent extraction and SAFE techniques. The volatile concentrate obtained by this sampling operation without excessive heating accurately reflected the characteristic aroma of this cheese product.

We found 28 compounds ($FD \geq 1$) that contributed to the kurut aroma according to AEDA; 26 were identified by MS, retention index, aroma character, and reference samples, and the intensities of the odor notes expressed as FD factors ranged between 1 and 256 (Table 2). Six compounds had the highest FD values ($FD \geq 64$; Table 2), and these compounds contributed 4 distinct aroma notes, described as cheesy, sweaty (butanoic acid); fruity (ethyl hexanoate); caramel, sweet,

Table 1. Terms and definitions used in the sensory evaluation of kurut

Term	Definition	Reference
Caramel	Aromatic associated with burnt sugar or syrup	Caramel pigment
Yogurt-like	Aromatic associated with yogurt	2,3-Butanedione (0.1% in water)
Fruity	Aromatic associated with different fruits	Fresh pineapple
Acid	Aromatic associated with sour, tangy, sharp	Acetic acid (0.5% in water)
Creamy	Aromatic associated with cream, milk	Fresh cream
Cheesy	Aromatic associated with cheese	Butanoic acid (0.1% in water)
Sulfur	Aromatic associated with cabbage, boiled potato	Boiled mashed egg

Table 2. Aroma compounds identified in an aroma distillate prepared from kurut¹

No.	Aroma compound	Odor quality ²	FD	Retention index (RI)		Method of identification
				DB-5	DB-WAX	
1	Butanoic acid	Cheesy, sweaty	256	802	1,629	RI, MS, O, Std
2	Ethyl hexanoate	Fruity	128	1,028	1,236	RI, MS, O, Std
3	Homofuraneol	Caramel, sweet	128	1,115	2,017	RI, MS, O, Std
4	Methional	Potato	64	911	1,448	RI, MS, O, Std
5	Furaneol	Caramel, burnt	64	1,051	1,932	RI, MS, O, Std
6	Unknown ³	Fruity	64	ND	ND	O
7	Acetic acid	Sour	32	603	1,468	RI, MS, O, Std
8	Acetoin	Creamy	32	659	1,275	RI, MS, O, Std
9	Ethyl butanoate	Fruity	32	797	1,038	RI, MS, O, Std
10	2-Heptanone	Banana	32	895	1,172	RI, MS, O, Std
11	Hexanoic acid	Sweaty, rancid	32	982	1,856	RI, MS, O, Std
12	Ethyl caprylate	Fruity	32	1,203	1,436	RI, MS, O, Std
13	Ethyl caprate	Wine-like	32	1,398	1,639	RI, MS, O, Std
14	Unknown ³	Wood-like	32	ND	ND	O
15	2,3-Butanedione	Creamy	16	572	951	RI, MS, O, Std
16	Hexanal	Green	16	801	1,073	RI, MS, O, Std
17	Furfural	Nutty	16	840	1,451	RI, MS, O, Std
18	2-Nonanone	Rose-like	16	913	1,389	RI, MS, O, Std
19	(E)-2-Nonenal	Green, fatty	16	1,153	1,526	RI, MS, O, Std
20	Phenethyl alcohol	Sweet, rose-like	8	714	1,092	RI, MS, O, Std
21	Nonanal	Musty	8	1,121	1,391	RI, MS, O, Std
22	Octanoic acid	Sweaty, cheesy	4	1,155	2,083	RI, MS, O, Std
23	3-Methylbutanoic acid	Rancid	4	877	1,665	RI, MS, O, Std
24	Limonene	Lemon, orange	2	1,025	1,178	RI, MS, O, Std
25	Methanethiol	Garlic	2	472	696	RI, MS, O, Std
26	Octanal	Soap, green	2	1,006	1,280	RI, MS, O, Std
27	2-Undecanone	Orange, fresh	1	1,096	1,543	RI, MS, O, Std
28	Dimethyl trisulfide	Cabbage	1	973	1,071	RI, MS, O, Std

¹FD = flavor dilution factor; MS = mass spectra; ND = not determined; O = odor; Std = standard compounds.

²Odor quality as perceived at the sniffing port during GC olfactometry.

³Unknown = not identified.

burnt (homofuraneol, furaneol); and potato (methional). The FD values of 14 compounds were between 8 and 32: acetic acid, acetoin, ethyl butanoate, 2-heptanone, hexanoic acid, ethyl caprylate, ethyl caprate, 2,3-butanedione, hexanal, furfural, 2-nonanone, (E)-2-nonenal, phenethyl alcohol, nonanal; they probably had an effect on the aroma of kurut and may contribute especially to the background aromas, including sour, creamy, fruity, sweaty, rancid, wine-like, green, nutty, rose-like, fatty, sweet and musty. Although detectable, the contributions of octanoic acid, 3-methyl butanoic acid, limonene, methanethiol, octanal, 2-undecanone and dimethyl disulphide were probably relatively small ($1 \leq \text{FD} \leq 8$). Two unknown compounds were also detectable with a fruity note (FD = 64) and wood-like note (FD = 32); their unknown status may have been due to their extremely low concentrates and thresholds.

Based on the results of AEDA, the key aroma compounds of kurut can be divided into several groups according to their chemical structure: carboxylic acids, furans, esters, lactones, methyl ketones, sulfur compounds, alcohols, and aldehydes. Carboxylic acids

(acetic acid, butanoic acid, 3-methyl butanoic acid, hexanoic acid, and octanoic acid) are all well known as important key aroma components of many cheese types, and they also serve as precursors of methyl ketones, alcohols, lactones, and esters. Apart from acetic acid, which is derived from the metabolism of lactose to lactate by lactic acid bacteria (McSweeney, 2004), most carboxylic acids originate from lipolysis of milk fat, and they could contribute a unique cheesy and sweaty flavor to kurut (Curioni and Bosset, 2002).

Furans are a series of lipophilic compounds with a sweet and caramel-like odor, and most of these are produced during thermal degradation reactions. As a result of their pleasant odor, furans have been widely applied as a synthetic flavoring agent during food manufacture (Morehouse et al., 2008). Furans have been reported at very low levels in low-fat Cheddar (Milo and Reineccius, 1997) or in Emmental (Preininger and Grosch, 1994), and these compounds could contribute caramel and nutty flavor to cheese. Furans identified in kurut (homofuraneol, furaneol, furfural) could be also responsible for its pleasant caramel-like note. They may come

from the manufacturing process of air drying, given the high local surface temperature (approximately 70 to 80°C) during the day.

Esters are common cheese volatile compounds, and some ethyl esters contain short-medium-chain fatty acids. Esterification reactions during cheese processing occur between short- to medium-chain fatty acids and primary and secondary alcohols derived from lactose fermentation or from amino acid catabolism (Bosset and Liardon, 1984). Esterase of lactic acid bacteria can also produce esters via a transesterification reaction, especially in hard cheese, where low water activity would increase the related reaction (Holland et al., 2005). The key esters we found in kurut were ethyl hexanoate, ethyl butanoate, ethyl caprylate, and ethyl caprate. These compounds were described as having sweet, fruity, creamy notes, and could provide a positive odor note to kurut.

Ketones, especially methyl ketones, are produced by partial β -oxidation of free fatty acids and are normally dominant in mold-ripened cheeses (Qian et al., 2002). Most methyl ketones identified in kurut with strong odor intensity—acetoin, 2-heptanone, 2,3-butanedione, 2-nonanone, and 2-undecanone—have a creamy and fruity flavor, and they may provide a pleasant milky note to kurut.

Volatile sulfur compounds are key flavor compounds in a variety of cheeses, and they originate mainly from the decomposition of sulfur-containing amino acids such as cysteine and methionine (Harper et al., 2011). Due to their low odor thresholds, they have very pronounced sensory properties, often related to notes of potato, garlic, or very ripe cheese, even at low concentrations (Fuchsmann et al., 2015). The most important of these compounds found by AEDA in kurut was methional, which could be produced by Strecker degradation (Christensen and Reineccius, 1995). Methional provides a boiled potato odor and has been found in many cheeses, such as Camembert (Karahadian et al., 1985) and Cheddar (Rehman et al., 2000). Methional could also be a precursor of methanethiol (FD = 2), which was also identified in kurut with a relatively lower aroma intensity (Landaud et al., 2008).

In addition to the volatile compounds above, we also found aldehydes, alcohols, and terpenes as aroma active compounds, but their relatively weak volatile intensities suggested that they have little effect on the sensory profile of kurut.

Quantification of Key Odorants in Kurut and Their OAV

The results of AEDA cannot provide information about the contribution of a single odorant to overall

flavor because they are based on the odor threshold in air, so the contribution of single volatile to a sample aroma profile might be overestimated. Moreover, possible losses of odorants during the sniff procedure of AEDA due to olfactory fatigue are not fully taken into account.

The olfactory intensity of each volatile compound in a sample is highly related to its concentration and odor threshold. To account for these 2 parameters, we applied OAV to describe the intensity of odorant compounds in sample (Grosch, 1993). To investigate the contribution of individual compounds to the kurut aroma, we further determined the OAV of 19 aroma compounds (FD $\geq$ 8) identified by AEDA.

Generally speaking, odor threshold values are taken from the literature or determined in a simple matrix, such as water (Bosse et al., 2013) or alcohol solution (Frank et al., 2011), using a dilution method. It is usually difficult to determine the thresholds of some volatile compounds in a complex matrix such as cheese. To get as close as possible to kurut consumption, we determined the thresholds of volatile compounds related to the kurut aroma profile in a kurut matrix, composed mainly of milk protein concentrate made into cheese curd, sunflower oil, and water. All thresholds were determined in kurut matrix for the first time. As shown in Table 3, odorants such as acetic acid had a fairly high threshold of 8,600 $\mu\text{g/kg}$ in the kurut matrix. However, the thresholds of some compounds present in tiny amounts in kurut were extremely low, such as methional and 2,3-butanedione. In our study, methional was determined to have a threshold value of 10 $\mu\text{g/kg}$, approximately 6 times higher than that reported in water (Grosch, 1994). The threshold value of 2,3-butanedione in kurut matrix was 15 $\mu\text{g/kg}$, 4.5 times higher than that in water (Van Gemert, 2011). These data revealed that the complicated matrix of kurut might lead to higher thresholds than in a simple matrix.

The findings for the OAV of 19 odorants in kurut are listed in Table 3, and most were similar to AEDA results. The highest OAV among the 19 odorants was ethyl hexanoate (9.0), followed by ethyl butanoate (6.9), butanoic acid (6.5), homofuraneol (6.4), and acetic acid (6.4). Further compounds with high OAV (>1) were acetoin (4.3), 2,3-butanedione (4.3), furaneol (3.4), ethyl caprylate (2.6), hexanal (2.5), nonanal (2.36), hexanoic acid (2.3), ethyl caprate (2.3), (E)-2-nonenal (2.18), methional (2.1), 2-heptanone (1.7), 2-nonanone (1.5), and phenethyl alcohol (1.32). Among the 19 volatile compounds (FD $\geq$ 8) identified based on AEDA, only furfural showed OAV less than 1 (0.96) so it is unlikely to contribute significantly to the kurut flavor profile. The main reason for the different results

Table 3. Standard curves, concentrations, and odor active values (OAV) of 19 odorants in kurut¹

Aroma compound	m/z^2	Calibration variable						Threshold ⁴ ($\mu\text{g/kg}$)	OAV
		Slope	Intercept	Linear range ($\mu\text{g/kg}$)	R^2	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Average ³ ($\mu\text{g/kg}$)	SD
Butanoic acid	41, 60, * 73	58.35	-1.22	3,551-40,563	0.9973	0.08	0.24	9,763.4	828.6
Ethyl hexanoate	29, 43, 88*	133.4	0.48	43-5,022	0.9886	0.35	1.05	343.7	130.2
Homofuranol	43, 57, 128*	13.8	0.36	20-794	0.9982	0.72	2.16	161.5	42.5
Methional	47,* 48, 104	51.6	-1.03	8-257	0.9734	0.03	0.09	21.8	6.1
Furanol	43,* 57, 85	4.93	-0.34	20-702	0.9997	0.47	1.41	121.3	12.7
Acetic acid	43,* 45, 60	137	2.85	8,503-149,855	0.9954	1.6	4.8	55,007.2	2,265.6
Acetoin	45,* 43, 88	7.23	-0.34	108-2,433	0.9853	0.2	0.6	433.9	65.2
Ethyl butanoate	43, 71,* 88	2.33	0.46	19-663	0.9993	0.35	1.05	278.2	47.4
2-Heptanone	43,* 58, 71	7.02	0.051	125-4,558	0.9974	2.11	6.33	852.3	92.4
Hexanoic acid	41, 60,* 73	1.08	-0.39	4,352-24,898	0.9996	0.76	2.28	10,421.2	566
Ethyl caprylate	88,* 101, 127	42	1.76	247-2,206	0.9957	0.37	1.11	572.4	395.5
Ethyl caprate	73, 88,* 107	0.412	-0.038	152-1,803	0.9992	0.45	1.35	421.3	283.7
2,3-Butanedione	42, 43,* 86	105	29.6	42-784	0.9587	0.56	1.68	64.8	84.6
Hexanal	41, 44,* 56	5.4	0.041	13-554	0.9968	0.69	2.07	123.9	23.4
Furfural	29, 39, 96*	5.38	0.422	1,038-6,133	0.9986	0.88	2.64	2,896.8	162.3
2-Nonanone	43,* 58, 71	72.5	14.2	20-839	0.9989	0.24	0.72	622.9	48.6
(E)-2-Nonenal	43,* 55, 70	31.6	3.22	43-454	0.9782	0.05	0.15	52.3	64.3
Phenethyl alcohol	77, 91,* 122	25.3	1.97	440-3,006	0.9985	0.12	0.36	1,250.6	96.8
Nonanal	41, 57,* 98	9.04	-1.89	37-1,533	0.9703	0.02	0.06	99.3	143.2

¹LOD = limit of detection; LOQ = limit of quantification.²Quantifying and qualifying ions; * = quantitation ion.³Average concentration of triplicates.⁴Threshold was evaluated in kurut matrix.

Figure 2. Aroma profile analysis of the kurut model and the reconstituted kurut model.

between FD factors and OAV is that the odorants present in aroma extract were vaporized during GC olfactometry analysis, whereas OAV were calculated using odor thresholds in matrix, in which only the amount of an individual odorant was present in the headspace above the matrix.

Combining the results of the AEDA and OAV analyses, the key odorants in kurut were fatty acids (butanoic acid, hexanoic acid, acetic acid), furans (homofuraneol, furaneol), esters (ethyl hexanoate, ethyl butanoate, ethyl caprylate, ethyl caprylate), ketones (acetoin, 2-heptanone, 2,3 butanedione, 2-nonanone), sulfur compounds (methional), alcohol (phenethyl alcohol), and aldehydes (hexanal, (E)-2nonenal, nonanal) ($FD \geq 8$ and $OAV \geq 1$); these 18 odorants were very important to the kurut aroma.

Aroma Reconstitution Experiments

It is difficult to predict the overall aroma of a complex mixture of compounds; aroma reconstitution experiments could show whether the kurut aroma profile can be mimicked by a mixture of key odorants in their natural concentrations. For this purpose, we mixed all 18 aroma compounds with $FD \geq 8$ and $OAV \geq 1$ in half the concentrations as they would occur in kurut. To build the RKM, we used the same kurut matrix we used for to threshold determination.

We conducted descriptive profile tests using the same sensory panel as in the thresholds test, and we further compared the odor attributes of the RKM and the KM. Figure 2 shows that the intensities of fruity, sulfur, and creamy notes were rated much higher and the intensities of caramel and yogurt-like attributes were rated slightly higher in the KM than in the RKM. The cheesy and acid attributes of the KM were rated lower than the RKM. Aroma is an extremely complicated olfactory sensation and is characterized by the synergistic work of multiple compounds, so it is difficult to create an identical flavor profile between the RKM and the KM. For the examined attributes, the aroma of the recombinant model was comparable to the KM. This result indicated success in simulating the typical aroma of kurut.

Aroma Omission Experiments

To undertake a deeper investigation of the aroma contribution of certain compounds, we prepared 16 aroma omission models by omitting either a group of odorants or a single odorant with higher OAV (Table 4). Each of the omission models was presented to the assessors in comparison with the RKM, using a triangle test. The results are shown in Table 4.

In the omission tests, all assessors were able to detect the omission model that lacked entire groups of acids

Table 4. Sensory evaluation for the aroma model of kurut as affected by the omission of compounds

No.	Compound omitted	N ¹	Significance
I	All acids	10	***
Ia	Butanoic acid	10	***
Ib	Acetic acid	7	*
II	All furans	10	***
IIa	Homofuraneol	8	**
IIb	Furaneol	7	*
III	All esters	9	***
IIIa	Ethyl hexanoate	8	**
IIIb	Ethyl butanoate	7	**
IV	All ketones	8	**
IVa	Acetoin	6	*
IVb	2-Heptanone	4	—
V	All aldehydes	6	*
Va	Hexanal	5	—
VI	Methional	7	*
VII	Phenethyl alcohol	5	—

¹Number of correct judgments from 10 assessors evaluating the aroma difference by means of a triangle test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(model I), furans (model II), and esters (model III), leading to highly significant differences ($P < 0.001$) between the omission model and recombinant model. We also observed a highly significant difference ($P < 0.001$) when butanoic acid (model Ia) was omitted. These results revealed the important role of these cheesy, sour, caramel, and fruity note odorants to the overall aroma, as shown in the results for AEDA and OAV, as well as in Figure 2.

The absence of all ketones (homofuraneol, ethyl hexanoate, and ethyl butanoate) was detectable with significance ($P \leq 0.01$) by sensory evaluation, suggesting the importance of these compounds to the aroma profile of kurut. Ethyl butanoate and ethyl hexanoate are traditionally regarded as potent aroma compounds for the fruity note in cheese because of their particularly low aroma thresholds, and in our study of their FD and OAV, the aroma contribution of ethyl hexanoate was obviously than that of ethyl butanoate. The omission experiment for these compounds indicated similar results for these components, which may have been due to the extremely low threshold of ethyl butanoate or the susceptibility of the assessors.

A difference was detectable when acetic acid, furaneol, acetoin, methional, and all aldehydes were individually omitted ($P \leq 0.05$). Due to their extremely low thresholds, volatile sulfur compounds have very pronounced sensory properties of cabbage-like and garlic aromas, even at low concentrations. Many volatile sulfur compounds have been reported as key flavor compounds in a variety of cheeses (Frank et al., 2011). It is interesting that although sulfur compounds have a unique and easily identified aroma, they had a comparably high FD

and low OAV in kurut. Compared with the omission experiment results, a flavor synergy effect among sulfur compounds and other volatiles might weaken the contribution of sulfur compounds in the final flavor profile of kurut.

The individual absence of 2-heptanone, hexanal, and phenethyl alcohol was correctly detected by fewer than 5 of 10 assessors in triangle tests. Therefore, we found no significant differences for these omission models compared to the reconstituted model and excluded these compounds as important aroma contributors.

CONCLUSIONS

The results of this study showed that responses to the sensory profile of kurut could be closely mimicked using a mixture of 18 volatile compounds in identical concentrations to those present in kurut. This study had limitations, including the relatively simplified kurut model matrix, which ignored the influence of nonvolatile compositions. The determination of key aroma compounds in kurut will contribute to a better understanding of this traditional Chinese cheese.

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ORCIDS

B. Wang <https://orcid.org/0000-0001-5407-7810>

J. Wang <https://orcid.org/0000-0003-0981-6033>